Food Bioactive Ingredients

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Editors

Nutraceuticals and Cancer Signaling

Clinical Aspects and Mode of Action

Springer

Food Bioactive Ingredients

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Preface

Cancer burden is the second leading cause of death at global level, after cardiovascular diseases. Nowadays, strategies to treat cancer include surgery, radiotherapy, chemotherapy and immunotherapy. In spite of significant advances in the last few years, surgery is still the most effective cancer treatment.

Hippocrates advocated 'Let food be thy medicine and medicine be thy food'. During the past 2000 years, from Hippocrates till modern medicines era, there was no distinction between food and medicine. Foods are a source of many nutrients and bioactive compounds which some of them are associated with cancer preventive properties. These include carotenoids such as lycopene, β-carotene and astaxanthin, polyphenols such as quercetin, resveratrol and (-)-epigallocatechin-3-gallate and phytoestrogens such as genistein and daidzein.

The present book addresses the role of different food components in chemoprevention and chemotherapy of cancer based on scientific evidence. Bioactive compounds and nutraceuticals can be of great significance in the prevention of cancer or for the effective treatment of cancer, mainly enhancing the effectiveness and/or minimizing the side effects of other therapies. In fact, some food components have already proven their effectiveness for the combat against cancer in clinical trials. This book aims to be an instrument for medical doctors, pharmacists as well as all health practitioners who wish to access scientific evidence on different bioactives and nutraceuticals in the prevention and treatment of cancer.

In the first part of the book, an overview of cancer is presented (Chap. 1) as well as the mechanisms involved in carcinogenesis process (Chap. 2). Chapter 1 addresses the impact of cancer worldwide, main causes of cancer and most common types of cancer. Moreover, the main treatments are also overviewed. Chapter 2 dedicates to the different mechanisms involved in carcinogenesis process. The elucidation of the molecular pathways of carcinogenic substances, including chemicals, radiations, viruses and parasites, offers a better understanding of how genetic manipulation influences the mechanism of cancer evolution.

The second part of the book is composed of 16 chapters dedicated to functional foods/nutraceuticals with potential as chemopreventive agents due to their composition of bioactive compounds. These include tomato (lycopene and β-carotene; Chap.

3), aromatic plants (essential oils; Chap. 4), bee propolis (caffeic acid phenethyl ester, Chap. 5), brown algae (fucoxanthin; Chap. 6), cruciferous vegetables (indole-3-carbinol, isothiocyanates; Chap. 7), crustacea (carotenoids namely astaxanthin; Chap. 8), curcuma (curcumin; Chap. 9), fruits and vegetables (flavonoids, namely quercetin and resveratrol; dietary fibres, carotenoids; Chap. 10), garlic (allylsulfur compounds; Chap. 11), ginger (gingerols and 6-shogaol; Chap. 12), saffron (crocins; Chap. 13), olive leaf (oleuropein; Chap. 14), honey (Chap. 15), soybeans and phytoestrogens-rich foods (genistein, daidzein; Chap. 16), tea (catechins; Chap. 17), yoghurt and fermented foods (probiotics; Chap. 18). The relevant chapters give information on the molecular targets of nutraceuticals, synthetic analogues of chemopreventive agents and clinical trials already performed.

The third part of the book is dedicated to the role of nutrients in the prevention of cancer including vitamins (namely C, D, E; Chap. 19), minerals (namely selenium; Chap. 20), dietary fibres/beta-glucan (Chap. 21) and omega-3 fatty acids (Chap. 22).

We are conscious of other functional foods/nutraceuticals and nutrients which could have been addressed. We look forward that this edition will be well received and hopefully in the close future, other functional foods/nutraceuticals and nutrients can be addressed in this emerging area.

We really appreciate the great cooperation of all authors of the chapters for taking time from their busy schedules to contribute to this project. Also, it is necessary to express our sincere thanks to all the editorial staff at Springer for their help and support throughout the project. Finally, special acknowledgement is to our family for their understanding and encouragement during the editing of this great project.

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Part I
An Overview of Cancer and Its Mechanisms

Chapter 1
A Brief Overview of Cancer, Its Mechanisms, and Prevention Methods

Ana Sanches Silva, Seyed Mohammad Nabavi, and Seid Mahdi Jafari

Abstract Cancer is a wide-reaching burden that affects differently diverse regions. In this chapter, the impact of cancer worldwide, main causes of cancer and most common types of cancer will be accessed. Moreover, the main treatments will also be addressed including surgery, radiotherapy, chemotherapy and immunotherapy. The understand of the causes of cancer, including environmental, genetic, and behavioral factors, will allow to better prevent cancer as well as to choose the most suitable treatment. In this regard, the use of some bioactives or nutraceuticals to prevent or combat cancer can be of great value. This book will update readers on the scientific evidence of different bioactive and nutraceuticals in the prevention and treatment of cancer.

Keywords Cancer · Prevention · Screening · Treatment · Radiotherapy · Chemotherapy · Immunotherapy · Bioactives · Nutraceuticals

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1 Cancer Over Time

Cancer, also called malignant tumour or neoplasm, is defined by the WHO as a "*large group of diseases that can start in almost any organ or tissue of the body when abnormal cells grow uncontrollably, go beyond their usual boundaries to invade adjoining parts of the body and/or spread to other organs*" (WHO 2020).

Cancer was firstly reported in the Edwin Smith Papyrus in 3000 BC, a surgical document. In this Papyrus, women with breast cancer were described (Kane et al. 2019). In 400 BC, Hippocrates has described different types of cancer. In the nineteenth century there was significant improvements in the treatment of cancer due to the better understanding of pathology at cellular level and the advances in antiseptics and anesthetics, which made possible the surgery involving the removal of the organ containing the tumour and lymph node drainage. The twentieth century replaced the complete removal of the affected organ by a combination of radiotherapy, selective surgery (just part of the affected organ was removed) and frequently adjuvant therapy (Sikora 2020).

The impact of cancer at pan level is impressive. Cancer is the second leading cause of death globally, accounting for an estimated 9.6 million deaths, or one in six deaths, in 2018 (WHO 2020). It is expected that in 2030, there will be around 25 million new cancer patients each year, most of them in countries with less resources for cancer control (Sikora 2020). According to WHO, 9.6 million people worldwide are estimated to die from cancer in 2018. It is the second leading cause of death globally. Although it affects more adults, 300,000 new cases of cancer are diagnosed each year among children aged bellow 19 years. It is estimated that about 30–50% of cancers could be prevented (WHO 2020).

The most common types of cancer differ between genders. In men the most prevalent are lung, prostate, colorectal, stomach and liver cancer. On the other hand, in women the most common are breast, colorectal, lung, cervical and thyroid cancer (WHO 2020). Over the past decade, the cancer incidence rate (2006–2015) was stable in women and declined by approximately 2% per year in men, whereas the cancer death rate (2007–2016) declined annually by 1.4% and 1.8%, respectively.

According to annual updates on cancer occurrence and trends annual report elaborated by the American Cancer Society (ACS), the Centers for Disease Control and Prevention (CDC), the National Cancer Institute (NCI), and the North American Association of Central Cancer Registries (NAACCR), between 2008 and 2014 the cancer incidence rate was stable in women and decreased about 2.2% per year in men in the United States (Cronin et al. 2018). The cancer death rate between 2007 and 2016 also decreased 1.4 and 1.8% in women and men, respectively, in the United States (Siegel et al. 2019). Cancer mortality is slowly decreasing, however there are more socioeconomic inequalities which are more notable in what concerns to preventable cancers (Siegel et al. 2019). The American Cancer Society also discusses the incidence and mortality of different types of cancer such as the colorectal cancer (Siegel et al. 2020) and specific age groups such as Adolescents and Young Adults (Miller et al. 2020).

Cancer does not only affect individuals physical and psychologically but also their families. Moreover, it has a high economic impact in the community and health systems. In fact, US$ 1.16 trillion is the estimated total annual economic cost of cancer in 2010 (WHO 2020). In high income countries the survival rates of cancer are higher than in low- and middle-income countries thanks to the string health systems that allow early detection, effective treatment and survivorship care (WHO 2020).

Feng et al. (2019) reviewed several reports on cancer, including the Global cancer statistics 2018 and Cancer statistics in China, 2015, along with the GLOBCAN 2018 online database, to discuss possible differences of cancer patterns among the United States of America (USA), the United Kingdom (UK) and China. Compared to the USA and UK, China has lower cancer incidence than USA and UK however it has higher cancer mortality (30% and 40% more than the UK and USA, respectively). In China about 40% of the cancer-related deaths were from the digestive tract cancers (stomach, liver, and esophagus cancer) while in EUA and UK digestive cancer deaths only represent less than 5% of the total cancer deaths. In addition to a high occurrence of infection-related and digestive cancers, in China there has been a rapid increase of colorectal, prostate, female breast cancers due to the westernized lifestyle (Feng et al. 2019).

To accelerate the progresses to gain the battle against cancer burden, it would be of utmost importance to enlarge the coverage of effective screening programs, specially to low income population groups, to allow their access to basic health care, vaccination programs and educational programs in order to have smoking cessation and education on healthier lifestyle. In the human body there is about 10^{13} cells and multiplication can face dysregulated cells that can multiply indefinitely (Sikora 2020). However, the biggest issue of cancer is not the local growth of tumour cells but their spread to other sites of the body through invasion or metastasis. Due to the impressive advances in the treatment of cancer, now it is possible the cure of this disease even if diagnosed in an advanced stage (Sikora 2020).

2 Cancer Prevention

Cancer prevention is better than cure. Although prevention of other deadly diseases has already been achieved, namely through the use of vaccines, the goal of cancer prevention is a tortuous path because many environmental, genetic, and behavioural factors contribute for different types of cancer (Bode and Dong 2009). In it generally accepted by the scientific community that to reduce the cancer risk worldwide is of utmost importance to avoid animal fat, reduce the intake of red meat, increase the consumption of fiber and of fruits and vegetables, eliminating exposure to carcinogens, such as alcohol and tobacco and to exercise in order to avoid obesity (McCullough and Giovannucci 2004; Umar et al. 2012).

Another cause of cancer are infections caused by virus, such as hepatitis B and human papilloma virus and bacteria such as *Helicobacter pylori* (Moore and Chang

2010; Moore and Chang 2017). The control of these infections can greatly reduce through the use of vaccines or through the control of the safety of all steps of food chain (Sikora 2020). The future of cancer prevention will lay on a personalized program based on lifestyle and environmental data.

Nowadays one of the tools to detect cancer in earlier stages is the use of screening tests. These are tests applied to an individual who does not require medical attention and should be simultaneously specific (avoid false results) and sensitive (detect all the cancers). Although the test can reduce cancer mortality due to allow earlier detection of the cancer, when the cancer is metastised does not prolong survival (Sikora 2020).

It is important to define rational screening programs that allow to improve survival rates in certain types of cancer. Some papers report the rational used for targeting of population groups and residential areas for cancer, namely colorectal cancer (Strömberg et al. 2019), breast cancer. These should be adapted to the reality of each country and should have a cost that corresponds to the gain in survival rates (Sikora 2020).

Screening targets only few organs that are affected by tumors of sufficient prevalence to show cost-effectiveness at population levels. Therefore, most types of cancer are not screened. Ahlquist defends a multi-organ cancer screening considering its great advantages including to provide an "universalized" value because it can detect all cancer types and an "individualized" value because indicated the likely organ of origin when it gives a positive result (Ahlquist 2018).

Some cancers present various symptoms from early stages but others are asymptomatic and are only detected when they are spread. The diagnosis of cancer as an emergency is generally accompanying with a markedly worse prognosis; though, there is still lack of data on this subject, being this limited to data from developed countries which have examined frequency and aetiology of cancer (Zhou et al. 2017).

The diagnosis of cancer has greatly improved thanks to imaging, namely computed tomography (CT) and magnetic resonance imaging (MRI) and biomarkers. Several biomarkers have been used to diagnosis cancer as prostate specific antigen (PSA) for the prostate cancer diagnosis (Sikora 2020).

Le Duff et al. (2019) reported a project that aimed to evaluate the impact of the provision of a test in pharmacies to screening for colorectal cancer in Corsica. The paper describes the procedure used to mobilize the pharmacists of the territory, provide them the test kits and accompanies the participants in the test. According to the authors the project had a very positive impact with a realized rate of 36% over 9 months (Le Duff et al. 2019). In one decade, it is likely that tests based on cancer biomarkers are available in the pharmacy to diagnose the major cancers or that implanted devises can detect cancer at early stages and be monitored through a computer or mobile phone (Sikora 2020).

3 Treatment of Cancer

The main focus of cancer treatments are surgery, radiotherapy, chemotherapy and immunotherapy. Although the progresses, surgery is the most effective cancer treatment. Surgery advances between the nineteenth century and nowadays are impressive. Surgery started to be radical, i.e., with complete removal of the affected organ and of the lymph node(s) of the area, but nowadays is more conservative allowing to maintain organs through minimally invasive surgeries (Sikora 2020).

Radioterapy has also evoluted significantly since its first use, over one century ago. This technique allows to destroy cancer cells and keep unaffected the normal cells through the use of radiation. The key factors for the effectiveness of the treatment with radiotherapy is the precise application of the radiation. This greatly improved thanks to computer-based imaging that allows deliver precisely the radiation according to the type, size and site of the tumour (Sikora 2020).

Radioterapy has many side effects and some of them can reduce the quality of life of patients. In this line intensity-modulated radiotherapy (IMRT) and image-guided radiotherapy (IGRT) are now routinely used, in combination, in order to reduce the side effects of radiotherapy (Bujold et al. 2012; Samuelian et al. 2012; Sikora 2020).

Another factor that can reduce the lack of effectiveness of radiotherapy is the resistance to radiation. According to scientific evidence microRNAs modulate key cellular pathways that mediate response to radiation, so microRNAs might have potential as targets for the development of new therapeutic strategies to battle cancer (Mueller et al. 2016).

Immunotherapy is a cancer treatment that mobilizes immune system of the individuals in order to recognize and induce cancer cell destruction. This will stop or slow down the growth of the tumor, avoid cancer cells spread (metastasis) or aid immune system to better detect and destroy cancer cells (Dougan and Dranoff 2012; Sikora 2020). There are different types of immunotherapies. These include non-specific immunotherapies like interferon and interleukins (Bassiony et al. 2020; Sikora 2020). Also cancer vaccines are used as immunotherapies (Banchereau and Palucka 2018; Grenier et al. 2018; Hollingsworth and Jansen 2019). These can prevent cancer like human papillomavirus vaccine. This vaccine avoids the infection with human papillomavirus that is responsible for cervical cancer and other types of cancer. Other vaccines are used to target cancer cells in order to treat cancer. Sipuleucel-T is a licensed vaccine for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer (Caram et al. 2019; Hu et al. 2016; Wei et al. 2018).

Many studies have combined radiotherapy with immunotherapy and they have proven to be effective (Wang et al. 2018). The synergy between these two therapies allows to increase the visibility of tumor antigens, to activate the cGAS-STING pathway, and to modulate the tumor microenvironment. Immunotherapy can act as a “radiation sensitizer” improving tumor local control but to increase the

effectiveness of this combination of therapies it is necessary to optimize radiation dose and time and to identify potential biomarkers (Wang et al. 2018).

Monoclonal antibodies are proteins that fight infection because they attach to specific proteins in the cancer cell and they allow immune system to destroy cancer cells, deliver drugs directly to cancer cells, or deliver radiation to cancer cells (radioimmunotherapy: a radioactive molecule is attached to monoclonal antibodies). Also therapies with monoclonal antibodies also present side effects these are generally mild and most of times similar to an allergic reaction. (Sikora 2020; Zahavi and Weiner 2020).

Many drugs are used to treat cancer. The number of the approved drugs increases every year and they have very different cellular targets. These include cell-surface receptors, signal transduction cog molecules, transcription factors, apoptosis- stimulating proteins, growth factors and cell-cycle control proteins. Some cancers such as Hodgkin disease, childhood leukemia and testicular cancer respond very well to chemotherapy (Morton 2020). However, other types have low cure rates such as colon, stomach and lung cancer.

4 Natural Food Compounds and Cancer

Many food components have been associated with chemopreventive properties. These include lycopene from tomatoes (Paur et al. 2017), resveratrol from grapes (Berman et al. 2017), curcumin from curcuma (Hesari et al. 2019), omega-3-fatty acids from fish (Eltweri et al. 2017), among others. The present book dedicates to the update and discussion of the role of different food components in chemoprevention and chemotherapy of cancer based on scientific evidence. Bioactive compounds and nutraceuticals can be of great value in the prevention of cancer or for the effective treatment of cancer, mainly enhancing the effectiveness and/or minimizing the side effects of other therapies. In fact, some food components have already proved their effectiveness the combat against cancer in clinical trials.

5 Remarking Conclusions

While the number of cancer patients will continue to rise, the knowledge regarding the causes and the number of effective therapeutics against this global burden is also expanding. This chapter overviewed impact of cancer worldwide, main causes of cancer, most common types of cancer and main treatments, including surgery, radiotherapy, chemotherapy and immunotherapy.

As stated by Sikora, the “molecular signatures” of the cancer will determine the treatment choice (Sikora 2020). The tendency on the treatment of cancer is to have available therapies able to have specific targets (more selective), less toxic and with less side effects. In this regard, bioactive compounds present in food can have a

major role in the prevention and treatment of cancer, mainly enhancing the effectiveness and/or minimizing the side effects of other chemotherapies. This book updates the scientific evidence of different bioactive and nutraceuticals as potential munitions in the effort to minimize the burden of cancer.

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Chapter 2
Mechanisms Involved in Carcinogenesis

Chandramohan Kiruthiga and Kasi Pandima Devi

Abstract The initiation of human cancer is primarily driven by carcinogenic substances including chemicals, radiations, viruses, and parasites. The carcinogenesis mechanism is a complex process in which cellular DNA mutations contribute to the initiation, which is the first step, and seems to be irreversible. The second stage is promoted over a long period and is largely reversible in initial stages. The key events for the carcinogenesis process tend to be epigenetic. Cancer genes are classified by their ability to regulate oncogenesis as the dominant oncogenes and recessive tumor suppressors. Activation of oncogenes may be due to the occurrence of mutations in these genes. Besides, a single sufficiently activated oncogene will initiate the entire process of the cancerous transition of a normal cell. Their function in cancer growth has been widely demonstrated in experimental studies involving viruses and chromosome translocations. Furthermore, micro-RNAs (miRNAs) are preserved throughout development and regulate gene expression during cell proliferation, growth, and even in cancer progression by an unidentified control mechanism. miRNAs also play a crucial function in malignancy. The discovery and elucidation of the carcinogenic molecular pathways of carcinogens provide a deeper understanding of how genetic manipulation influences the mechanism of neoplastic development. The current chapter explains the different mechanisms involved in the carcinogenesis process.

Keywords Cancer · Influential factors · Mechanisms · Carcinogenesis · Tumors · Signalling pathways

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1 Introduction

Cancer is a debilitating and life-threatening disease. 5–10% of human tumors are believed to be induced by virus and bacteria, and the remaining 90–95% by environmental factors due to alterations in genes. Among these, an additional 30% were induced with the consumption of tobacco-related products and the remaining by food, and environment-related chemicals. Cancer cells are generated by our own tissues, but several internal and external causes can be connected to the risk of getting cancer for a lifetime (Yokota 2000). Although cancer as such is not contagious, certain infections may serve as a stimulus to induce and facilitate the proliferation of cancer cells. In the 1970s, cancer was defined in a pathology text by Cappell and Anderson, who presented malignancy by describing a tumor as "an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the surrounding tissue, and that continues to grow in the same excessive manner after cessation of the stimulus that caused it". The basis of cancer is monoclonal, so genetic mutations may arise on it, in order for a regular cell to alter its shape and become a neoplastic cell. Such genetic mutations change the proteins which the gene will codify under normal conditions and ultimately cause cancer (Mendelsohn et al. 2008; Vancheri 2016). Carcinogenesis may result from anyone or a mixture of chemical, physical-biological, and genetic disruptions to single cells in a multicellular animal. The analysis of the general carcinogenesis process takes into account the vast number of factors concerned, the prolonged period between the function of a cause and the clinical occurrence of the disease, which makes it hard to accept the pathophysiological importance of certain microorganisms. The carcinogenic substance is nucleophilic whether it functions directly or indirectly. The target of the carcinogenic component is chromosomal DNA, where a lesion can be replicated or reversed (Murphy and Charnay-Sonnek 2019).

The carcinogenesis theories can be grouped as follows: the theory of genetic mutation, the theory of aberrant differentiation, viral theory, and the theory of cell selection. A theory which is unanimously accepted is the multi-stage theory (Hart and Turturro 1988). Carcinogenesis is a complex process because there are several phases between the initial carcinogenic stimulation and the final cancer manifestation. The time between the exposure of a carcinogen on chromosomal genes and the emergence of a neoplastic cell population can be categorized into the following phases: initiation, promotion, and progression (Barrett 1993).

2 Phases of Carcinogenesis: Initiation, Promotion, and Progression

The incidence of tumors in humans and animals will rise in many different types of carcinogenic exposure, but it usually takes a long time before the carcinogenic risk of exposure is manipulated. Berenbaum and Schubik first introduced the concept of multi-stage carcinogenesis in 1948 (Berenblum and Shubik 1949), and later confirmed by studies. Foulds, L. (Neoplastic Development, Academic, New York, 1969), had the

insight of its stage development in the evolutionary history of cancer and Berenblum pointed out three distinct stages: the phase of initiation, the phase of promotion, and the phase of progression (Rubin 1994; Weiss 2004). Certainly, the firsttwo phases help to explain the cell transformation mechanism, the third level dictates the conversion of a benign tumor into a malignant type, with malignancy sustaining and evolving (Fig. 2.1).

The established multi-stage carcinogenic paradigm typically involves more than 80 alterations or modifications in the cancer genome, which are the key players for cancer growth pathways. Carcinogenesis hallmarks involve genetic alterations comprehending: maintaining proliferative signalling; preventing growth suppressors; suppressing apoptosis; facilitating replicative longevity; triggering angiogenesis; initiating invasion and metastasis; implanting energy metabolism, and preventing immune depletion (Hanahan and Robert 2017).

2.1 Initiation

During research on skin carcinogenesis in mice, the pathogenesis of initiation and promotion were initially identified and have since been extended to a range of other tissues and organisms (Abel et al. 2009). A regular cell endures an irreversible transition during the initiation phase of carcinogenesis, represented by an intrinsic ability for autonomous growth. For weeks to years, this potential for autonomous development persists latent, during that period the activated cell can be genetically differentiated from entire parenchymal cells in a particular tissue region. Spontaneous initiation will arise when the operation of DNA polymerase throughout normal cell proliferation or DNA repair becomes abnormal. Operational activation infers that cellular DNA alteration occurs at one or more locations inside the genome (Stratton et al. 2009; Vogelstein et al. 2013). This modification reflects a genetic mutational phenomenon. Within limited hours of exposure, there is metabolic activation of a carcinogen and its subsequent reaction to target DNA bases. Most tissues have the capacity over days or weeks to repair this damage. Currently accepted theory indicates that, if not initially restored by natural cellular processes, the carcinogen compromised DNA is transformed into a permanent genetic lesion through DNA replication. Therefore, the genetic lesion is then believed to be "secure" if a round of cell division occurs until the DNA damage is corrected. This effect can clarify the high prevalence of neoplasms in multiplying tissue, in which the cell turnover rate correlates with exposure to a carcinogen. Contrary to the initiation stage, the conversion of an initiated cell to a completely malignant neoplasm is typically a protracted phase, in animals lasting months and in human's years (Oliveira et al. 2007). Depending on the possibility that most initiators are mutagenic or genotoxic, the changes that arise during initiation, trigger a permanent and inherited existence. Initiators associate in specific patterns with host cellular macromolecules and nucleic acids, usually entails the production of reactive species or free radicals that covalently attach in crucial cellular macromolecule nuclear sites.

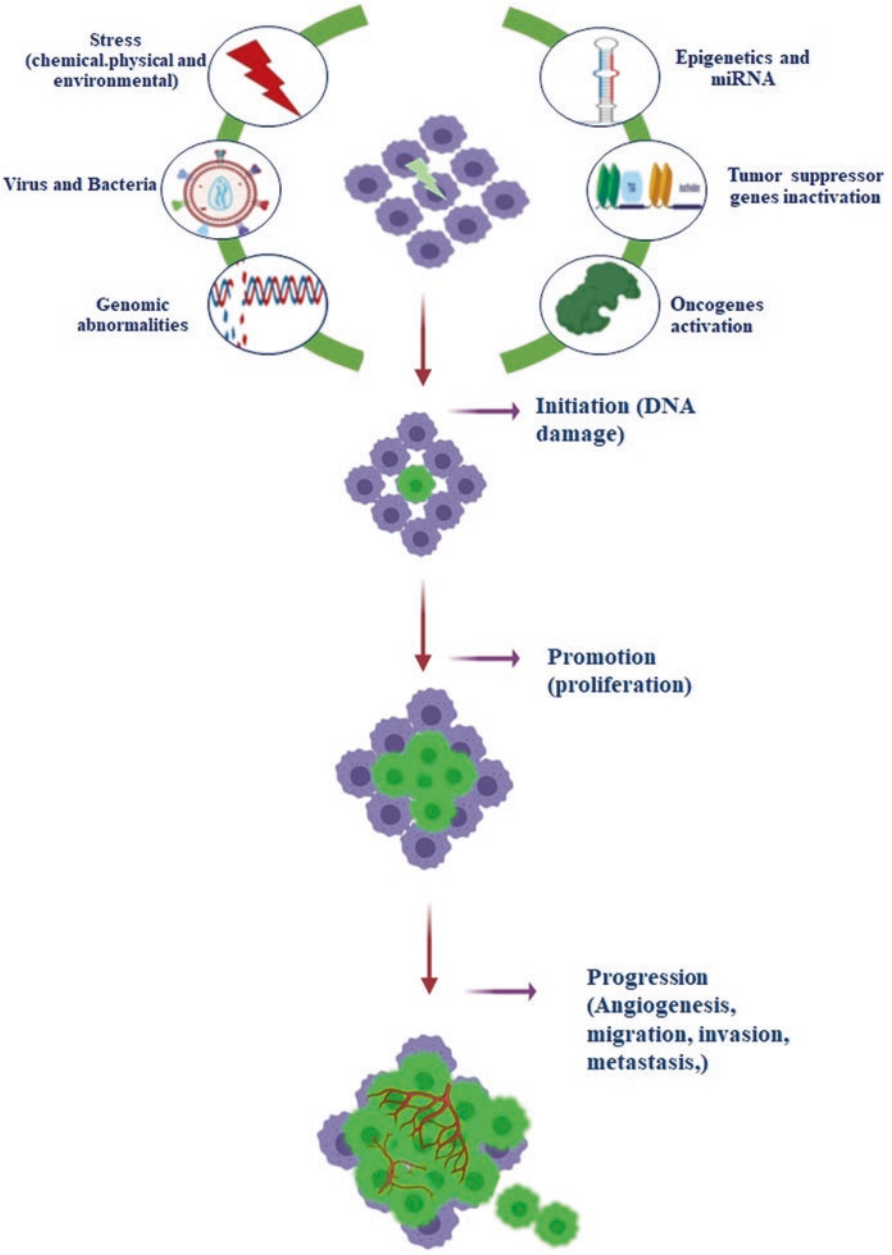

Fig. 2.1 Phases of carcinogenesis: Carcinogenesis phases: initiation, promotion, progression. The effect of anyone or a combination of factors such as chemical, physical, biological, environmental, and/or genetic alterations on cells may eventually lead to carcinogenesis. Such modification initiates the cell which acquires/loses different functional aspects (proliferation conduct, cell death pathway modified, etc.). In the Promotion process, the activated clone is intensified, and the cell acquires metastatic potential through development as well as through mutations

Several pieces of evidence propose that contact of laboratory animals to chemicals with initiating operation inevitably leads to several neoplasms caused in a specified tissue. That specific neoplasm is regularly shown to have initially been monoclonal, started to emerge from a particular induced cell(Vincent and Gatenby 2008; Abel and DiGiovanni 2011). In addition to that, initiation is exponential and neoplasm yield is carcinogen concentration-dependent. Adding the dosage of initiator improves the frequency and abundance of resultant neoplasms, and decreases the time to neoplasm appearance. Since a round of cell proliferation will repair the initiating case, It is evident that initiation relies on the cell division (Barrett 1993; Grizzi et al. 2006). Yet the optimal dosage for maximum and minimum initiator response may differ between individuals.

2.2 Promotion

Many recognized carcinogens have both initiating and promoting action and, if consistently delivered, may cause neoplasms rapidly and with high yield. A cell that has experienced an irreversible transition enabling its eventual neoplastic transformation conversion may possibly be phenotypically identical from the neighboring standard parenchymal cells. It has, nevertheless, inherent potential for autonomous development if adequately stimulated. General characteristics of the initiator and promotor are summarized in Table 2.1.

Classically, promotion is called a portion of the multi-stage carcinogenic mechanism where particular substances, referred to as promoters, facilitate the production of neoplasms from the context of induced cell population. Typically, after initiation, a promoter is administered at some point, and the concentrations of the promoter used will be inadequate for cancer development. However, when the promoters are delivered at relatively high concentrations, and for over long periods, neoplasia can occur with no prior initiation. Under such circumstances, a promoter must be treated as a carcinogen. Further, when an agent is supplied concurrently with an initiator, which results in the production of neoplasms being accelerated, it is known to be a co-carcinogen instead of a promoter (Hecker 1978). Although certain promoters,

Table 2.1 Common characteristics of carcinogenesis initiators and promoters

S.no	Initiator	Promotor
1.	Mutagenic	Usually non-mutagenic
2.	Irreversible	Reversible
3.	Additive	Non-additive
4.	Can induce in all type of cells	Cell-specific and active only after initiation
5.	Dose-dependent	Dose-dependent
6.	Act as carcinogen	Act as co-carcinogen
7.	Development of electrophiles and covalent binding to DNA	No electrophiles development and no covalent binding to DNA

like phorbol esters, maybe co-carcinogenic, not all promoters such as phenol, phenobarbital contain co-carcinogenicity and, alternatively, not that all co-carcinogenic are promoters. Promoters involve compounds such as drugs, phytochemicals, and hormones that are not genotoxic but somehow affect the transcription of the cellular DNA encoded genetic information. It has been proposed thatgene manipulation and instability can be induced by fostering agents. Many experimental evidences show that gene manipulation is specific to the feature of the treated promoter(Derelanko 2001; Cohen and Arnold 2011). Several promoters are assumed to achieve their results by association with receptors present in the cell surface, cytosol, or nucleus. Conversely, certain hydrophilic and hydrophobic promoters impose their activity at the cellular interfaces by their molecular configuration. Some promoters are mitogenic, promoting transcription of DNA and enhancing the proliferation of the cells. This can happen explicitly or, similarly, obliquely by manipulating cells with a shorter G1 process, thereby granting them a proliferative selective advantage. Tissue culture experiments have shown that such promoters hinder intercellular interaction (Loeb and Harris 2008).

Empirical evidence reveals that the molecule as a whole can influence the promotional impact and the compound activity is defined by the molecular settings. If the promoter undergoes metabolism, it inevitably results in the inactivation of the promoter. Promoters tend to have a fairly strong sensitivity to the tissue. For example, phenobarbital acts as a promoter in rat liver carcinoma, although not in the urinary tract. In comparison, 12-0-tetradecanoylphorbol-13-acetate is a strong neoplasm promoter for the skin and forestomach, which has no significant liver function. 3-tert-butyl-4-methoxyphenol and 2,6-ditert-butyl-4-methoxyphenol that serve as promoters in any one organ, act as an anti-promotor in second organ, and shows no impact in the other organ (Frenkel et al. 1993). Therefore, a promoter's functional description may provide the description of the responsive tissue.

Experimental proof of the function of relatively high-fat food in fostering mammary cancer has been reported in rats subjected to mammary carcinogen 7,12-Dimethylbenz[a]anthracene (DMBA)(Zarbl et al. 1985). Likewise, bile acids are recognized as promoters in rat liver carcinogenesis, since they are modulated by fat intake(de Gerlache et al. 1987). Based on clinical epidemiology research, demographic-and gender-associated modulations of hormone rates of progesterone, estrogens, and androgens are inferred as possible promoters of breast cancer. Laboratory findings have shown consistently that these hormones help to facilitate mammary cancer in rats conducted with mammalian carcinogens along with pituitary prolactin (Clevenger et al. 2003). Hyperplasia and/or inflammation are induced by certain promoters. It is particularly valid in studies of epidermis initiation–promotion utilizing phorbol esters used for promotion activity but often seen in hepatocyte hyperplasia after treatment with mutagenic agents like phenobarbital. Phenobarbital induces temporary hepatocyte hyperplasia in the rat liver. It should be noted that certain substances can cause hyperplasia and inflammation which may occur without the promotion process (Lewis and Adams 1987).

2.3 Progression

The process of carcinogenic development is an extension of the tumor promotion step and proceeds from the fact that cell proliferation caused by stimulating factors enables the spread of cell damage acquired by initiation. Morphological characteristics prevail that the activated cells are clonally dispersed, consisting of constant clonal replication of the transformed cells, during which there is no modulation of growth and escape from the host resistance pathways (Ruddon 2010). The progression phase is demonstrated by karyotypic destabilization and the development of aneuploid, permanent, malignant cells (Olson 1992). During the progression process, some genetic and epigenetic changes may occur, frequently involving proto-oncogene stimulation and the suppression of tumor suppressor genes to act. Further, two major pathways also induce protooncogenes: where *RAS* gene family, point mutations can be found in specific genomic regions and *MYC*, *RAF*, *HER2*, and jun multigene families may be over-expressed, often contributing to chromosome segments containing such genes being amplified (Harris 1991). The presence of a genetic alteration in the former genome and the lack of quantifiable systemic changes in the latter differentiates the progression from the promotion. The emerging new technologies focused on histochemistry and on-site hybridization, will represent both structural genomic modifications and biochemical changes specific to tumor growth. Furthermore, oncogenic proteins allow us to distinguish between benign to malignant neoplasms in the various stages of development (Elder 2016). In certain scenarios, symptoms of more advanced malignancy may be identified before the neoplasm reaches macroscopic size; in other circumstances, well-defined slow-growing tumors may persist for years until a reasonably rapid transition to more destructive behavior (Conti 2010). Both cases of acceleration or retardation by extrinsic causes are prone to progression. Initiating agents tend to decide the direction and stage of progression and their prolonged invasion may accelerate the progression outcome beyond the minimum needed to cause a tumor; however, progression is independent of such carcinogenic agents until the initiation phase is sufficiently advanced (Polonara et al. 2012).

3 Mechanisms of Carcinogenesis

With the advancement of the latest developments of molecular biology, such as profiling of gene expression, systems biology, microRNAs, gene exploration, and pathway research, carcinogenesis is becoming even more complicated than merely being a clonal mutation of a cell that suffered twin genetic “hits” from a carcinogen. Such molecular changes result from the accumulation of genetic programs modifications that regulate the proliferation of cells and its lifespan, relations with adjacent cells, and the ability to hide from the immune response. That process ends to result in a mass of deregulated cells being produced. For a longer period, such a

mass might be asymptomatic. It will also expand and disrupt the physiological processes, resulting in different manifestations of position and relative magnitude of the mass and the distribution of cancer cells throughout the body.

3.1 Oncogenes Activation

The cancer-targeted genes are found in hundreds that are distributed throughout the human genome. Human DNA is thought to contain around 23,000 genes. Thousand of those genes (3000–5000) encode for proteins that are implicated in cancer deregulated genetic processes. A defective gene may result in the development of excessive amounts of a vital protein, the production of an aberrant protein, or the complete lack of the protein (Croce 2008; Hartl and Bister 2013). A proto-oncogene is a natural gene that, after a genetic modification (mutation), can become an oncogene, resulting in enhanced transcription. Normally, proto-oncogenes code for proteins that regulate cell proliferation by transducing signals and conducting mitogenic signals. The oncogene protein is a tumor-inducing agent when activated. Best recognized proto-oncogenic sources include *RAS*, *ERK*, *MYC*, *WNT*, and *TRK* (Botezatu et al. 2016). The other oncogene BCR-ABL gene was located on the Philadelphia chromosome, a genetic mutation in chronic myelogenous leukemia caused by chromosome 9 and 22 t translocation (Pane et al. 2002).

Oncogene activation through structural alteration such as mutation, gene fusion, chromosomal rearrangement, and genome amplification or epigenetic change such as gene promoter hypomethylation, the microRNA expression gives an enhanced or deregulated expression; cells containing these modifications also have continuous growth or an enhanced rate of survival. For instance, mutation in KRAS gene transforms a protein located right inside the cell membrane into a signaling multiplier for cell development. This protein generally works as a signaling intermediate between surface growth factor receptors and molecular wiring systems that deliver growth signals to the nucleus for the cell replication to take effect. When the KRAS gene is mutated, the corresponding protein acts as a switch locked in the "on" position, generating a permanent division signal for the cells. *KRAS* mutations are common in many cancers, such as colorectal cancers (about 40% of cases), or lung adenocarcinomas (about 30% of cases). This triggered gene is considered an "oncogene," since it facilitates the proliferation of cells (Jančík et al. 2010; Fearon 2011; Karachaliou et al. 2013) (Table 2.2).

A few cancer syndromes are triggered by hereditary proto-oncogene mutations that enable the oncogene. However, most oncogene mutations that develop cancer are inherited, not genetic. They usually enable oncogenes through chromosome rearrangements which leads to shifts in chromosomes that cause one gene to trigger the other and gene duplication which leads to extra copies of a gene that may contribute to the abundant generation of a certain protein.

Table 2.2 List of known human cancer oncogenes. Cancer results from genetic modifications of key oncogenes that regulate cell proliferation, differentiation, and survival. *PIK_3CA*-Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha. *KRAS*- Ki-ras2 Kirsten rat sarcoma viral oncogene homolog. *BRAF*- v-Raf murine sarcoma viral oncogene homolog B. *HRAS*- v-ha-rasharvey rat sarcoma viral oncogene homolog. *NRAS*- Neuroblastoma RAS viral oncogene homolog. *RET*- Proto-oncogene tyrosine-protein kinase receptor Ret. *B-CATENIN*-Catenin beta-1. *EGFR3*- Epidermal growth factor receptor 3. *FLT_3*- class III receptor tyrosine kinases. *KIT*- tyrosine-protein kinase

Oncogenes	Cancer type	Reference
PIK_3CA,KRAS,BRAF	Cervical cancer	Ma et al. (2000), Janku et al. (2011)
HRAS,NRAS,KRAS	Prostate cancer	Abate-Shen (2000), Baca et al. (2013)
BRAF,NRAS,KRAS,BRAS	Melanoma	Cicenas et al. (2017)
HRAS,NRAS,BRAF,RET	Thyroid cancer	Quiros et al. (2005)
BRAF,KRAS,PIK_3CA,B-CATENIN	Colorectal cancer	Baldus et al. (2010), Therkildsen et al. (2014)
BRAF, KRAS	Biliary tract cancer	Chang et al. (2014)
B-KATENIN,KRAS	Pancreatic cancer	Eser et al. (2014), Kamisawa et al. (2016)
BRAF,KRAS,NRAS,EGFR3	Lung adenocarcinoma	Paik et al. (2011), Seo et al. (2012)
KRAS,NRAS,FLT_3,KIT	Acute myeloid leukemia	Schlenk et al. (2008)
KRAS,NRAS,HRAS	Hepatocellular carcinoma	Hou et al. (2014)
KIT,KRAS	T cell lymphoma	Foss et al. (2011)
KRAS,NRAS	Acute lymphoblastic leukemia	Tomizawa and Kiyokawa (2017)

3.2 Tumor Suppressor Gene Inactivation

Tumor suppressor genes (TSG) are the reverse hand of cell growth regulation, usually functioning to prevent cell proliferation and production of tumors. Such genes are defective or inactivated in several cancers, thus suppressing negative cell proliferation regulators and leading to excessive tumor cell proliferation. TSG operates to control cell growth and proliferation within the genome. They also assist with pathways for the repair of DNA and other essential cellular signals including the apoptosis pathway (Wang et al. 2018). The very first insight into the role of TSG resulted from studies concerning somatic cell hybridization, pioneered in 1969 by Henry Harris and his colleagues (Harris et al. 1969). There is a large chance of disordered cell development which can contribute to malignant tumour without the activated tumor suppressor genes. Loss of function mutations in TSG has also been reported in several forms of cancer comprising ovarian, kidney, colorectal, head and neck, pancreatic, uterine, breast, and bladder cancer.

In cancer, the failure of TSG activity happens, according to Knudson's two-hit model theory, by removing or inactivating two alleles. It is now apparent that alterations in TSGs are suppressive at a specific cell level; thus, a point mutation in a TSG is not necessary to induce cancer. Some experiments, however, have described

Table 2.3 List of tumor suppressor genes and their role

Gene	Gene function	Reference
pRB and p16	Intracellular proteins, that control cell cycle progression	Leiderman et al. (2007)
Transforming growth factor (TGF)-β and adenomatous polyposis coli (APC)	Receptors or signal transducers that inhibit cell proliferation	Smith et al. (2012)
Breast cancer type 1 susceptibility protein (BRCA1), p16, and p14	Checkpoint-control proteins that trigger cell cycle arrest in response to DNA damage or chromosomal defects	Savage and Harkin (2015)
p53	Proteins that induce apoptosis	Rahman and Scott (2007)
p53 and DNA mismatch repair protein 2 (MSH2)	Proteins involved in repairing mistakes in DNA	Tomlinson et al. (2002)

candidate TSGs that do not follow this normative description, including genes that are inactivated through epigenetic silencing rather than deletion. In addition, the inactivation of TSGs often includes proteasomal degradation by ubiquitination, irregular cellular localization, and transcriptional control (Wang et al. 2018). For eg, the TP53 gene encodes a protein that normally functions as an "emergency stop" to prevent the improper division of the cells. Mutation in this gene interferes with the protein, which is unable to resist cell proliferation when required. Mutations in TP53 occur in almost all types of cancer. Such a gene that contributes to the production of cancer by losing its role is called a tumor suppressor since its active products serve as a brake under normal conditions to subdue the cancer cell growth (Gariglio 2012) (Table 2.3).

3.3 Association Between Infectious Agents and Carcinogenesis

3.3.1 Oncogenic Virus

The carcinogenic mechanism includes multiple influencing factors that involve external conditions, diet, host characteristics, hereditary genetic features, and infectious agents. Infectious agents are essential because they reflect a major and preventable source of cancer from a public health perspective. The frequency of infection-attributable cancer was recorded in the global occurrence of cancer in 2018 as 18.1 million new cancer cases (17.0 million except nonmelanoma skin cancer) and 9.6 million cancer deaths (9.5 million except nonmelanoma skin cancer) (Bray et al. 2018). The International Agency for Research on Cancer (IARC) identifies seven viral factors which have been known to be carcinogenic which include Hepatitis B Virus (HBV) and Hepatitis C Virus (HCV), Epstein-Barr Virus (EBV), Human Papilloma Virus (HPV), Kaposi-Sarcoma Herpes Virus (Human Herpes Virus 8), Human Immunodeficiency Virus type 1 (HIV) and Human T-cell leukemia virus type I (HTLV-1). The inductionof cancer formation by viruses includes sustained invasion of the organism. Long-term infection is hypothesized to cause

cellular changes that predispose to the progression of cancer. Examples of recurrent infections correlated with ongoing inflammation include HBV and HCV infections. HBV and HCV are responsible for 54% and 31% of the worldwide reports of clinical hepatocellular carcinoma (HCC)(El-Serag 2012). Such a hepatotropic virus causes cirrhotic livers that may trigger the development of HCC.

Individuals with HIV have a slightly greater chance of some tumors relative to those of the same sex that are uninfected. Such tumors are considered malignancies associated with AIDS which include Kaposi sarcoma (a mesenchymal tumor, originates from lymphatic endothelial cells), cervical cancer, and Non-Hodgkin Lymphoma (Braoudaki and Tzortzatou-Stathopoulou 2011). However, certain forms of cancer, such as Hodgkin's disease (HD), anal cancer, lung cancer, and testicular germ cell tumors, tend to occur frequently in HIV-infected individuals relative to the common population and are referred to as AIDS-associated cancers. HIV is a family of Retroviridae, RNA lentivirus. The viruses that belong to this group merge into the host genome and thus have the ability to induce direct induction mutations or cellular oncogene activation. Many members of the Retroviridae family, such as Mouse mammary tumor virus (MMTV), has a very well-defined association with mice's tumors that are possibly mediated by the insertion of cellular genes in the breast tissue via hormone-response elements in the MMTV promoter (Hacein-Bey-Abina et al. 2008).

In addition, EBV, Human herpesvirus 8, HTLV-1, and HPV, some of the carcinogenic viruses that have been identified and recognized, are tumor viruses that develop oncogenic viral proteins for carcinogenesis. Oncogenic viruses can transform cells by transferring viral oncogenes to a cell or by inducing cell proto-oncogenes (Zheng 2010). Virally mediated oncogenes release manipulating signaling molecules that deregulate regulation and proliferation of development, resulting in malignant transformation. Oncogenic viruses categorized into DNA and RNA tumor viruses are given below.

DNA Tumor Virus

EBV is a Herpesviridae family with double-stranded DNA that induces contagious mononucleosis. EBV induces a life-long persistent infection for other herpesviruses, so EBV is the main source of B-cell development in Burkitt's lymphoma (Orem et al. 2007). This became the first human tumor diagnosed with an infectious agent. EBV has also become implicated in a variety of other cancers. The presence of the viral oncogene, latent membrane protein-1 (LMP1), in the case of EBV-lymphoma, turns cells into lymphoblasts by blocking cellular signal transduction. By contrast, the BamHI-A viral read frame-1 (BARF1) gene is expressed in most Nasopharyngeal Carcinoma (NPCs). In NPC pathology, BARF1 was established as an essential oncogene. Therefore, EBV has multiple oncogene expression profiles that are consistent with specific cancers. The incidence of EBV is highly prevalent impacting more than 90% of the world's population, and only a limited percentage of affected people develop an EBV-attributable disease (Raab-Traub 2002; Brennan 2006).

RNA Tumor Virus

HCV is an RNA virus of the genus Flaviviridae family of hepaciviruses. HCV is not incorporated within the host genome and some key proteins have been identified as possible oncogenic candidates *in vitro*, including nonstructural (NS) protein 3, NS protein 4B including NS5A. It has been shown, that the HCV NS5A protein binds and sequesters the cellular p53 protein to the perinuclear membrane, which could be crucial to HCC growth (El-Serag 2012). HTLV-I is an HIV-related retrovirus that is associated with adult T-cell leukemia. Just 1% of HTLV-I contaminated people can experience leukemia, and only after a long delay time of 20–30 years. In comparison to HIV, HTLV-I infections are not linked with immunosuppression. However, HTLV-I encodes an oncogenic protein (Tax), which is known to bind to several cellular genes involved in the cell growth and control of cell cycle production, such as NFkB and p53. By encouraging synthesis and progression of the cell cycle, Tax is proposed to create a self-stimulating loop that induces increased proliferation of contaminated T-cells, and eventually leukemia (Shuh and Beilke 2005; Martin et al. 2016).

3.3.2 Oncogenic Bacteria

It is commonly thought that bacterial infections cause chronic infections and diseases, including cancer (Vogelmann and Amieva 2007). The involvement of bacteria in carcinogenesis is due to chronic inflammation triggered by recurrent bacterial infections and secondary metabolites (bacterial toxins) generated by chronic carcinogenic bacterial infections. Hence comprehending the carcinogenesis stimulated by bacteria could allow us to prevent and treat certain forms of cancers (Lax and Thomas 2002).

There could be different carcinogenic mechanisms caused by chronic bacterial infections. The presence and abundant release of inflammatory mediators is a common characteristic of chronic infections. Transcription factors like the nuclear factor-κB (NF-κB) family have been linked to inflammatory response-driven carcinogenesis (Karin and Greten 2005). Bacterial pathogens and even pro-inflammatory cytokines like TNF-α and IL-1 activate the mechanism for NF-κB. Further, the mentioned pathway involves the activation of IKK (inhibitor of nuclear factor kappa B) complex and the destruction of NF-κB inhibitors, thus trying to free NF-κB to reach the nucleus and mediate the intended transcriptional activity. Some of the genes related to apoptosis inhibition pathways, like p21, p53, and pRb, are found to be decreased in expression, while the genes associated with cell cycle regulation, such as cycline D1, CDK2 kinase, c-myc (cell cycle regulators), are significantly upregulated by NF-κB. NF-κB often upregulates various cytokines, such as IL-1β, IL6, Vascular endothelial growth factor (VEGF) (proinflammatory and proangiogenic), but decreases TNF, thus promoting tumor development. In addition, the genes related to invasion and metastasis are also upregulated by NF-κB (Van Antwerp et al. 1996).

Table 2.4 List of bacterial toxins known for causing human cancer

S.No	Bacteria	Potential toxin/ Pro-carcinogenic toxins	Mechanism	References
1.	*Haemophilusducreyi, Helicobacter hepaticus, Salmonella typhi. Actinobacillus*	Cytolethal distending toxin (CDT)	DNA damage and cell cycle inhibitor	Faïs et al. (2016)
2.	*Salmonella typhi*	Toxin B	DNA lesions	Martin and Frisan (2020)
3.	*Pasturella multocida*	Pasturella multocida toxin	Modifies Gq (a heterotrimeric G protein) proliferation	Banu et al. (2020)
4.	*Helicobacter pylori*	Vacuolating cytotoxin A	Upregulation of vascular endothelial growth factor	Caputo et al. (2003)
5.	*Bacteroides fragilis*	Bacteroides fragilis toxin	Cleaves E- cadherin proliferation	Wu et al. (1998)
6.	*Escherichia coli, Campylobacter jejuniand Salmonella typhi, Helicobacter hepaticus*	Cytotoxic necrotizing factor-1	Modifies rho family proteins, inflammation and inhibition of cell cycle, blocks cytokines	Boquet (1999), Travaglione et al. (2008)
7.	*Escherichia coli*	Cell cycle inhibiting factor	Inhibit cell cycle at G_2-M transition	Samba-Louaka et al. (2009)
8.	*Citrobacterrodentium*	Mitochondrial associated protein (Map)	Multifunctional effectors protein that target disruption of epithelial barrier function	Ma et al. (2006)
9.	*Bartonella species*	Bartonella effector proteins (BepA–G)	Angiogenesis and proliferation	Kempf et al. (2001)

A limited list of possible bacterial toxins implicated in carcinogenesisis listed in Table 2.4. The toxins could either destroy the cells or manipulate the cellular processes that govern cell division, DNA damage, apoptosis, and differentiation. Such toxins interact either with the cell signaling factors or specifically with DNA. Harm to host cells may be caused by an enzymatic attack, by influencing DNA damage repair mechanisms or triggering persistent inflammatory reactions and generating free radicals (Herrera et al. 2005; Nath et al. 2010).

3.3.3 Oncogenic Parasites

Parasitic infections also have been known for years to be associated with human carcinogenicity. Helminth parasite infections such as schistosomiasis, opisthorchiasis, and clonorchiasis are extremely carcinogenic, however, malaria doesn't seem to

Table 2.5 List of parasitic pathogens associated with human cancer

Parasitic pathogens	Associated cancer	Reference
Schistosoma haematobium	Urinary bladder cancer, adenocarcinoma, squamous cell carcinoma	Palumbo (2007), Mitreva (2012)
Schistosoma japonicum	Colorectal cancer, rectal cancer, squamous cell carcinoma, membranous nephropathy, metastatic lung cancer	Ishii et al. (1994), Zanger et al. (2010)
Schistosoma mansoni	Adenocarcinoma, colorectal cancer, hepatocellular carcinoma	Scholte et al. (2018)
Opisthorchis viverrini	Cholangiocarcinoma	Sripa et al. (2011)
Clonorchissinensis	Cholangiocarcinoma	Kim et al. (1989)
Opisthorchis felineus	Cholangiocarcinoma	Lim (2011)
Trypanosoma cruzi	Gastrointestinal cancer, uterine leiomyoma	Matsuda et al. (2009)

be causative to carcinogenesis. Whereas, the protozoan *Trypanosoma cruzi*, the causative agent of Chagas disease plays a dual role as a carcinogenic and an anti-cancer agent. *Plasmodium falciparum* involves additional transition events caused by the Epstein-Barr virus (EBV) driven Burkitt lymphoma. When the red blood cells which are infected with the *P. falciparum* interact (via *P. falciparum* erythrocyte membrane protein 1's CIDR1 domain) with the B cells that are infected with EBV, it leads to the proliferation of the infected B cells and also the activates the EBV. The interaction between iRBCs and EBV-infected B cells is also the result of an enhanced expression of Activation Mediated Cytidine Deaminase (AID). In specific, AID contributes to the breakdown of host DNA resulting the activation of oncogenes (*c-Myc*) (van Tong et al. 2017). Most of the parasitic infection is associated with carcinogenesis through inflammation and oxidative stress caused by parasite-derived molecules. Some of the parasites and the associated cancer types are listed in Table 2.5.

Chronic inflammation caused during infections with *Opisthorchis*, *Clonorchis*,and *Schistosoma* contributes to the stimulation of signal transduction pathways, including NF-κB, p53, Jak/Stat, and Rb, which may induce somatic mutations and/or trigger oncogenes. Further, the parasite metabolites secreted to the recipient microenvironment may induce various metabolic functions, especially oxidative stress, which promotes disruption to the chromosome DNA of proximal epithelial cells, particularly urothelial and cholangiocytes cells of the liver (van Tong et al. 2017). In addition, the physical disruption to the host infected cells during the growth of parasites, along with the successful tissue repair cycle, contributes to enhanced cell regeneration and proliferation, which is also correlated with DNA damage. Coupled parasitic organism-host association events like chronic inflammation, parasite-derived metabolites, and nuclear DNA damage contribute to a shift in cell differentiation, proliferation, and viability that, in turn, initiates and encourages malignancy (Vennervald and Polman 2009). However, thorough observations into such interactions and/or recognizing the functional implications of both parasite and host

influences have not yet been obtained. Studies based on the detection of carcinogenic parasite influences through increasing the processes of host signal transduction pathways or oncogenes resulting in the activation of cancer propagation are also needed.

3.3.4 Oncogenic Fungi

The cancer causing mycotoxins could be exposed through absorption or by inhalation and also through the food that is infected. *Asergillus flavus* and *Aspergillus parasiticus* fungi species produce mycotoxins, and these mycotoxins which are termed as aflatoxins have been identified to be highly toxic (Gourama and Bullerman 1995). When the aflatoxins penetrate the cells, the cytochrome P450 metabolizes them, results in the production of aflatoxin-8, 9-epoxide. It is extremely reactive and unpredictable and involves attachment to DNA or to a cluster of protein with high affinity in order to be more stable and it forms aflatoxin-N7-guanine, which cause transverse mutation. It further influences the cell cycle directly by manipulating the p53 genome (Kew 2013).

Human beings are regularly exposed to mycotoxin, such as aflatoxins, ochratoxins, primarily from plant and animal sources. The health threats resulting through mycotoxins could be due to their potential toxicity, in specific their carcinogenicity potential. Mycotoxins, particularly aflatoxins, ochratoxin A (OTA), citrinin (CIT), patulin, fumonisin B, ochratoxin A, zearalenone, have been identified to induce cancer, which are summarized in Table 2.6. New knowledge of the genotoxicity of mycotoxin (formation of mycotoxin-DNA adducts), the function of mycotoxin in oxidative damage and the discovery of epigenetic modifications involved in mycotoxin carcinogenesis provide compelling evidence that mycotoxin carcinogenicity is driven by various signaling mechanisms that exists in humans (Ostry et al. 2017).

3.4 Involvement of MicroRNA in Cancer

Small regulatory RNAs may be classified into two main classes: microRNAs (miRNAs) and small RNAs interfering (siRNAs). miRNAs are short 22–25 long noncoding nucleotides that are retained throughout development, which regulate gene expression in multicellular organisms, plants, viruses, and bacteria mainly at transcription and post-transcription processes, although the yeast genome is considered to lack miRNA genes. miRNAs control specific gene transcription by breaking down the associated mRNA and/or inhibiting its translation process. Presently, miRNA ‘s vital mechanisms have been established to regulate the immune function, cell growth, differentiation, cell cycle, and carcinogenesis (Ahmad et al. 2013). In the human genome, miRNAs are likely to be present at least 400 numbers, and possibly as high as around 1000. Concerning complex evolution, the wide estimated number of miRNAs found in higher mammals may indicate their possible role in

Table 2.6 List of fungi and their related human cancer-associated substances

Fungi	Accountable substances	Mechanism	Associated cancer	References
Malassezia spp.	Glycans	Mannose binding lectin attaches to fungal cell wall glycans and stimulates the chain reaction-oncogenic development	Pancreatic ductal adenocarcinoma	Aykut et al. (2019)
Candida albicans	Hyphae	Dysplastic modifications contributing to cancer infiltrate the oral epithelium with fungal hyphae	Oral cancer	Alnuaimi et al. (2015)
Aspergillus flavus	Alfatoxin	Induce DNA adducts	Hepatocellular carcinoma	Kew (2013)
Penicillium, Aspergillus, Monascus	Ochratoxin A (OTA) and/or citrinin (CIT)	Genotoxic activity	Urinary tract cancer, liver cancer	Pitt (2000), Knasmüller et al. (2004), El Adlouni et al. (2006)
Penicillium pabulum	Patulin	Trigger G1/S aggregation and cell cycle arrest with apoptosis induction, PARP cleavage and ATF3 protein expression	Colon cancer	Kwon et al. (2012)
Fusarium verticillioides	Fumonisin B	Induced hepatotoxicity and preneoplastic abnormalities	Hepatocarcinoma	Gelderblom et al. (2001)
Aspergillus ochraceus	Ochratoxin A	Induces adducts in testicular DNA	Testicular cancer	Schwartz (2002)
Fusarium graminearum	Zearalenone	Abberations in hormonal activity andenhance tumor cell proliferation	Breast cancer	Belhassen et al. (2015)

regulating more precise gene expression (Esquela-Kerscher and Slack 2006; Bushati and Cohen 2007). Annotation of miRNAs genome locations suggests that most miRNAs genes are situated in intergenic domains, they are often present inside exonic or intronic areas but in either context or anti-sense direction. Localized miRNAs have been referred to as ‘mirtrons’, present inside protein-encoding introns or non-encoding genes. miRNAs may be grouped as a single gene or placed as clusters containing a family of miRNAs typically linked in sequence and function. miRNAs are transcribed predominantly by RNA polymerase II (RNA pol II) out of their own promoter or from the promoter of the host gene they live in. miRNAs impose their genetic regulation activity mainly by defective base pairing to the 3′ UTR of its

target mRNAs, resulting in depletion or translational suppression of mRNA. In cancer, miRNAs are frequently disordered with their patterns of expression being associated with clinically important tumor characteristics (Peng and Croce 2016).

miRNAs have recently been shown to function specifically in the development and advancement of cancer. The first proof of miRNAs being associated withhuman cancer results from chronic lymphocytic leukemia (CLL) research. The key chromosome region 13q14, which is regularly lost in CLL, but two miRNA genes like miR-15a and miR-16-1 are expressed within polycistronic RNA (Calin et al. 2004). Growing research indicates that human carcinogenesis may include an archetypal miRNA, let-7. The research documented the regular incidence of substantially decreased expression of family members of the let-7 miRNA genes in lung cancers. Such ideas of the possible biological activities of altered miRNA in human cancers are also strengthened by the detection of RAS as a target gene for let-7 (Yanaihara et al. 2006). In *C. elegans*, the let-7 family negatively controls the encoding of let-60 genes in tiny GTPases (*RAS* oncogenes homologs), while let-60/*RAS* deficiency suppresses the let-7 mutant phenotype. It has been found that the human *RAS* gene also comprises of various complementary let-7 sites and is controlled by let-7, which provides clues to a mechanistic explanation for let-7 changes in human lung cancer. Another archetypal miRNA, lin-4 could also contribute to carcinogenesis in humans (Hristova et al. 2005). Lin-14, the lin-4 target, is a transcription factor that regulates several downstream processes. miR-125b -mediated downregulation of lin-28 was indicated to lead to neuronal carcinoma, while miR-125b depletion was shown to have significant inhibitory effects on the proliferation of adult differentiated cancer cells rescued by co-transfected, mature miR-125b (Lee et al. 2005). However further studies need to be carried out to validate the significance and potential roles of miRNA signalling in carcinogenic processes.

3.5 Role of Epigenetics in Cancer

Epigenetic variations have a pertinent impact on cancer. Considerably, earlier this century, science and clinical associates specifically reported that epigenetics dysregulation leads to structural and inheritable changes in chromatin function impacting the whole epigenome without modifying the DNA sequence. This involves DNA methylation, post-translational histone alteration, and microRNA interference with RNA, and inactivation of primary cell regeneration pathways involved in carcinogenesis and its progression (Lee et al. 2005; Jones and Baylin 2007). These epigenetic changes will be stable to preserve the same cell lineage or dynamic to retaliate to the development and the environment signals of the cell (Jones and Takai 2001). A different kind of epigenetic mechanisms is sometimes diversified in different types of cancer, including the silencing of tumor suppressor genes (TSG) and stimulation of oncogenes by different patterns of CpG island methylation, histone modifications, and DNA binding protein impairment.

3.5.1 DNA Methylation

DNA methylation is possibly one of the most extensively studied epigenetic modification in mammals. It is quite stable and acts as a specific epigenetic memory of particular cells during the cell cycle throughout all generations. It can also control histone code expression and activity. DNA methylation mainly emerges in mammals by the covalent alteration of cytosine(C) residues which is bound to a guanine(G) by a phosphodiester bond in CpG dinucleotides. CpG dinucleotides are not uniformly dispersed throughout the human genome but rather focus in short CpG-rich DNA stretches called 'CpG islands' and wide repetitive sequence regions (Saxonov et al. 2006; Klose and Bird 2006; Sharma et al. 2010). Extensive hypomethylation of DNA by DNA methyltransferase enzymes such as DNA methyltransferase 1 (DNMT1), DNMT3a, and DNMT3b occurs during tumor formation in repetitive DNA elements and intergenic regions. Methylatable genomes forfeit sequences of CpG owing to mutability by the addition of methyl group to cytosine that will suddenly deaminate to thymine. For example, it can prevent transcriptional activity by inhibiting transcription factors from entering target-binding sites such as c-myc and Membrane-bound lytic mureintransglycosylaseF (MLTF). This tends to result in chromosomal aberrations, genomic instability, mutagenesis, and perhaps carcinogenicity (Jones 2003).

Consequently, DNA hypomethylation may result in the activation of growth-promoting genes such as R-Ras, cyclin D2, and mapsin (a member of the serpin family of serine protease inhibitors) in stomach cancer, S-100 in colorectal carcinoma, and MAGE (melanoma-associated antigen) in melanoma, and loss of imprinting (LOI) in carcinomas. In Wilms' cancer, the hypomethylation-induced LOI of insulin-like growth factor 2 (IGF2), a significant autocrine growth factor, leads towards its pathological expression of biallelic, which is also associated with an elevated risk of colon cancer. Besides, altering gene-specific methylation can result in alterations in gene expression and the transformation of the malignant cell. Besides hypomethylation which influences genomic instability and stimulates proto-oncogenes, site-specific hypermethylationalsoleads to carcinogenesis by silencing genes that suppress tumors. From the early observation of the Rb promoter (a retinoblastoma-associated TSG) on CpG island hypermethylation, several other TSG, particularly p16 in non-small cell lung cancers, breast, prostate, and several other tumors, MLH1 1 in colorectal and uterine carcinomas and BRCA1 in breast cancer, has also been reported to endure in tumor-specific silencing by hypermethylation which further allows the cells to accrue additional genetic lesions resulting in a rapid progression of cancer. Hypermethylation of TP53, APC, and RASSF1A (Ras association domain-containing protein 1) promoter regions is identified as crucial epigenetic markers to detect cancer development (Coyle et al. 2007; Kanwal and Gupta 2012; Sanchis-Gomar et al. 2012).

3.5.2 Histone Modifications

Anomalous histone modifications are reported to serve as a crucial factor in the pathogenesis of many human diseases including cancer, neurodegenerative and inflammatory diseases. Histone proteins that constitute the nucleosome core have a C-terminal globular domain as well as an unstructured N-terminal tail. Several post-translational covalent modifications, including methylation, phosphorylation, acetylation, ubiquitination, will be carried out by histone N-terminal tails, the well-studied and most significant in chromosomal structure regulation and function contexts. The tendency of the protein to acetylate non-histone transcription factors, p53 and BCL6, is an aspect of the function of histone acetyltransferase (HAT) found by the different mutations in CBP and EP300. In addition to the absence of p53 and BCL6 acetylation, their transcriptional activator and repressor functions abrogate, making the subsequent cells very tumorigenic via aberrant pathways that sustain DNA damage duringapoptosis and cell cycle arrest (Sawan and Herceg 2010; Pasqualucci et al. 2011). H3tre11 is a particular substrate for tumor-specific pyruvate kinase M2 (PKM2) in transcription initiation mediated by Epidermal growth factor (EGF) and acetylation of histone 3 lysine 9 (H3K9), ensuing in tumor cell proliferation. H2Bser32p exists prevalently in human cells nevertheless, it is also comprehensively phosphorylated in skin cancer cells by RSK2 kinase (an RSK family kinase AGC). Janus kinase 2 (JAK2) is often shown to phosphorylate H3tyr41, further obstructing the heterochromatin protein 1α (HP1α) binding with chromatin. HP1α has been reported to associate directly with H3 via their chromo-shadow domain. The removal of HP1α from chromatin consequently results in constitutive activation of the JAK2 signaling pathway, including oncogene imo2, contributing to carcinogenesis (Shanmugam et al. 2018).

3.5.3 Dysregulation of miRNAs Expression

Transforms in miRNAs expression might be processed in several mechanisms involving chromosomal anomalies, binding of the transcription factor, and epigenetic modifications. During carcinogenesis, certain tumor suppressor miRNAs targeting growth-promoting genes are silenced. Likewise, miR-15 and 16 targeting BCL2, an antiapoptotic gene are suppressed in chronic lymphocytic leukemia while let-7 targeting oncogene, *RAS* is decreased in lung cancer (Sharma et al. 2010). BCL6, an oncogene is a major target of miR127 which performs as a TSG, so that the intense epigenetic regulation of its expression is an essential mechanism for bladder cancer (Bandres et al. 2009). Repression of miR-29 family through various epigenetic regulations was found to be reported in several carcinogenesis processes which include B-cell lymphomas, rhabdomyosarcoma, acute myeloid leukemia, chronic lymphocytic leukemia. For instance, some other downregulated miRNAs include let-7a-3 in lung cancers, miR-31 in several cancer progression, miR-23a in human leukemic Jurkat cells, miR-200b in prostate and hepatocellular carcinoma (HCC). In contrast, certain upregulated miRNAs also play a vital role in

carcinogenesis namely miR-615 in prostate cancer, miR-224 in HCC, and miR-155 in breast cancer (Liu et al. 2013; Moutinho and Esteller 2017). Thus several studies have indicated that epigenetic regulation is responsible for most of the miRNome changes found in human cancer, which were eventually involved both in carcinogenesis and the development of metastases. Hence, it significantly elucidates that cancer cells undergo systemic alterations in the structure of chromatin involving the entire epigenome and that a whole mechanism pertinent to cell renewal is epigenetically dysregulated.

4 Conclusion

The prevalence of cancer in animals and humans can be increased by several different forms of carcinogenic exposure, but a longer period of time period is typically needed. Observations can be explained by the conversion of a normal cell into neoplasm due to complicated mechanisms and heritable alterations in multiple or single gene products. For chemical carcinogenesis, the three-stage model of initiation, promotion, and progression has established a framework, which is not sufficient to explain the carcinogenic method. Accumulation of data shows that almost 10 genetic trials in humans are implicated in common adult malignancies. The relevance and specific functions of known cancer-causing factors in many biological processes, including differentiation, proliferation and apoptosis, and carcinogenesis, have now become evident. Two distinct groups of genes, namely oncogenes (which may be activated) and tumor suppressor genes (which may be inactivated) are implicated in the development of cancer. The discovery of genes reponsible in carcinogenesis and the understanding of pathways for their stimulation or inhibition makes it possible to understand how carcinogens affect the phases of neoplastic evolution. In the form of mutagenic processes, carcinogens can heritably change cells by epigenetic modification and enhance the clonal growth of altered cells. Most carcinogens work by a variety of mechanisms, and their primary mode of action can differ based on the targeted tissue. With the understanding of specific gene manipulation, cellular response, events of biological activities in the spread of cancer cells, there are now new insights on some of the discoveries in the detection, prognosis, and treatment of cancer. Nevertheless, it is satisfying to notice some of the significant developments in this crucial field of cancer science. While immense obstacles exist, it is expected that all these lines of research will continue to clinical research.

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Part II
Functional Foods/Nutraceuticals as Chemo Preventive Agents

Chapter 3
Tomato (Lycopene and β-Carotene) and Cancer

Rim Gheribi and Khaoula Khwaldia

Abstract Consuming fruits and vegetables regularly is able to decrease the risks of cancer, increase longevity and improve the quality of life. Among them, tomatoes are particularly interesting for their potential anticancer activity. In fact, the main carotenoids in tomatoes, namely lycopene and β-carotene, are responsible for the anti-cancer and anti-tumoral activities, particularly against prostate and gastric cancers. These carotenoids had an anti-proliferative activity against cancer cells by inhibiting cell viability and angiogenesis, activating apoptosis and decreasing metastasis. This chapter highlights the biochemical properties and mechanisms of action of lycopene and β-carotene and their preventive and curative effects on different types of cancer and discusses the main findings of epidemiological, animal and clinical studies.

Keywords Tomato · Lycopene · β-carotene · Anticancer activity · Antioxidant · Bioavailability · Pro-vitamin A

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1 Introduction

To guarantee a balanced diet, it is mandatory to ensure a regular daily intake of fruits, vegetables and non-refined cereals with a modest consumption of red meat, poultry, fish, sugar and dairy products (De Alvarenga et al. 2018). In fact, it has been proved that consuming fruits and vegetables can significantly decrease risks related to chronic diseases, increase longevity and improve the quality of life. A diet based on fruits and vegetables could be effective to prevent cardiovascular diseases, osteoporosis, diabetes and various types of cancer (Rao and Rao 2007). Several cancer institutes and health organizations have recommended to increase the dietary intake of citrus, fruits and vegetables (mainly yellow and green ones and those with high contents of vitamins A and C) in order to reduce cancer risks (Barber and Barber 2002). Fruits and vegetables owe their beneficial role to health promoting compounds such as vitamins, minerals and phytochemicals, particularly polyphenols and carotenoids. Tomatoes are one of the richest fruits in carotenoids and lycopene is considered to be the most prevalent one, being pursued by β-carotene. Several studies based on *in vitro*, animal and clinical investigations, with considerable results, revealed that carotenoids are in charge for the biological activity of tomato fruit (Desmarchelier et al. 2018). These activities are related to the provitamin A activities characteristic of some carotenoids like β-carotene, α-carotene and β-cryptoxanthin and the well-established antioxidant effect of tomato carotenoids, mainly lycopene. Carotenoids also show biological activities which are not related to their antioxidant power, such as modulator agents of inflammation or ligands for nuclear receptor (Desmarchelier et al. 2018). The biological activities of tomatoes and tomato-based products were essentially associated with lycopene, in combination with other carotenoids present in fresh tomatoes and the other nutrients and molecules present in processed tomato-based products and meals (Frohlich et al. 2006; Desmarchelier et al. 2018).

Thanks to their various biological activities, carotenoids provided by a tomato rich diet, whether consumed directly or through tomato-based products, act as a natural drug for curing and preventing cancers. The Mediterranean diet, for example, is abundantly based on tomatoes and derivatives due to their availability throughout the year and their affordability. This diet has proven health benefits, especially in preventing chronic and degenerative troubles due to its high content on phytochemicals. This observation was approved by a prospective cohort study dealing with the prevention of cardiovascular diseases using the Mediterranean diet (De Alvarenga et al. 2018). Moreover, Canene-Adams et al. (2007) studied the effect of tomato intake on preventing and treating cancer and found that rats consuming tomatoes 5 to 7 times per week had a decrease of 30 to 40% in risk related to prostate cancer. Even though the beneficial effect of tomato consumption on human health was evidenced through several studies, the results of clinical assays remain controverted and this may be related to many factors such as sampling method, subject attributes (age, gender, chronic diseases, etc.) and the method used to quantify the anticancer activity in the clinical assay.

In this context, the present chapter aims at highlighting the anticancer activity of tomato fruit with a special focus on lycopene and β-carotene, the two entities mostly responsible for this biological activity. The mechanisms of action of these phytochemicals on curing and preventing cancers are particularly developed. The results of mainly epidemiological, animal and clinical studies are summarized and their limits are highlighted.

2 Tomatoes: Botanical Aspect, Composition and Main Properties

Tomato (*Lycopersicon esculentum* Mill., from its old nomenclature *Solanum lycopersicum* L.) is a member of the *Solanaceae* family, the genus of *Solanum* and the section of *Lycopersicon*. Tomato is characterized by relevant traits like its fleshy fruit, sympodial branching and compound leaves (Naika et al. 2005; Costa and Heuvelink 2018). Fruit shape could be round, oval or flattened depending on the variety and the fruit color can change from green to red as a function of ripeness stage. Tomato is an annual plant that grows in various climate conditions from temperate to tropical but it requires cool and dry climate for high quality fruits. Tomato is originated in South America and was brought to Europe by Spanish then introduced to Asia, Africa and Middle East (Naika et al. 2005). Tomato has immense economic importance as it is a short duration crop with high production yield, leading to a continuous expansion of tomato cultivation area. This latter reached 4.3 million hectares in 2014, mostly held in China, India, USA, Turkey and Egypt. In the same year, the global production of tomatoes amounted to 171 million tons and this production was mostly carried out by China, European Union, India, USA and Turkey (Costa and Heuvelink 2018).

Tomatoes are considered to be the second most valuable crop in the world, after potatoes, and are essential for human nutrition and diet. In fact, tomatoes can be eaten in different forms whether fresh or processed (puree, juice, paste, pickled or dried). They can also be consumed as tomato-based products such as ketchup, sauce, soup or complex dishes. The largest worldwide tomato consumer is China, followed by European Union, Mediterranean Africa, USA, Mexico and Canada (Costa and Heuvelink 2018).

Tomatoes owe their nutritional attributes to their composition on nutrients and phytochemicals. They present high amounts of vitamins, minerals and fibers and are considered as the main source of carotenoids. In fact, tomato is a natural source of vitamin A, thiamine (B1), riboflavin (B2), pantothenic acid (B3), ascorbic acid (C), folic acid, α-tocopherol (E), biotin, and niacin (Ibrahim et al. 2019).

Today, various types of tomato fruits are available (grape, cherry, plum, cocktail, round and salad) with different colors (red, yellow, green, orange, brown, black, pink, purple) and with different amounts of phytonutrients. Carotenoids and nutrients contents in tomato fruits depend on their variety and the environmental conditions where they have been cultivated. It has been proved that cherry, cluster and

round tomatoes contain the highest amount of lycopene among 40 varieties of tomato. Moreover, orange tomatoes present higher amounts of vitamin A and carotenoids when compared to red ones. However, lycopene content in yellow tomatoes is 10 times lower than that in red tomatoes (Dorais et al. 2008). Till today, traditional and molecular methods are used by researchers and companies to enhance lycopene and other carotenoids contents in tomato fruit (Dorais et al. 2008; Bogacz-Radomska and Harasym 2018).

Processing and cooking of tomatoes have an effect on the content of phytochemicals whether in a positive way by improving their bioavailability or in a negative way because of thermal degradation, oxidation, loss of nutrient and the formation of toxic compounds (De Alvarenga et al. 2018). Tomato phytochemicals, mainly carotenoids, polyphenols and ascorbic acid, confer to this fruit an interesting antioxidant activity responsible for the observed health benefits. In fact, the mentioned antioxidants help the human body fight free radicals which may lead to undeniable biological activities principally preventing and curing cancers and several neurodegenerative and cardiovascular diseases (Ibrahim et al. 2019).

3 Carotenoids

Carotenoids are hydrophobic molecules synthesized by plants as secondary metabolites or by some microorganisms and algae. Animals are unable to synthetize carotenoids and thus need a daily intake in their diet (Jaswir et al. 2011). Carotenoids are generally characterized by their pigmentation which can range from red to yellow, nevertheless, there are also some colorless carotenoids (like phytoene and phytofluene) (Desmarchelier and Borel 2017). Carotenoids are responsible for light absorption and prevention of cells photo-oxidation during photosynthesis and regulation of membrane fluidity (Barber and Barber 2002; Jaswir et al. 2011). There are more than 750 carotenoids naturally available but, in human diet, we can find about 40 kinds of carotenoids which are basically lycopene, β-carotene, lutein, β-cryptoxanthin, α-carotene, and zeaxanthin (Desmarchelier and Borel 2017; Reboul 2019).

The monomer of carotenoids is isopentenyl diphosphate, which contains 40 atoms of carbon, and carotenoids are represented by the formula $C_{40}H_{56}O_n$, where n ranged from 0 to 6. These chemical entities are derived from phytoene, where geranylgeranyl pyrophosphate (GGPP) undergoes a reductive dimerization following dehydrogenation, cyclization, hydroxylation, oxidation and epoxidation reactions (Jaswir et al. 2011). Most of carotenoids are all-*trans* molecules, but heat treatment may induce *cis*-trans isomerization (Desmarchelier and Borel 2017; Reboul 2019). A mixture of methanol, hexane and acetone solvents can extract carotenoids (Ibrahim et al. 2019). Carotenoid extraction from fruits and vegetables progresses through the steps described in Fig. 3.1. Sometimes, the raw material can be subjected to fermentation to increase the efficiency of carotenoids extraction (Bogacz-Radomska and Harasym 2018). Although their extraction process is simple and easy, the yield of carotenoids extraction is too low and not profitable. In addition, natural carotenoids are very sensitive to external factors so it is very hard to

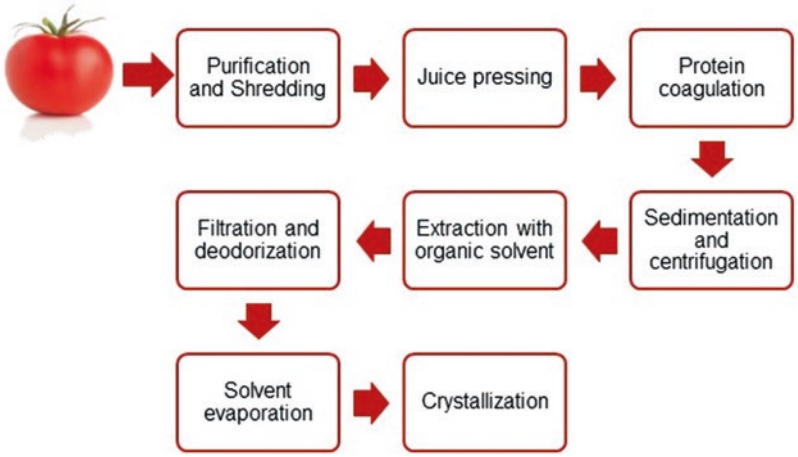

Fig. 3.1 Carotenoid extraction steps from tomato fruit

standardize the dye tones and composition. These drawbacks could be avoided using synthetic carotenoids which are more resistant thanks to pure colorless sugars, proteins or mineral salts, and more commercially viable as they require a small amount of raw material (Bogacz-Radomska and Harasym 2018).

Colored fruits and vegetables contain significant amounts of carotenoids, and tomatoes are particularly an interesting source of these phytochemicals. Among tomato carotenoids, lycopene and β-carotene are the most prevalent ones and are the most effective antioxidant and active compounds. Other carotenoids are significantly available in tomatoes, namely lutein, α-carotene, δ-carotene and γ-carotene. Carotenoid contents vary significantly among tomato species and cultivars. Moreover, the techniques used for cultivation, the location, the period of harvest and the stage of ripeness may highly influence the nutrient and phytochemical composition of tomatoes (Desmarchelier et al. 2018; Ibrahim et al. 2019). Carotenoids are considered as intracellular products and can be detected in the membranes of mitochondria, chloroplasts or endoplasmic reticulum where they are generally associated with lipids or found as hydrophobic structures like membranes (Jaswir et al. 2011).

Carotenoids are distinguished by their antioxidant activity which could be involved in other biological activities such as the prevention of cellular damage. Carotenoids take part in the human antioxidant defense system and can quench singlet oxygen in the same way as tocopherols (Jaswir et al. 2011). In fact, carotenoids present an intrinsic mechanism of defense using oxidative weapons with an unpaired electron called free radicals. This mechanism includes two enzymes, which are glutathoine peroxidase and superoxide dismutase. In this case, carotenoids transfer the unpaired electron of oxygen free radicals putting it into an excited triplet state. Afterward, energy in excess may be converted into heat and, in this case, the carotenoid remains intact and will be able to participate in other reactions of free radical scavenging. Otherwise, the energy in excess may be dissipated by discoloring the carotenoid leading to its decomposition. This process depends on how many double bonds are present in carotenoid structure and the higher is the

carbon-carbon double bonds the more efficient is the carotenoid (Barber and Barber 2002).

The biological activities of carotenoids depend on their intake and circulating levels as well as their bioavailability. Carotenoid bioavailability is a complex phenomenon including several factors such as diet features (intake of fats, fibers, etc.), processing methods and the characteristics of the concerned subjects (age, sex, diseases, etc.). Previous studies demonstrated that single-nucleotide polymorphisms are involved in tomato carotenoids bioavailability (Desmarchelier et al. 2018). Carotenoids are particularly sensitive to high temperatures which is an important factor that induce oxidation, in addition to light and oxygen. Oxidation is considered to be the major cause of carotenoids loss. Nevertheless, cooking process can improve the bioaccessibility of carotenoids by disrupting carotenoid-protein complexes, modifying the integrity of the matrix, increasing carotenoid extraction from food matrix and promoting carotenoid isomerization (De Alvarenga et al. 2018). Carotenoids can be combined with other nutrients and compounds such as coix seed oil and olive oil in order to enhance their bioavailability and bioactivity. However, it has not yet been proven that the mentioned combined systems could be economically viable and suitable for food applications.

Carotenoids belong to lipid family and, once ingested by the body, their destiny follows the gastrointestinal digestion, absorption by enterocytes and distribution in blood circulation of the other lipidic molecules (Desmarchelier et al. 2018). First of all, carotenoids are degraded from food matrix under the effect of digestive enzymes, then transferred using pancreatic lipase, to mixed micelles. This step is called micellization. Secondly, bioaccessible carotenoids are assimilated by enterocytes where this assimilation is facilitated by several proteins. For example, scavenger receptor class B member 1 is a protein, which participate in provitamin A, lycopene, lutein, zeaxanthin, phytoene and phytofluene uptake. These proteins could favor chylomicrons assembly and secretion and thus increase the gradient of carotenoids between the intestinal lumen and absorptive cells (Desmarchelier et al. 2018).

Today, carotenoids are not only exploited as food, feed and nutraceutical ingredients but also in pharmaceutical field after developing structurally diverse carotenoids. From industrial and commercial point of view, carotenoids are applied as natural colorants, dietary supplements, animal feed supplements and even as nutraceuticals for cosmetic and pharmaceutical applications (Jaswir et al. 2011).

4 Lycopene

4.1 Chemistry

The most prevalent carotenoid within red ripe tomatoes is obviously lycopene, which constitutes about 80–90% of its total carotenoids (Dorais et al. 2008). This phytochemical confers to tomato and several other fruits namely guava, watermelon, pink grapefruit and papaya their red color (Barber and Barber 2002). Lycopene content ranges from 0.1μg/g in green tomatoes to 50μg/g in red ripe

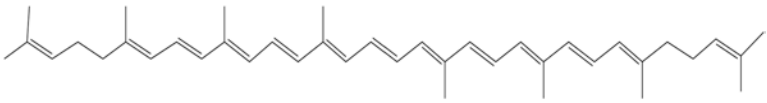

Fig. 3.2 All-*trans* isomer of lycopene

tomatoes and can reach 70μg/g in overripe softened tomatoes (based on fresh weight) (Dorais et al. 2008).

Lycopene is an extremely hydrophobic carotenoid with a linear chemical structure containing 13 conjugated double bonds (Fig. 3.2). Lycopene structure is free from β-ionone ring, which make it deprived of provitamin A activity (Barber and Barber 2002). Lycopene double bonds allow it to isomerize and various *cis* and *trans* isomers can consequently be obtained. Lycopene is considered to be stable during cooking, even though susceptible to isomerization. All-*trans* isomers, the most commonly found in raw materials, are considered to be the most thermodynamically stable (Barber and Barber 2002).

4.2 Bioavailability

In the human body, lycopene is absorbed and then distributed in the plasma lipoproteins. The limited bioavailability of this lipophilic carotenoid is mainly attributed to the high resistance of chloroplasts, where lycopene is found, to gastric and intestinal digestion (Schweiggert et al. 2014). Lycopene serum levels vary among populations and individuals (e.g. dietary intake, structure of the food matrix source, age, food processing, mastication, dietary composition, hormonal and pharmaceutical status) (Clinton 2005). In fact, thermal processing may increase the lycopene availability by improving the accessibility of lipophilic compounds to form lipidic micelles with dietary lipids and bile acids (Jaswir et al. 2011). It has been proven that lycopene presents as *cis*-isomers in processed tomato products (e.g., catsup, tomato juice, tomato puree) is more bioavailable and better absorbed by humans than lycopene from fresh and unprocessed tomatoes (Tan et al. 2010). Indeed, lycopene contents in cooked tomatoes, tomato sauce, ketchup, and fresh tomatoes are 3.7, 6.2, 9.9–13.4, and 0.8–7.4 mg lycopene/100 g (Barber and Barber 2002). Lycopene absorption and bioavailability was improved when consumed with fat as it is a fat-soluble compound (Clinton 2005).

Lycopene is found in *trans*-form in crystalline aggregates in fresh red tomatoes, while it is present as tetra-*cis* isomers in lipid dissolved globular matrices in tangerine tomatoes. The accumulation of tetra-*cis* form of lycopene in place of all-*trans* form in tangerine tomatoes is due to the absence of specific isomerase able of isomerizing poly*cis* to all-*trans*-lycopene and confers orange color to tomatoes (Isaacson et al. 2002). As demonstrated by clinical studies, *cis*-isomers of lycopene were easily absorbed by human intestinal cells and then more bioaccessible than the all-*trans* lycopene, probably due to the higher solubility of the former in the bile acid micelles, their presence in lipid-dissolved globular structures and their better intracellular

stability (Boileau et al. 2002; Unlu et al. 2007; Cooperstone et al. 2015). Cooperstone et al. (2015) reported in a randomized, crossover clinical trial that lycopene bioavailability in orange tomato juice was 8.5 times greater than that in red tomato juice and concluded that orange tomatoes are a rich source of lycopene, causing great levels of lycopene in plasma. More recently, Cooperstone et al. (2017) confirmed the higher lycopene availability from orange tomatoes in an animal model study where mice fed with orange tomato powder, which contained about three time less total lycopene than red tomato powder, displayed increased plasma and skin lycopene concentrations reaching 286–500 nmol/L and 0.23–2 nmol/g, respectively.

4.3 Biological Activities and Modes of Action

Lycopene is known as the most effective antioxidant among tomato carotenoids especially because of its potent singlet oxygen quenching activity and peroxyl radicals scavenging. In its excited state, lycopene has not enough energy to set off the excitation of other molecules leading to the generation of reactive species. Thus, a single lycopene molecule is able to quench more than one free radical (Jaswir et al. 2011). Lycopene is obviously an effective antioxidant and singlet oxygen quencher because of its unsaturated structure (Rao and Rao 2007). Lycopene owe these particularly interesting activities to the double bonds present in large number within its chemical structure, which is correlated to the antioxidant activity efficacy (Dorais et al. 2008; Barber and Barber 2002). For this reason, lycopene was found to be two times more effective than β-carotene as a nitrogen dioxide scavenger (Barber and Barber 2002).

Thanks to its antioxidant activity, lycopene is particularly efficient against cancer incidence and many carcinogenesis studies have been performed with the purpose of bringing out the anticancer activity of this phytochemical and highlighting its mechanisms of action (Fig. 3.3). The presence of lycopene in human blood at high concentrations was revealed to be effective in fighting oxidative damage and mutagens occurring to DNA, cellular proteins and lipids and leading to several types of cancers (Barber and Barber 2002; Ibrahim et al. 2019). Lycopene prevents oxidative damage of cells through the scavenging of oxygen free radicals, like peroxyl radicals, and through the interaction with reactive oxygen species, like hydrogen peroxide and nitrogen dioxide (Barber and Barber 2002). In case of gastric cancer, it has been shown that lycopene prevented oxidative injury through the stimulation of both levels and activities of glutathione (GSH), glutathione-S-transferase (GST) and glutathione peroxidase (GPx) enzymes and thus, the anticancer activity of lycopene can be linked to the enhancement of the antioxidant activity of enzymes as well as the reduction of oxidative damage in gastric mucosa (Kim and Kim 2015). Moreover, lycopene potential anticancer activity may be associated with the regulation of cell-cell communication and the alteration of cell signaling (Barber and Barber 2002).

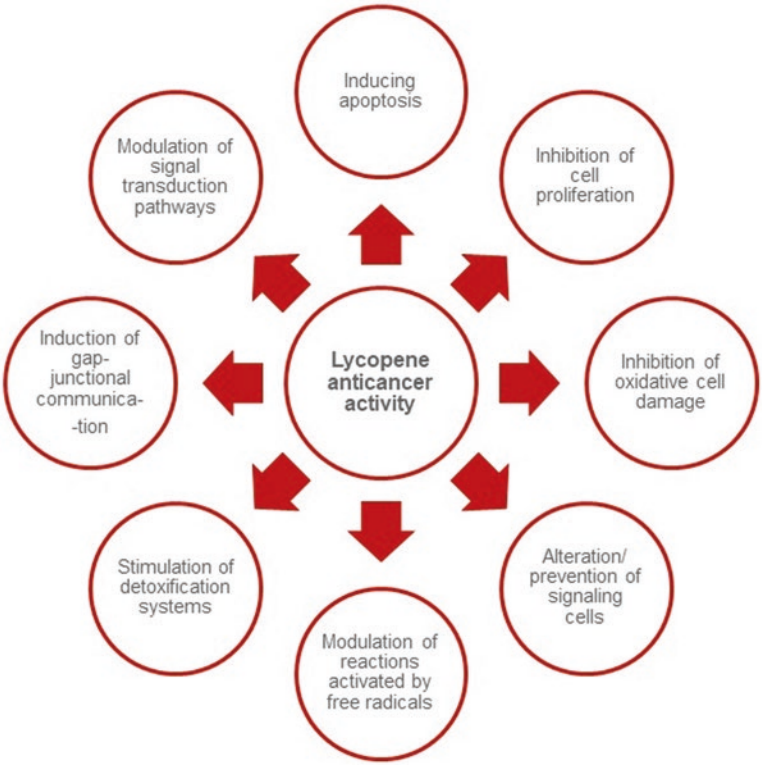

Fig. 3.3 Some proposed mode of action underlying the lycopene anticancer activity

In fact, lycopene may inhibit cancer development by limiting cell proliferation through the inhibition of regulatory proteins phosphorylation, making the cell cycle stop at G0/G1 phase (Palozza et al. 2004; Gupta et al. 2018). In addition, lycopene demonstrated an efficient effect of blocking insulin-like growth factor type 1 (IGF-1) responsible for the proliferation of various cell lines of tumors, and the lower levels of IGF1 were related to higher intake of tomatoes (Karas et al. 2000; Barber and Barber 2002). The inhibition of IGF-1-induced cell growth was linked to the inhibition of IGF signaling, as confirmed by the reduction in IGF-I stimulation of tyrosine phosphorylation of insulin receptor substrate 1 (IRS-1) and binding capacity of activating protein-1 (AP-1). The effect of lycopene on inhibiting IGF-1 was correlated with the enhancement of the number of cell surface-associated insulin-like growth factor-binding proteins (IGFBPs) which negatively modulate the receptor function (Karas et al. 2000). Moreover, lycopene decreased the incidence of mammary tumors whether induced spontaneously or chemically in animal models. In fact, it has been proved that lycopene prevents AP-1 signaling in mammary cells (Gupta et al. 2018). Lycopene is also effective in preventing and inhibiting cancers by inducing cell apoptosis. It has been shown that lycopene as well as its auto-oxidant

products caused apoptosis in human leukemia cells. In fact, lycopene is involved in apoptosis through B-cell lymphoma 2 (Bcl-2) and this action is somehow linked to the antioxidant power of lycopene (Karas et al. 2000; Palozza et al. 2004; Gupta et al. 2018).

Clinton (2005) concluded that, even though the consumption of lycopene based products may only decrease overall cancer risk by 30, 20 or even 10%, the efforts dedicated to establish complex array of risk factors and preventive interventions could be motivated by the cost of health care system particularly in screening, diagnosis and therapy and also by the pain caused by the disease and its involvements. The same author suggested that combining tomato products with efficient chemopreventive and dietary compounds could effectively intervene in the prevention of cancer.

5 β-Carotene

5.1 Chemistry

β-carotene is a lipid soluble secondary metabolite, presenting the structure of a tetraterpene. It is synthesized by plants and presents a molecular weight of about 536 Da (Zahra et al. 2016). It is derived from acyclic structure and presents a long chain made of conjugated double bonds (Bogacz-Radomska and Harasym 2018). β-carotene is generally present in all-*trans* isomer, shown in Fig. 3.4. The high temperature induces the isomerization of β-carotene double bonds and lead to the enhancement of the resulting color (Bogacz-Radomska and Harasym 2018).

β-carotene is present in fruits and vegetables, particularly in orange carrots, pumpkins, spinach, and tomatoes. This carotenoid accounts for around 7% of total carotenoids present in tomato fruit (Dorais et al. 2008).

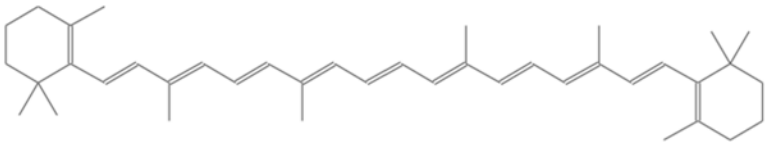

Fig. 3.4 All-*trans* β -carotene

5.2 Bioavailability

β-carotene is considered as a provitamin A carotenoid and its bioavailability depends on many factors such as the food matrix nature, the presence of other nutrients in the meal, β-carotene dose, processing, and genetic, health, and nutritional status of individuals. Desmarchelier et al. (2018) revealed that six genes were shown to have single nucleotide polymorphisms, which may explain the observed differences in β-carotene bioavailability between persons. High β-carotene intake led to a decrease the conversion of this carotenoid to vitamin A in the intestines. Indeed, a two-fold increase in β-carotene dietary dose produced an increase in plasma vitamin A not exceeding 36% (Novotny et al. 2010). Dietary fat intake and ultra-processed foods promote intestinal absorption of β-carotene (Haskell 2012). β-carotene bioaccessibility and bioavailability in tomato paste was greater than that of lycopene due to the higher solubility of β-carotene into micelles and their various structural location in tomato matrix. Incorporation of tomato peels in tomato paste increased β-carotene bioavailability (Reboul 2019). Bugianesi et al. (2004) reported in a crossover clinical trial that human plasma β-carotene levels remained unchanged after ingestion of two test meals based on fresh or cooked cherry tomatoes.

5.3 Biological Activities and Modes of Action

β-carotene is considered to be a highly efficient scavenger of singlet oxygen and is a potent dietary precursor of vitamin A as it is converted into retinal through enzymatic reaction, and ultimately into retinol, namely vitamin A (Jaswir et al. 2011; Zahra et al. 2016). Thanks to its chemical properties and biological activities, β-carotene is applied in food, cosmetic and pharmaceutical fields where it is used as orange-red pigment or as active ingredient against oxidation and UV radiation (Bogacz-Radomska and Harasym 2018).

As an antioxidant agent, β-carotene quenches the free radicals present in human cell membranes, high-density lipoprotein (HDL) and low-density lipoprotein (LDL) and thus inhibits the oxidation of some fats under certain conditions (Ringer et al. 1991). In fact, this phytochemical scavenges free radicals following the process including radicals' incorporation into carotenoids, extraction of hydrogen and transfer of electron (Krinsky 2001). Thus, β-carotene protects lipidic membranes especially through its synergy with vitamins C and E (Zahra et al. 2016). However, it has been shown that β-carotene is not always effective in LDL protection against oxidative agents and this finding is depending on population characteristics, diet and other unknown factors (Bogacz-Radomska and Harasym 2018).

Thanks to its role as a vitamin A precursor, β-carotene undergoes a symmetrical cleavage by β-Carotene-15,15′-oxygenase (BCO1) leading to the production of two molecules of retinaldehyde. β-carotene cleavage can also be ensured by another enzyme called β-carotene-9′, 10′-oxygenase (BCO2) but this cleavage is performed

in an asymmetric manner and produces apocartenal and β-ionone ring, which will be further converted into retinaldehyde (Zahra et al. 2016). Retinaldehyde is later oxidized by enzymes from retinaldehyde dehydrogenase family and produces all-*trans* retinoic acid, which is the vitamin A active form (Zahra et al. 2016).

β-carotene is considered as a health promoting phytochemical with antioxidant and anticancer activities (Bai et al. 2019) and low β-carotene plasma levels could lead to death (Zahra et al. 2016). β-carotene could increase longevity, reduce osteoporosis risks and prevent oxidative stress on bone (Rao and Rao 2007). High consumption of β-carotene may decrease the incidence of different diseases namely cataract formation, macular degeneration, cardiovascular diseases and several types of cancers (Zahra et al. 2016). It also presents an antimutagenic activity and could provoke apoptosis and stop cell cycle.

In fact, some studies reported that β-carotene could be efficient in blocking the growth of malignant cells thanks to its pro-apoptotic effect. In case of human cervical cancer, β-carotene may cause chromatin condensation, which is a characteristic phenomenon of apoptosis. Moreover, it has been reported that the initiation of the mentioned cancer can be prevented thanks to the induction of cervical dysplastic cells apoptosis through the down-regulation of the protein related to the receptor of epidermal growth factor (EGF) (Muto et al. 1995; Palozza et al. 2004). In human leukemia, undifferentiated HL-60 cells were more vulnerable to β-carotene pro-apoptotic action than the differentiated ones. Thus, it has been confirmed that the cell type may influence the pro-apoptotic properties of β-carotene due to the difference in carotenoid incorporation by the cells. In addition to leukemia cells, β-carotene was effective in inducing apoptosis of adenocarcinoma of human colon. However, carotenoid inhibitory concentrations were very different in these two types of cancer cells. It has been stated in previous studies that β-carotene apoptotic activity could be perceived at the concentration range of 2–20μM (Palozza et al. 2004).

Even though consuming fruits and vegetables particularly rich in β-carotene was linked to a significant decrease in cancer risks, many researches have not associated cancer prevention to β-carotene anticancer activity. Some studies have demonstrated that subjects consuming β-carotene from non-natural sources showed an increased mortality rate with an increased cancer incidence. In fact, it has been confirmed that β-carotene should be taken in a balanced diet based on fruits and vegetables and not through supplements containing only β-carotene because the beneficial effect of this phytochemical requires the presence of other compounds, which can facilitate its absorption by the body and enhance its biological activity. β-carotene interacts with the other chemicals present in natural fruits and vegetables synergistically and thus, an increased longevity and decreased cancer incidence were observed. However, it is difficult to quantitatively measure these observed health benefits and prove the synergistic relationship among the different dietary phytochemicals (Dickman 2019).

6 Epidemiologic Studies

Epidemiologic studies have linked increased fruit, vegetable and carotenoid intake with decreased risk of cancer development in the lung (Neuhouser et al. 2003), the cervix (Goodman et al. 2007), the prostate (Tan et al. 2016), the pancreas (Chen et al. 2016), the breast tissue (Tamimi et al. 2009), the anal canal (Shvetsov et al. 2010), the liver (Montella et al. 2011) and the ovaries.

Lung cancer is among the most serious cancer types with approximately two million cases in 2018 (World Health Organization 2018). Wright et al. (2010) reported an important increase in lung cancer rates in male smokers supplemented with β-carotene and attributed this result to tumor development in the airway epithelial cells of these smokers. Likewise, in a multicenter, double-blind chemoprevention trial, Neuhouser et al. (2003) reported that persons with increased risk of getting lung cancer and supplemented with β-carotene and retinol did not take advantage of *active substances* in fruits and vegetables and no protective effect on risk for developing lung cancer was found. Many hypotheses including the modulating activity of phase 1 enzymes by high dose of supplemental β-carotene and its potential adverse effect on the bioavailability of other bioactive phytochemicals were given. Recently, Hashim et al. (2014) performed a mortality analysis, using a database, which comprises clinical measurements of asbestos exposed workers, to evaluate the relation between β-carotene concentrations in serum without supplementation and mortality in these workers. Serum β-carotene concentration, strongly correlated with fruit and vegetable consumption, is considered as a nutritional status or a healthy diet indicator. Despite the reducing effect of high serum β-carotene concentrations on overall mortality of asbestos-exposed workers, no correlation was found with overall cancer or lung cancer mortalities.

The results of a systematic review including 10 studies found a negative but insignificant relation between lycopene consumption and risk for developing ovarian cancer in elderly women (Li and Xu 2014). The presumed protective effect of lycopene is related to its great ability to quench singlet oxygen molecules and to scavenge peroxyl radicals. The authors attributed the insignificant obtained results in the meta-analysis to postmenopausal status of participants, histological types of ovarian cancer in included studies and the difficulty in quantifying lycopene intake. Furthermore, Tamimi et al. (2009) reported that high concentrations of α-carotene, lycopene, lutein and β-cryptoxanthin in plasma decreased breast cancer risk by up to 40% in women with high mammographic density.

A meta-analysis of the impact of β-carotene supplements on cancer prevention revealed no protective effect on cancer prevalence and mortality (Jeon et al. 2011). Likewise, no effect of β-carotene intake was found on prostate cancer survival, while α-tocopherol supplementation was linked to improved prostate cancer survival (Watters et al. 2009). Inconsistent findings on the correlation between dietary lycopene intake and prostate cancer were obtained from meta-analysis studies (Etminan et al. 2004; Chen et al. 2015). Haseen et al. (2009) claimed in their systematic review that lycopene reduced cancer-related symptoms in the few

intervention studies on the impact of lycopene on prostate cancer progression. In a prospective cohort study among male health practitioners, a strong inverse relation was found between high lycopene consumption and lethal prostate cancer and the authors hypothesized that lycopene-rich diet can inhibit angiogenesis by regulating vascular permeability factor, preventing aggressive prostate cancer (Zu et al. 2014). Although this trend was not observed in the meta-analysis conducted by Chen et al. (2015), an exposure-effect relationship was found between lycopene supplementation and circulating concentration and prostate cancer incidence.

7 Animal Studies

In an *in vitro* study performed by Elgass et al. (2012), the anti-angiogenic effect of lycopene using rat aortic rings was revealed. Indeed, a lycopene concentration of 1.15 mmol/l significantly decreased tubule length and network branching by 25 and 44%, respectively. In another study, Tan et al. (2016) demonstrated that tomato powder and lycopene beadlets, which produced lycopene serum concentrations in mice comparable to those detected in human serum, inhibited prostate oncogenesis in the transgenic adenocarcinoma of the mouse prostate system in a BCO2 genotype-specific manner. Indeed, loss of BCO2 gene might decrease the chemopreventive effect of lycopene, supporting the potential anticancer activity of lycopene cleavage metabolites. It was established that lycopene interacts with fructose-amino acids synergistically to inhibit the growth of rat prostate adenocarcinoma cells by more than 98% and the highest survival rate from prostate tumorigenesis was obtained in rats fed with tomato paste/ketosamines (>50 weeks) (Mossine et al. 2008). Likewise, combined administration of lycopene, selenium and vitamin E effectively inhibited prostate cancer cell growth and progression in lady transgenic mice (Venkateswaran et al. 2009). In male nude mice supplemented with lycopene, a significant inhibition of metastasis of lung cancer induced by human hepatoma cells was observed by Huang et al. (2008) who attributed this result to significant reduction in matrix metalloproteinase-2 and vascular endothelial growth factor levels as well as increase in interleukin-12 production. According to Luo and Wu (2011), lycopene provided to male Wistar rats with gastric cancer exhibited an anticancer activity, which may be related to improvement of immunity function and stimulation of antioxidant enzymes. In another study, lycopene prevented oxidative stress and polychlorinated biphenyls-induced apoptosis in Sertoli cells from albino rat testes. Indeed, lycopene supplementation decreased Bad and Bid expression and increased anti-apoptotic Bcl2 protein in testicular cells (Krishnamoorthy et al. 2013). Moreover, tomatoes had a defensive effect against UVB-induced skin tumors by reducing DNA inflammation in mice skin (Cooperstone et al. 2017).

8 Human Clinical Trials

Associations between tomatoes intake and decreased human cancers risk have been established in many epidemiological and clinical studies (Ford et al. 2011; Venier et al. 2012; Chen et al. 2015). Many mechanisms have been advanced to investigate the chemopreventive effects of bioactive constituents in tomato, such as lycopene and various phenolic compounds, against many cancers. These mechanisms could include inhibition of Reactive Oxygen Species (ROS), activation of apoptosis pathways, activation of cell growth arrest, increase of gap-junctional communication and inhibition of cell proliferation and viability (Sharoni et al. 2016). The considerable increase in prostate lycopene content in cancer patients may in part justify its protective action in lowering damage of prostate DNA and prostate cancer biomarkers (Basu and Imrhan 2007).

Although the relation between consumption of β-carotene and risk of prostate cancer was not established, high lycopene intake decreased this risk by 26% (Sharoni et al. 2011). Many clinical trials with high tomatoes consumption or high lycopene plasma levels have documented lycopene antioxidant potential and anticancer properties against cancer cells in prostate (Ford et al. 2011; Venier et al. 2012; Chen et al. 2015). Using different lycopene concentrations, Soares et al. (2013) showed that 10 μM of lycopene increased human malignant prostate cancer cell death by more than twofolds after 96 h treatment with significant increase in expression of BAX and CK18 genes and decrease in expression of Bcl-2 gene. In a recent study, Soares et al. (2019) treated two human cancer cells in prostate with lycopene extracted from various tomato-based products and evaluated its effect on viability, cycle progression and death of cells. They found that lycopene exhibited a significant anticarcinogenic activity against prostate cancer cell lines by inhibiting cell viability, inducing cell cycle arrest and increasing apoptosis. After 4 days of treatment, lycopene promoted growth arrest in G0/G1 and G2/M phases and achieved the highest increase rate in apoptotic cells. *In vitro* assays using three different human prostate cancer cells demonstrated that lycopene enhanced the anticancer properties of capsaicin and these two dietary agents were able to promote apoptosis by regulating Bax/Bcl-xl ratio and stimulating caspase cascade in androgen sensitive cells (Venier et al. 2012). Likewise, Jeong et al. (2019) demonstrated that lycopene promoted apoptosis in human pancreatic cancer cells by reducing intracellular and mitochondrial reactive oxygen species and downregulating NF-κB activity and NF-κB regulated genes. Moreover, Huang et al. (2013) demonstrated that lycopene anti-angiogenic activity may be explained by modulation of cytokine secretion and decrease of matrix metalloproteinase-2 activity in human peripheral blood mononuclear cells.

Recently, Navarro-González et al. (2018) assessed gene expression variation of human hepatocytes obtained from liver tissues of overweight patients supplemented daily with 200 ml of tomato juice and found that this latter induced cellular apoptosis and regulated cell cycle progression due to lycopene accumulation in the liver. Moreover, tomato intake was found more effective in preventing hepatocarcinoma

than the intake of pure tomato compounds separately, suggesting the synergistic action of different tomato phytochemicals in prevention of liver cancer. Furthermore, Tanambell et al. (2019) reported that extracts from high-β-carotene tomatoes promoted proliferation of prostate cancer cells, while those from orange tomatoes (rich in tetra-*cis* lycopene) had anti-proliferative activity against prostate cancer cells. Moreover, tangerine tomatoes had greater anti-inflammatory activity than red and orange-colored tomatoes. It is worth noting that the observed *in vitro* anti-proliferative and anti-inflammatory properties should be validated with in vivo assays.

In a 24-month, randomized, double-blind trial, Keefe et al. (2001) evaluated the effect of daily β-carotene supplementation for women on the decrease of cervical intraepithelial neoplasia 2 and 3 lesions and measured levels of vaginal and serum micronutrients. β-carotene intake did not result in an improvement in cervical lesion regression, particularly for women infected with human papillomavirus, and the authors suggested that complex interactions between micronutrients might explain the obtained results. Li et al. (2005) reported in a randomized, double-blind clinical trial that β-carotene supplementation prevented prostate cancer in the participants with AA genotype, versus a placebo during 7 years.

Skin cancer is the most frequent cancer in the U.S. and worldwide, representing a major public health and economic problem. Every hour, more than 396 people are diagnosed with skin cancer and more than 2 people die of skin cancer in the U.S. (American Cancer Society 2019). Keratinocyte carcinomas, known as non-melanoma skin cancers, are the most frequent skin tumours that are associated with exposure to solar ultraviolet radiation. Human clinical results demonstrated that dietary tomato paste rich in lycopene prevented sunburn due to the photoprotective effect of carotenoid compounds stored in human skin (Stahl et al. 2001; Cooperstone et al. 2017). Consumption of a whole tomato provided a better photoprotective effect that supplement intake (Stahl et al. 2005).

9 Limitations of Cancer Studies and Future Trends

The obtained results of many epidemiologic and clinical studies lack consistency and coherence because of many factors including:

- Low statistical power of performed studies resulting mainly from small sized samples and effects
- Variability in dietary assessment methods and concerns related to their reproducibility and validity
- Supplements Interference with each other and with cancer treatments
- Heterogeneity in cancer screening among different populations or within the same patient
- Overdiagnosis of indolent cancers resulting in low impact on cancer prevalence and death rate reductions as well as in needless harmful treatments

– Late diagnosis of some cancers, such as lung cancers, until they are far advanced may result in undetected carcinomas in persons classified as noncases
– Wide variation of cancer risks among individuals depending on lifestyle, dietary and genetic factors.
– Variation of results with the form, dosage, composition, metabolism, bioavailability and timing of the administrated supplement, association with other supplements or phytochemicals as well as stage and molecular characteristics of cancer
– Incomplete control of some confounding factors (i.e., physical activity, alcohol
– intake levels, age, cancer subtypes)
– Biased data on self-reported nutrient intake (food frequency questionnaires)

10 Conclusion and Future Trends

Several *in vitro* studies have underlined the protective effects of lycopene and β-carotene on cancer. However, many others have been uncapable to successfully convert these results in related preclinical/clinical model systems. In fact, cancer and anticancer activity are complicated mechanisms that cannot easily be explained or underlined by simple models of biological reactions and mechanisms. This may be due to the fact that properties related to the biology, pharmacology and bioavailability of carotenoids, especially lycopene and β-carotene, are still not well understood and further studies on their pharmacokinetic properties are required.

Up today, several researches have shown the health advantages of lycopene and β-carotene intake, especially when brought naturally within an equilibrated diet. For this reason, the intake of tomato and tomato based products such as sauces and tomato puree have been widely encouraged. In addition, it has been established that the consumption of tomato in complex dishes increases lycopene and β-carotene availability. Thus, future research could be conducted to develop new formulations that guarantee an optimal carotenoid intake where several types of oils can be added to enhance intestinal absorption of carotenoids. As the beneficial effects of lycopene and β-carotene, when consumed within an equilibrated diet were established, more researches need to be performed in order to enrich food matrices with these carotenoids and to increase their bioavailability in natural food products where they already exist.

Even though the consumption of lycopene and β-carotene from food sources is highly recommended by the scientific community to reduce cancer risks, the health benefits of purified forms of lycopene and β-carotene remain poorly understood and further research should be designed to enhance our knowledge on the anticancer effects of these specific purified supplements. Moreover, molecular mechanisms and genetic interactions associated with the impact of tomato consumption on several types of cancer need to be refined.

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Chapter 4
Essential Oils from Aromatic Plants in Cancer Prevention and Treatment

Patricia Reboredo-Rodríguez and Alfonso Varela-López

Abstract Food and other industries recently focused on natural additives rather than synthetic due to health hazards, therefore essential oils (EOs) are one of the most important alternatives under study which are abundantly and easily extractable natural safe oils used worldwide. EOs are natural, volatiles and aromatic liquids extracted from special plants. Their biological properties include antioxidant, antimicrobial and anti-inflammatory activities. Recently, available studies indicate their anticancer potential. As a summary, EOs and their constituents act as antiproliferative agent on cancer cells by multiple mechanisms involving apoptosis induction and cell cycle arrest, oxidative stress induction, mitochondrial function impairment and different pathways and gene expression modulation, as well as, changes in some hormonal levels and immunomodulatory effects.

Keywords Essential oils · *In vitro* studies · *In vivo* studies · Citotoxicity · Cell cycle arrest · Apoptosis · Mitochondrial function impairment

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1 Introduction

Plants are capable of synthesizing two kinds of oils: fixed oils and essential oils (EOs, volatile oils). Fixed oils consist of esters of glycerol and fatty acids (triglycerides or triacylglycerols), meanwhile EOs are complex mixtures of secondary metabolites originating from a single botanical source that determines the specific aroma and the flavor and fragrance of the plants (Sirousmehr et al. 2014; Tisserand and Young 2013).

All parts of aromatic plants may contain EOs, specifically flowers, leaves, rhizomes, seeds, fruits, wood and bark (Dhifi et al. 2016). In nature, the particular aromatic and chemical characteristics of EOs play many important functions in the plant, such as, (1) attract beneficial insects and pollinators, (2) shield the plants from some environmental stress factors (heat, cold, etc.) and (3) protect the plants from pests and/or microorganisms (Burt 2004; Dhifi et al. 2016). EOs are volatile, usually liquid and colorless at ambient temperature. They are poorly soluble in water but highly soluble in alcohol, organic solvents, and fixed oils (Falleh et al. 2020). Moreover, EOs exhibit a very characteristic odor and, as a consequence, they are responsible for the specific scents that aromatic plants emit (Dhifi et al. 2016; Valderrama and Ruiz 2018).

EOs have a very high variability of their composition, both in qualitative and quantitative terms. The factors responsible for this variability can be grouped into two categories: intrinsic factors related to the plant, interaction with the environment, the maturity of the plant, even the harvest time during the day; and extrinsic factors related to the extraction method and the environment (Dhifi et al. 2016). EOs are worldwide known for their proven biological activities including antimicrobial, antifungal, antioxidant, antiviral, antimycotic, antiparasitic and insecticidal properties (Burt 2004; Calo et al. 2015; Dhifi et al. 2016). They are used in cosmetics (perfumes and make-up products), in agriculture (biopesticide and repellent), in sanitary products (fragrances for household cleaning products) and as natural remedies in aromatherapy (Falleh et al. 2020).

2 Chemical Composition of Essential Oils

EOs are a complex mixture of volatile compounds characterized by a strong smell and flavor depending on their chemical composition. The constituents of plant EOs fall mainly into two distinct chemical classes: terpenes and phenylpropanoids. Terpenes are characterized by their basic structural element (isoprene unit) and are formed by the condensation of two or more isoprene units (Pavela 2015). Considering the number of isoprene subunits, terpenes are classified in different subgroups, such as hemi- (C5), mono- (C10), sesqui- (C15), di- (C20), sester- (C25), tri- (C30), tetra- (C40) and polyterpenes (C5)n (isoprene units >8) (Pavela 2015). It should be highlighted that the monoterpenes (C10) are frequently the predominant molecules in EOs, and they may reach up to 90% of the whole EO. The aromatic and aliphatic

groups are the less abundant in EOs and its compounds correspond to alcohols, aldehydes, phenols, heterocycles, and methoxy derivatives (Dhifi et al. 2016; Pavela 2015). The pathway involved in terpenoids are the mevalonate and mevalonate-independent (deoxyxylulose phosphate) pathways, whereas phenylpropanoids are originated through the shikimate pathway (Dewick 2002; Lichtenthaler 1999).

3 Main Studied Essential Oils

The most studied EOs in cancer research are summarized in Fig. 4.1 and discussed below.

3.1 Essential Oils from Boswellia spp.

The frankincense tree belongs to the family of the Burseracea and its genus is denoted as Boswellia, which is widespread in the dry areas of the Horn of Africa (Somalia, Sudan, Ethiopia, and Eritrea), the Arabian Peninsula (Oman and Yemen), and in India (Hussain et al. 2016). Other members of the genus *Boswellia* include *Boswellia serrata* Roxb. ex Colebr. (India), *Boswellia ovalifoliolata* Bal. & Henry (India), *Boswellia pirottae* Chiov. (Ethopia), *Boswellia dalzielii* Hutch. (West Africa), *Boswellia frereana* Birdw. (Somalia), *Boswellia neglecta* S. Moore (Kenya), *Boswellia papyrifera* (Del.) Hochst. (Ethopia), *Boswellia rivae* Engl. (Ethopia), *Boswellia hildebrandtii* Engl. (Somalia), *Boswellia ogadensis* Vollesen (Ethopia), *Boswellia popoviana* Hepper (Yemen), *Boswellia nana* Hepper (Yemen), *Boswellia bullata* Thul. & Gifri (Yemen), *Boswellia dioscorides* Thul. & Gifri (Yemen), *Boswellia elongata* Balf. f. (Yemen), *Boswellia ameero* Balf. f. (Yemen), *Boswellia odorata* Hutch. (Tropical Africa), *Boswellia dalzielli* Hutch. (Tropical Africa), and *Boswellia socotrana* Balf. f. (Yemen) (Hussain et al. 2016).

The Boswellia plants are deciduous trees that can reach a height of up to 5 m and even more but mostly are dependent on the particular species and the growing area to determine their final height. Generally, the frankincense tree is wounded in March or April and the resin is harvested through the summer and autumn. It has been reported that a Boswellia tree can produce this exudate in high quality only for three consecutive years, after which the quality of the resin decreases (Hussain et al. 2016). Traditionally, gum resins from frankincense (known as Ru Xiang in Chinese) have been used as a major ingredient in Ayurvedic and Chinese medicine to treat a variety of health-related conditions. Its biologically active ingredients are usually extracted by hydrodistillation. The antitumoral effect of EOs obtained from different Boswellia species (*Boswellia carterii*, *Boswellia sacra* and *Boswellia serrata*) as well as their gum resins have been tested in several cancer cell lines of human bladder cancer (J82), breast adenocarcinoma (MCF7, T47D and MDA-MB-231), chronic myelogenous erythroleukemia (K562), neuroblastoma (SH-SY5Y), pancreatic cancer (MIA PaCa-2, Panc-28, BxPC-3, and DANG), cervical cancer (HeLa),

Boswellia spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Cell cylcle arrest

Cinnamomum ssp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Cell cylcle arrest
- Oxidative stress induction

Citrus spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Cell cylcle arrest

Curcuma spp.

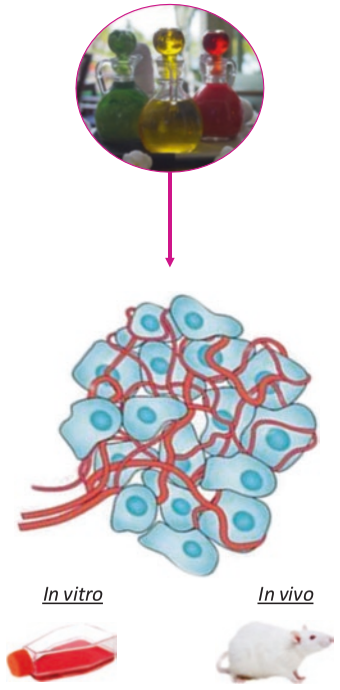
- Cytotoxic/antiproliferative effect
- Programmed cell death

Cymbopogon spp.

- Cytotoxic/antiproliferative effect

Lavandula spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Cell cylcle arrest



Origanum spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Lipid metabolism alteration

Pinus spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Cell cylcle arrest
- Oxidative stress induction

Salvia spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Cell cylcle arrest
- Oxidative stress induction

Thymus spp.

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Oxidative stress induction
- Immunomodulation

Zataria multiflora

- Cytotoxic/antiproliferative effect
- Programmed cell death
- Oxidative stress induction
- Immunomodulation

Fig. 4.1 *In vitro* and *in vivo* effects of EOs and their constituents on cancer research

as well as the immortalized normal bladder UROtsa cells and the immortalized normal breast epithelial MCF-10-2A cells showing cytotoxic effects (Al-Otaibi et al. 2018; Dozmorov et al. 2014; Najar et al. 2020; Ni et al. 2012; Ren et al. 2018; Suhail et al. 2011). Such effect was associated with pro-apoptotic activities (Al-Otaibi et al. 2018; Dozmorov et al. 2014; Ni et al. 2012; Ren et al. 2018; Suhail et al. 2011) and/or cell cycle arrest in the same studies (Dozmorov et al. 2014; Ni et al. 2012; Suhail et al. 2011). In addition, a *Boswellia sacra* EO fraction also showed these anti-proliferative and pro-apoptotic activities against pancreatic tumors in a heterotopic xenograft mouse model (Ni et al. 2012). Consistent with the *in vitro* activities, frankincense EO was effective in inhibiting tumor growth and inducing tumor cell apoptosis in a human breast cancer mouse model (Ren et al. 2018).

3.2 Essential Oils from Cinnamomum spp.

The genus *Cinnamomum* (Laureaceae family) consists of 250 species of trees and shrubs distributed in south-east Asia, China, and Australia. It is a small, evergreen tree, 10–15 m tall, whose bark is widely employed as a spice (Cardoso-Ugarte et al. 2016). They are usually found in tropical rain forests, where it grows at various altitudes from highland slopes to lowland forests and occurs in both marshy places and on well-drained soils. However, in latitudes with seasonal climatic conditions, they become exceedingly rare (Cardoso-Ugarte et al. 2016). The cinnamon EO composition varies depending on the geographical origin of the species and the processing conditions. The most important cinnamon oils in word trade are those from *Cinnamomum zeylanicum*, *Cinnamomum cassia*, and *Cinnamomum camphora*.

In the context of cancer research, the most studied specie is *Cinnamomum zeylanicum* whose EO presents as dominant metabolites cinnamaldehyde, cinnamaldehyde dimethyl acetal, cinnamyl acetate, eugenol, linalool, eucalyptol, limonene, *o*-cymol, and α-terpineol (Kubatka et al. 2020). The EOs obtained from this plant have shown antitumoral activities *in vitro* (Kubatka et al. 2020; Najar et al. 2020; Yang et al. 2015; Zu et al. 2010) in human cancer cells of breast adenocarcinoma (MCF7, T47D and MDA-MB-231), chronic myelogenous erythroleukemia (K562), neuroblastoma cell lines (SH-SY5Y), human prostate carcinoma cell (PC-3), human lung carcinoma (A549), head and neck squamous cell carcinoma (HNSCC, FaDu, Detroit-562 and SCC-25) and colon cancer (HCT116) (Alkhatib et al. 2020; Kubatka et al. 2020; Najar et al. 2020; Yang et al. 2015; Zu et al. 2010). In breast adenocarcinoma cells and colon cancer cells, such effect was associated with apoptosis induction (Alkhatib et al. 2020; Kubatka et al. 2020) predominantly by activating mitochondrial-dependent pathway (Kubatka et al. 2020). Moreover, it suppressed growth tumor in a Hep-2 cell xenograft model (Yang et al. 2015). Likewise, a bark powder of *Cinnamomum zeylanicum* administered in the diet reduced the incidence of chemically-induced mammary carcinomas in rats that also showed a significant decrease in the ratio of high-/low-grade carcinomas. A similar treatment also

decreased tumor volume in a syngeneic 4T1 mouse model that showed a significant decrease in mitotic activity index (Kubatka et al. 2020). In treated rat carcinomas, it has been found caspase-3 and Bax expression increase confirming the pro-apoptotic activity found in cancer cell lines. Moreover, a decrease in Bcl-2, Ki67, VEGF, and CD24 expressions and MDA levels as well as several epigenetic changes were observed (Kubatka et al. 2020). The results of the *in vivo* study in the Hep-2 cell xenograft model and *in vitro* assays in HNSCC cells also have suggested that mechanism underlying its anticancer action was the suppression of EGFR-TK (Yang et al. 2015).

Regarding *Cinnamomum cassia, v*arious biological functions including antioxidant, anti-microbial, anti-inflammation, anti-diabetic and anti-tumor activity have been attributed to EO obtained from its bark whose predominant active components are cinnamic aldehyde and cinnamyl aldehyde. EO from its bark, also have been tested showing similar antitumoral properties *in vitro* in human tongue squamous carcinoma cells (HSC-3) and a mouse melanoma model (Chang et al. 2017; Kwon et al. 2010). In HSC-3 cells, these properties were associated with G2/M cell cycle arrest and apoptosis induction. Moreover, it promoted an increase in cytosolic Ca^{2+} levels, induced mitochondrial dysfunction and activated cytochrome c release supporting the role of the intrinsic apoptosis pathway (Chang et al. 2017). In a mouse melanoma transplantation model, the anti-tumor effect of oral administration of the extract were linked with an enhanced pro-apoptotic activity by inhibiting the activities of the transcription factors NF-*kappa*B and AP1. Indeed, it strongly inhibited tumor cell proliferation *in vitro* and induced active cell death of tumor cells by upregulating pro-apoptotic molecules while inhibiting NF-*kappa*B and AP1 activity and their target genes such as Bcl-2, BcL-xL and survivin (Kwon et al. 2010). In addition, other authors have reported that cinnamaldehyde, the main chemical component of the EO isolated from *Cinnamomum cassia* EO also act as an efficient cytotoxic agent against three types of non-small-cell lung carcinoma (NSCLC) (Wu et al. 2017) and human oral squamous carcinoma (HSC-3) cells (Chang et al. 2017) promoting an increase in cytosolic Ca^{2+} levels and inducing cytochrome c release. This could be consequence of the mitochondrial dysfunction also observed which correlated with changes in intracellular redox status including an increased ROS production and a reduction of cellular glutathione content and GPX activity demonstrating an association with oxidative stress also reflected by oxidative damage marker TBARS rise (Chang et al. 2017). Moreover, docking experiments showed that *trans*-cinnamaldehyde was proficiently fitted into the inner grove of the active site of EGFR by making close inter-atomic contacts with key catalytic residues suggesting that it could be responsible for the mentioned suppression of EGFR-TK observed Hep-2 cell xenograft model (Yang et al. 2015). Cinnamaldehyde is able to inhibit NSCLC cell growth by inducing apoptosis and reverse EMT through terminating Wnt/β-catenin pathway, which might supply further insight into cinnamaldehyde-mediated anti-tumor effect against NSCLC for better prognosis (Wu et al. 2017).

Lastly, camphor white oil (CWO) from *Cinnamomum camphora* (Moayedi et al. 2019) has been tested for anti-tumor activity in a mouse model of

keratinocyte-derived skin cancer. Daily topical treatment with CWO induced dramatic regression of pre-malignant skin tumors and a twofold reduction in cutaneous squamous cell carcinomas. In cultured keratinocytes, CWO stimulated calcium signalling, resulting in calcineurin-dependent activation of nuclear factor of activated T cells (NFAT) (Moayedi et al. 2019). *In vivo*, CWO induced transcriptional changes in immune-related genes resulting in cytotoxic T cell-dependent tumor regression that would constitute an additional mechanism through which a plant-derived EO diminishes established tumor burden (Moayedi et al. 2019).

3.3 Essential Oils from Citrus spp.

Citrus fruits are the most common subtropical crops in the world; there is a great amount of variation among citrus species and cultivars as a result of frequent bud mutation, interspecific and intergeneric hybridization, apomixes, and a long history of cultivation (Azar et al. 2011). Among the citrus species, *Citrus sinensis* L. Osbeck, commonly known as sweet orange, is the most horticulturally important and widely known worldwide (Grosser et al. 2007). The EOs obtained from several species belonging to Citrus genus including *Citrus x aurantium*, *Citrus limon* and *Citrus sinensis* showed *in vitro* cytotoxic activity on human cancer cell lines of breast adenocarcinoma (MCF7, T47D and MDA-MB-231), chronic myelogenous erythroleukemia (K562), neuroblastoma (SH-SY5Y), lung (A549), prostate (PC-3) and colon (HCT116) (Mitoshi et al. 2012; Najar et al. 2020; Yousefian Rad et al. 2020; Zu et al. 2010). In particular, EO from *Citrus limon* also has been shown to induce apoptosis in A549 cells and decreased angiogenesis in chick embryo chorioallantoic membrane when was applied in EO-nanoemulsion droplets to improve the drugs' biocompatibility in aqueous conditions (Yousefian Rad et al. 2020).

Additionally, the by-products of several Citrus fruits, namely its peels, also has been used as source of EOs which, in turn, have been investigated in this context. In particular, EOs and extracts obtained from the peels of *Citrus reticulate* present volatiles (EOs, limonoids) and non-volatiles (mainly polymethoxy flavones) components. *Citrus reticulate* peel water extract showed *in vitro* cytotoxic activity against DLA cell line inducing cell cycle arrest in G0/G1 phase and apoptosis (Nair et al. 2018). *In vivo* experiments, *Citrus reticulate* peel extract protected from DLA tumor growth in pre-treated mice compared to post-treated mice (Nair et al. 2018). Likewise, the oil from the peel of *Citrus reticulata Blanco cv. Dancy* (mandarin) that contains EOs and carotenoids, causes a dose-dependent growth inhibition of NSCLC cells (A549) by inducing cell cycle arrest mainly at the G0/G1 phase and reduced the amount of membrane-bound Ras protein along with apoptosis induction. Moreover, the effect on tumor growth of these cells also was confirmed *in vivo* when were implanted in nude mice fed with supplemented diets (Castro et al. 2018).

3.4 Essential Oils from Curcuma spp.

3.4.1 *Curcuma longa*

Turmeric (*Curcuma longa*) is a rhizome and herbaceous perennial aromatic plant belonging to the family Zingiberaceae and it is native to south-east India. This plant is an old perennial plant that has been used by Asians for thousands of years. The whole turmeric rhizome has a rough, segmented skin. Dried rhizomes are slightly acrid in taste. It is a major part of the Siddha system and it has recommended turmeric for medicine. Rhizomes measure about 2.5–7.0 cm in length and 2.5 cm in diameter with small tubers branching off (Das 2016). Curcuminoids and EO from turmeric have shown various bioactivities and promising results in various research investigations. Hence, the oil has been in high demand since ancient times and recently finds extensive application in flavor, perfumery, cosmetic, food products, beverages, and the pharmaceutical industry. The nature and versatile applications of this oil highlight the importance in the global market and India is one of the top most commercial growers for it. Food and other industries recently focused on natural additives rather than synthetic due to health hazards, therefore turmeric is one of the most important alternatives which is abundantly and easily extractable natural safe oil used worldwide. Turmeric EO exhibited antiproliferative activities against some cancer cell line in their neat oily state and in water-based microemulsions where the EOs exist as nanoparticles (Abd-Rabou and Edris 2017). In HeLa cells exhibited potent cytotoxic activity associated with apoptosis induction markers (Santos et al. 2016). Likewise, three curcuminoid-containing turmeric extracts differing with respect to the inclusion of additional naturally occurring chemicals (EOs and/or polar compounds) inhibited human breast cancer MDA-MB-231 cells growth and secretion of osteolytic PTHrP. While curcumin and bis-demethoxycurcumin were equipotent to each other and to the naturally occurring curcuminoid mixture, demethoxycurcumin did not have any effect on cell growth. However, each of the individual curcuminoids inhibited PTHrP secretion to the same degree as the curcuminoid mixture. Degradative curcuminoid metabolites (vanillin and ferulic acid) did not inhibit cell growth or PTHrP, while reduced metabolites (tetrahydrocurcuminoids) had inhibitory effects on cell growth and PTHrP secretion but only at concentrations ≥tenfold higher than the curcuminoids (Wright et al. 2013).

3.4.2 *Curcuma zedoary*

Zedoary (*Curcuma zedoaria* Rosc. syn. C. zerumbet Roxb.) closely resembles turmeric (*Curcuma longa*) in appearance. It is a native of northeast India and is widely cultivated in many parts of China, Sri Lanka, and India. The plant bears green leaves with brownish-purple veins and grows up to a height of 50 cm. The rhizomes, which are large and fleshy, are cut into small pieces and dried. Dried slices have a bitter, strong and camphoraceous taste and are used for commercial purposes. The rhizomes have aromatic, stimulant, and carminative properties. Besides its use as

spices, the rhizomes are also used for the preparation of shoti starch (flour), a substitute for arrowroot and barley. It is highly valued as a dietary item, especially for infants and convalescents. Steam distillation of the rhizome yields a light-yellow oil. The odoriferous constituent is said to be a sesquiterpene alcohol belonging to the tricyclic group. Zedoary was an important spice in the past, but these days, it is usually used only for flavoring liqueurs and curries (Wilson 2015)**.** The EO obtained from *Curcuma zedoary* has shown efficient cytotoxic effects on NSCLC cells and causes cell apoptosis and cell cycle arrest. Notably, *Curcuma zedoary* EO led to the release of apoptosis-inducing factor, endonuclease G, and cytochrome c into the cytosol and increased levels of p53 in lung cancer H1299 cells. Furthermore, decreases in the levels of Bcl-2 and Bcl-xL and an increase in the Bax/Bcl-2 ratio was observed. Mechanistically, phosphorylation of ERK1/2 and enhanced the phosphorylation of JNK1/2 and p38 was inhibited (Chen et al. 2013). Moreover, intraperitoneal administration of *Curcuma zedoary* EO significantly suppressed the growth of H1299 cells *in vivo* (Chen et al. 2013).

3.5 Essential Oils from Cymbopogon spp.

3.5.1 *Cymbopogon citratus*

Lemongrass is a tall perennial C4 grass belonging to the family Poaceae (Gramineae), commonly known as the "sweet grass family." Cymbopogon is a genus of about 55 species indigenous to the tropical and semitropical areas of Asia and cultivated in South and Central America, Africa, and other tropical countries. Lemongrass plants do not typically produce flowers or flowering panicles (Abdulazeez et al. 2016). EOs extracted from the culm and leaf from *Cymbopogon citratus* exhibited cytotoxic activity against various human lung cancer cell lines, breast adenocarcinoma (MCF7, T47D and MDA-MB-231), chronic myelogenous erythroleukemia (K562), neuroblastoma (SH-SY5Y), prostate cancer (LNCaP and PC-3) and glioblastoma (SF-767 and SF-763) (Bayala et al. 2018; Najar et al. 2020; Trang et al. 2020). *In vivo*, a reduction in tumors as well as necrosis and mitosis in female rats with 7,12-dimethylbenz [a] anthracene (DMBA)-induced breast cancer consuming 50, 100 or 200 mg/kg per day during 14 weeks (Rojas-Armas et al. 2020). *Cymbopogon citratus* EO contained 15 compounds and the major ones were geranial/citral A and neral/citral B, so citral is its major component (Bayala et al. 2018). Interestingly, it has been found that the activity of EO of *Cymbopogon citratus* was statistically equal to that of its major component, citral, in LNCaP, PC-3, SF-767 and SF-763 cell lines (Bayala et al. 2018). An additional activity found for EO obtained from *Cymbopogon citratus* leaves was that doxorubicin-resistant ovarian carcinoma cells resulted sensitize to doxorubicin effects, an effect that was related to P-glycoprotein efflux pump inhibition (Viktorová et al. 2020). Citral was no able to sensitize doxorubicin-resistant ovarian carcinoma cells in contrast to complete leaf EO (Viktorová et al. 2020).

3.6 Essential Oils from Lavandula spp.

Lavenders are a diverse group of plants in the Lamiaceae family. Generally native to the Mediterranean region, lavenders are now a significant commercial crop around the world with over 2000 metric tons of lavender EO produced every year, primarily in Europe (Erland and Mahmoud 2016). The oil of Lavandula genus has been used for centuries as a therapeutic and aromatic agent in traditional medicine due to its carminative, sedative, and antidepressant properties and has gained popularity in the flavor and fragrance industries. Lavender EO is composed of mainly mono- and sesquiterpenes, aromatic 10- and 15-carbon compounds, with backbones formed from the condensation of five-carbon isoprene units (Erland and Mahmoud 2016). Different species and cultivars of lavender have unique oil chemotypes, and to date one of the most accurate ways to identify an individual is to characterize its oil composition (Erland and Mahmoud 2016). Due to its antimicrobial, antioxidant, antifungal, insecticidal, and insect repellent properties, lavender EO is becoming a popular target product to replaced traditional, synthetic treatments. *Lavandula angustifolia* or English Lavender, is composed of over 50 cultivars and is native to the Mediterranean region. As the name suggests, lavender grows readily in England, Europe, North America, and Australia often being found in ornamental and herb gardens, or commercially cultivated for its high quality EO. Some of the most popular cultivars include Lady, Munstead, and Hidcote; all vary slightly in EO composition and plant morphology. Blooming in late spring and early summer, though flowering will continue through to the end of August, lavender produces fragrant flowers from which EO is distilled. Although both leaves and flowers produce EO, only the floral EO is of commercial value, since flowers produce significantly more EO than leaves. Floral oils are richer in desirable EO compounds such as linalool and linalyl acetate and have lower amounts of undesirable compounds, such as camphor (Erland and Mahmoud 2016). *In vitro* antitumor activities of *Lavandula angustifolia* EO has been confirmed against human prostate cancer cell lines (PC-3 and DU145) (Zhao et al. 2017) as well as HeLa and MCF-7 cell lines (Tayarani-Najaran et al. 2014). Moreover, lavender EOs cytotoxicity on human prostate carcinoma cell (PC-3) was significantly stronger than on human lung carcinoma (A549) and human breast cancer (MCF-7) cell lines (Zu et al. 2010). Interestingly, only a marginal cytotoxicity to non-malignant human fibroblasts was found inducing a sub-G1 peak in flow cytometry histogram of treated cells compared to the control (Tayarani-Najaran et al. 2014). Western blot analysis demonstrated that EtOH and *n*-hexane extracts upregulated Bax expression, also it induced cleavage of PARP in HeLa cells compared to the control, thus apoptosis induction could be the possible mechanism of action explaining their anti-tumor actions (Tayarani-Najaran et al. 2014). Likewise, treated cells were arrest in the G2/M phase was also reported in PC-3 cells (Zhao et al. 2017). The antitumor effect was confirmed in subcutaneous xenograft tumors of nude mice inoculated with PC-3 cells and it was associated with cell proliferation inhibition and apoptosis induction (Zhao et al. 2017). The major constituents of *Lavandula angustifolia* EO, linalool, and linalyl acetate has been also

tested on human prostate cancer cells showed stronger inhibitory effect on PC-3 cells than on DU145 cells, although they led to lower values of apoptotic cell populations. In case of linalool, its anti-tumor effect was also confirmed in the xenograft model with PC-3 cell transplantation (Zhao et al. 2017). In addition, *Lavandula stoechas* EO has shown antitumor activity against different cancer cell lines including gastric adenocarcinoma (AGS), melanoma (MV3), and breast carcinoma (MDA-MB-231) (Boukhatem et al. 2020).

3.7 Essential Oils from Origanum spp.

3.7.1 *Origanum vulgare*

Origanum vulgare L. (oregano-*O. vulgare*) is a perennial and herbaceous plant growing from 20–80 cm tall belonging to the Lamiaceae family widespread across Europe and Asia. It is native to temperate western and Mediterranean region. *Origanum vulgare* EO showed *in vitro* antiproliferative effect against human breast adenocarcinoma (MCF-7), and especially human colon adenocarcinoma (HT-29) (Begnini et al. 2014) as well as hepatocarcinoma HepG2 cells (Elshafie et al. 2017). EO have apoptotic effects in human stomach cancer cell lines (AGS) and altered colony forming characteristics and migration ability (Balusamy et al. 2018). The calculated inhibition concentration values for HepG2 were lower than healthy renal cells HEK293, indicating the sort of selectivity of the studied substances (Elshafie et al. 2017). Induction of apoptosis correlated with an increase of BAX expression and downregulation of BCL2 expression indicated that oregano EO induced mitochondrial mediated apoptosis (Balusamy et al. 2018). Expression of genes involved in fatty acids and cholesterol biosynthesis pathway including 3-hydroxy-3-methylglutaryl-coenzyme A reductase, Acetyl CoA synthase, sterol regulatory element-binding protein and fatty acid synthase decreased and protein accumulated with the inhibition of cell growth (Balusamy et al. 2018). Consumption of a low uptake dose of EO with drinking water for three months in F1 DBA C57 Black hybrid mice inoculated with Lewis carcinoma decreased tumor engraftment, its size and significantly suppressed the development of tumor in sick mice (Misharina et al. 2013). EO main constituents include carvacrol, thymol, citral and limonene. These compounds alone also reduced cell viability percentage of treated HepG2 when compared to untreated cells *in vitro*. However, citral is not potentially recommended as an anticancer therapeutic agent, since there are no significant differences between IC50 values against both tested cell lines (Elshafie et al. 2017).

3.7.2 *Origanum majorana*

Origanum majorana (known as Sahtar or Zaatar in traditional medicine) currently named sweet marjoram, is a medicinal plant of the Lamiaceae family, a perennial herb of Origanum genus, with a self-supporting growth habit, it is a photoautotroph. This plant is distributed around the Mediterranean regions, in particular, Morocco, Algeria, Egypt, Spain, and Portugal (Bouyahya et al. 2021). *Origanum majorana* EO has shown antiproliferative activities against some cancer cell lines in their neat oily state and in water-based microemulsions (Abd-Rabou and Edris 2017). Its EO also inhibited the cellular viability and colony growth of human colorectal cancer cells (HT-29) (Athamneh et al. 2020). In this study was also found that the EO induced protective autophagy, associated with downregulation of the mTOR/p70S6K pathway, and activated caspase-8 and caspase-9-dependent apoptosis. Blockade of autophagy with 3-methyladenine and chloroquine, two autophagy inhibitors, potentiated the apoptotic cell death induced by the treatment with the EO. Inversely, inhibition of apoptosis with the pan-caspase inhibitor, Z-VAD-FMK, significantly reduced cell death, suggesting that apoptosis represents the main mechanism of cell death induced by this EO. Mechanistically, this effect would occur via the activation of the p38 MAPK signalling pathway since pharmacological inhibition of p38 MAPK by the p38 inhibitors SB 202190 and SB 203580 not only significantly decreased apoptotic cell death, but also reduced the autophagy level in EO treated cells. Strikingly, this EO also induces p38 MAPK-mediated caspase-dependent cleavage of p70S6K, a protein reported to be overexpressed in colon cancer and associated with drug resistance which result very interesting to use this EO as coadjuvant treatment (Athamneh et al. 2020).

3.8 Essential Oils from Pinus spp.

3.8.1 *Pinus densiflora*

Pinus densiflora is a pine tree widely distributed in Asian countries commonly called Japanese red pine that has been used as a traditional medicine. Common name is in reference to the attractive orange-red bark that exfoliates with age. It will soar to 100′ in the wild, but in cultivation is more likely to top out at 40–60′ tall. It is noted for its irregular but frequently graceful form and its orange-red bark. The anticancer activity of EO, extracted by steam distillation, from the leaf of *Pinus densiflora* strongly inhibited proliferation and survival YD-8 human oral squamous cell carcinoma cells. Notably, this treatment increased ROS generation phosphorylation of ERK-1/2 and JNK-1/2 in YD-8 cells indicating that it induced apoptosis. However, it did not affect the expression of Bax, XIAP and GRP78 in this cell line. Importantly, pharmacological inhibition studies demonstrated that treatment with the antioxidant vitamin E or Z-VAD-FMK (a pan-caspase inhibitor), but not with PD98059 (an ERK-1/2 inhibitor) or SP600125 (a JNK-1/2 inhibitor), strongly

suppressed the pro-apoptotic effect in YD-8 cells and reduction of their survival and blocked activation of caspase-9 and Bcl-2 down-regulation (Jo et al. 2012). Therefore, antiproliferative effects of this EO would be largely due to the ROS-dependent activation of caspases in this cell line.

3.8.2 *Pinus koraiensis*

Pinecone from *Pinus koraiensis*, an abundant plant in the northeast of China, is a traditional folk herb which has been used in China for many years. The pine nuts are edible and available commercially throughout Europe and North America. Extensive research has shown that pinecone from *Pinus koraiensis* have various bioactive substances such as flavonoids, polysaccharides, and phenolic compounds that have anti-tumor, anti-inflammatory, anti-bacterial, and antioxidant activities (Zhang et al. 2021). In the EO from *Pinus koraiensis* pinecones (PEO), obtained by hydrodistillation, 41 compounds have been identified mainly including α-pinene (40.91%), limonene (24.82%), and β-pinene (7.04%). Anti-tumor experiments *in vitro* showed PEO could significantly inhibit the proliferation and migration of human gastric carcinoma MGC-803 cells, and it also could arrest the cell cycle in the G2/M phase, decrease the mitochondrial membrane potential, and induce apoptosis (Zhang et al. 2019). In the same way, the EO obtained from the leaves of *Pinus koraiensis* significantly decreased colorectal cancer cells (HCT116) cell proliferation and migration, and induced G1 arrest without affecting normal cells. Mechanistically, this was related to the suppression of PAK1 expression that led to inhibition of ERK, AKT, and β-catenin activities (Cho et al. 2014).

3.9 Essential Oils from Salvia spp.

Salvia is the largest genera of the Lamiaceae family containing over 900 species of herbaceous, perennial, biennial, annual and aromatic plants. This genus inhabits several regions of the world including the Pacific Islands, Central Asia, Mediterranean, tropical Africa, and America. Iran is a center of origin for the genus Salvia, with 60 species, among which 17 are endemic. The common Persian name of Salvia is "Maryam-Goli" and is mainly used as a flavoring agent, perfume additive and condiment (Askari et al. 2021). In relation to cancer, various species have been studied including *Salvia acetabulosa*, *Salvia aurea*, *Salvia judaica*, *Salvia lerifolia*, *Salvia officinalis* and *Salvia viscosa.*

Salvia acetabulosa and *Salvia leriifolia* EOs exerted a strong antiproliferative activity against Cellosaurus cell line (COR-L23) which was much higher than comparing with the anti-cancer drug vinblastine effect (Loizzo et al. 2010). *Salvia aurea*, *Salvia judaica* and *Salvia viscosa* EOs reduce the growth of human prostate cancer cells (DU-145) *in vitro* activating an apoptotic process and increasing ROS generation (Russo et al. 2018). *Salvia officinalis* EO showed antiproliferative

activity on three human colon cancer cell lines, which correlates with cell cycle arrest, but it has no effect on the viability of normal colonic epithelial cells (Luca et al. 2020). In the same sense, EOs obtained by hidrodestillation from *Salvia officinalis* exerted cytotoxic effects on amelanotic melanoma (C32), renal cell adenocarcinoma (ACHN), hormone-dependent prostate carcinoma (LNCaP), and breast cancer cell lines (MCF-7) (Loizzo et al. 2007). Lastly, the three main compounds of *Salvia officinalis* EO, α-thujone, 1,8-cineole (eucalyptol) and camphor alone showed effect in the same sense (Luca et al. 2020).

3.10 Essential Oils from Thymus spp.

The thyme plant, *Thymus vulgaris* L., is a perennial subshrub with a lifespan of approximately 10–15 years belonging to the Lamiaceae family. Its stem becomes woody with the age, and it has both horizontal and upright habits. *Thymus vulgaris* blooms in June to July and it is cultivated all over the world (Mandal and DebMandal 2016). The leafy parts of thyme and its EO have been used in foods for flavor, aroma, and preservation and are added to meat, fish, and food products. The use of thyme in the cosmetic industry has increased the economic importance of this medicinal crop worldwide; the plant thus has changed from a traditional herb to a serious drug in rational phytotherapy. Importantly, *Thymus vulgaris* has generally recognized as safe (GRAS) status, its oil should be reserved for topical use; internally, it may lead to dizziness, vomiting, and breathing difficulties (Mandal and DebMandal 2016). Thyme EO exhibited a strong cytotoxicity towards three human cancer cells (PC-3, A549 and MCF-7 tumor cell lines) (Zu et al. 2010), chronic myelogenous erythroleukemia (K562) and neuroblastoma (SH-SY5Y) (Najar et al. 2020), HNSCC (Sertel et al. 2011) and on breast cancer (MCF-7 and MDA-MB-231) cells (Kubatka et al. 2019; Najar et al. 2020) with evidenced proapoptotic activity in these last cell lines (Kubatka et al. 2019). In HNSCC, thyme EO was confirmed to modulate expression of genes involved in the cell cycle, cell death and cancer and three most significantly regulated pathways by thyme EO were interferon signalling, N-glycan biosynthesis and extracellular signal-regulated kinase 5 signalling (Sertel et al. 2011). Anti-cancer effects of *Thymus vulgaris* were confirmed also *in vivo*. Dried *Thymus vulgaris* (as haulm) administered in the diet reduced the volume of 4 T1 tumors in a syngeneic 4 T1 mouse model. Moreover, treated tumors showed a substantial decrease in necrosis/tumor area ratio and mitotic activity index. Likewise, a similar treatment decreased the tumor frequency in a chemically-induced rat mammary carcinomas model whose carcinoma cells showed a CD44 and ALDH1A1 expression decrease and Bax expression increase in parallel to an increase in MDA levels and a decrease in VEGFR-2. Moreover, multiple epigenetic changes were detected. In this sense, it has been found a decrease in the lysine methylation status of H3K4me3 in both treated groups (H3K9m3, H4K20m3, and H4K16ac were not changed); up-regulations of miR22, miR34a, and miR210 expressions (only at higher doses); and significant reductions in the methylation

status of four gene promoters-ATM serin/threonine kinase, also known as the NPAT gene (ATM); Ras-association domain family 1, isoform A (RASSF1); phosphatase and tensin homolog (PTEN); and tissue inhibitor of metalloproteinase-3 (TIMP3) (the paired-like homeodomain transcription factor (PITX2) promoter was not changed) (Kubatka et al. 2019).

EOs obtained from other species of this genus less evaluated include *Thymus broussonettii (*Moroccan*), Thymus capitatus, Thymus caramanicus Jalas* (Iran)*, Thymus citriodorus* and *Thymus revolutus Célak. Thymus capitatus* EOs have exhibited *in vitro* cytotoxic activity on human cancer cells of breast adenocarcinoma (MCF7, T47D and MDA-MB-231), chronic myelogenous erythroleukemia (K562) and neuroblastoma (SH-SY5Y) cell lines (Najar et al. 2020). *Thymus broussonettii* EO showed a similar activity in human ovarian adenocarcinoma IGR-OV1 parental cell line, OV1/P, and its chemoresistant counterparts OV1/adriamycin (OV1/ADR), OV1/vincristine (OV1/VCR), and OV1/cisplatin (OV1/CDDP) (Ait M'barek et al. 2007). *Thymus revolutus Célak* EO and its two main constituents, γ-terpinene and *p*-cymene, are potential oxidative agents against lung cancer (H1299) and epidermoid carcinoma (A431) cells, an activity related to changes in G6PD, GST, GRx, and GPx enzyme activities (Özkan and Erdoğan 2017). *Thymus caramanicus Jalas* EO has shown a potent cytotoxic effect on human oral epidermoid carcinoma KB cells. Surprisingly, cytotoxic effects of EO and extract of this plant on KB cancer cells were greater than those on normal gingival HGF1-PI1 cell line (Fekrazad et al. 2017). *Thymus citriodorus* EO has shown HepG2 cells growth inhibition (Wu et al. 2013). In case of *Thymus broussonettii* EO, the anti-cancer activity was also confirmed in a DBA-2/P815 (H2d) mouse model receiving intra-tumoral injection of EO (Ait M'barek et al. 2007).

3.11 Essential Oils from Zataria multiflora

Zataria multiflora also called *Zataria bracteata* Boiss. and *Z. multiflora* var. elatior is a member of the Lamiaceae family. The plant genus name is derived from the Arabic word *"Za'atar"*, a generic name for some Middle Eastern herbs like thyme, oregano, and savory. *Zataria multiflora* Boiss. is a thyme-like plant known as *"Avishan-e-Shirazi"* in Iran. This name comes from Avishan meaning thyme, and Shiraz, a state of Iran. Geographically, it grows wild only in central and southern Iran (Shiraz, Isfahan, Yazd, Dezfol, Kerman, and Khorasan), Pakistan, and Afghanistan (Basti et al. 2016). It is an aromatic bush, 40–80 cm height, and its young branches are white with dense glandular, spreading, pilose indumentums while mature ones are woody and without any leaves. The aerial parts are collected, depending on flowering time, in late May up to early November. The leaves and flowers of this plant are used for medicinal purposes including inflammation, spasm, pain, and cancer symptoms reduction. The aerial branch of this plant contains at least 0.6% EO, fatty acids, oleanolic acid, β-sitosterol, and betolin. In spite of high variation in chemical composition of the EO from different ecotypes of this plant, it

has been well documented that oxygenated monoterpenic compounds, mainly thymol and carvacrol, are major components of this EO; these two compounds constituted 30–86% of total EO composition evaluated in different experiments. Carvacrol is a monoterpenic phenol isomer of thymol that presents as the main component in the dried plant, where thymol was found to be the most abundant constituent in fresh plant. Other reported main compounds include ρ-cymene, γ-terpinene, caryophylene, linalool, and α-pinene (Basti et al. 2016).

Anticancer activity of EOs from this plant have been tested *in vitro* both, alone in mouse mammary carcinoma 4 T1, mouse cervical cancer TC1 (Azadi et al. 2020); and human colorectal tumor cell lines (HCT116 and SW48) (Ahani et al. 2020) or loaded into chitosan nanoparticles on breast cancer cells (Salehi et al. 2020) showing antiproliferative activities which correlated with proapoptotic effects (Ahani et al. 2020; Azadi et al. 2020; Salehi et al. 2020) and elevated markers of oxidative stress without harming normal cells (Ahani et al. 2020; Salehi et al. 2020). Moreover, the increase in ROS production was associated with mitochondrial membrane permeabilization as well as DNA damage, which constitutes a link with apoptosis induction (Salehi et al. 2020). In addition, the induction of apoptosis in HCT116 and SW48 cells lines has been suggested to occur via UCP2-related mitochondrial pathway as consequence of the increase in intracellular ROS production (Ahani et al. 2020). Many of these properties have been confirmed *in vivo*. In this type of studies, EO of this plant was effective in decreasing the tumor weight compared to the control. Additionally, it was effective in tilting the balance of cytokines in favour of T helper 1 through the increase in the secretion of TNF-α, IFN-γ, IL-2 and decrease in IL-4. This immunomodulatory role could be an additional beneficial mechanism against cancer (Azadi et al. 2020).

4 Concluding Remarks

Essential oils (EOs) have been under study for their use in cancer therapy. In the present study, EOs from Boswellia spp., Cinnamomum spp., Citrus spp., Curcuma spp., Cymbopogon spp., Lavandula spp., Origanum spp., Pinus spp., Salvia spp., Thymus spp. and *Zataria multiflora* as well as their constituents have been reviewed. As a summary, they act as antiproliferative agent on cancer cells by multiple mechanisms involving apoptosis induction and cell cycle arrest, oxidative stress induction, mitochondrial function impairment and different pathways and gene expression modulation, as well as, changes in some hormonal levels and immunomodulatory effects. These interesting and promising findings encourage our knowledge about the chemopreventive effects of EOs and their constituents and could be useful for further *in vivo* studies against cancer. In addition, further explorations are necessary to improve and standardize the pharmaceutical preparations.

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Chapter 5
Bee Propolis (Caffeic Acid Phenethyl Ester) Against Cancer

Maqsood Ur Rehman, Abdullah, Fazlullah Khan, and Kamal Niaz

Abstract Caffeic acid phenethyl ester (CAPE) is the vital natural phenolic chemical composite present in a variety of plants which is also abundantly present in the honeybee hives as propolis. It is the combination of caffeic acid and phenethyl alcohol. There are many studies reported the pharmacological and physiological activities of CAPE in a variety of *in-vitro* and *in-vivo* models. CAPE has shown clinical significance as anti-mitogenic, anti-inflammatory, anti-carcinogenic, antibacterial, antiviral, antifungal, and immunomodulatory activities. CAPE or bee propolis leads to an abrupt cell cycle, which mitigates NF-kB signals, induces apoptosis, and inhibits angiogenesis in cancer cells. CAPE has the ability of anti-angiogenic substances that may reduce tumor invasion and neovascularization. The purpose of this chapter was to summarize the anticancer potential of bee propolis but other activities such as neuroprotective, and hepatoprotective, along with the nutraceutical application of CAPE will be discussed.

Keywords Caffeic acid phenethyl ester · Anti-tumour · Anti-microbial · Angiogenesis · Nutraceutical

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1 Introduction

In Greek the word propolis means “Defense of the city”. In common words it is called “Bee glue”. It is a resin containing substance formed by honeybees from plant’s buds and processed in the hives to cover its walls (Cayuela and Serrano 2003). Propolis provides physical support to shelter holes and cracks, gives protection against the pathogenic fungi and bacteria to hive, regulates the temperature inside the hive, and acts as a barrier to the entrance against other creatures (Castaldo and Capasso 2002; Pietta et al. 2002). Propolis is soft, very sticky and pliable at high temperature; however, on cooling, it converts a hard and brittle material especially on freezing. Propolis after this treatment remains brittle. At the temperature of 60–70 °C, it liquefies but not completely as few samples have higher melting points up to 100 °C (Krell 1996). Propolis’s colour of differs from green to reddish and brown, depending on its origin. Propolis has a distinguishing and pleasing aromatic odour. Propolis is collected by honeybees from different species of plants including *Salix alba*, Betula spp., *Populus alba*, *Fagus sylvatica*, *Ulmus glabra*, *Aesculus hippocastanum*, *Alnus spp*, and *Taxus baccata* (Ghisalberti 1979).

Historically, propolis has been used for different purposes but the scientists in history have been able to prove its medicinal benefits. The Egyptians used it to embalm corpses due to its anti-rotting activity. The Romans and Greeks used propolis as an oral disinfectant and to heal the wounds. In pre-Columbian America, it was used as antipyretic, while it was included in the list of official medicine in English Pharmacopoeia in the 1700 AD (Castaldo and Capasso 2002).

Recently, it has attained status as a potential health food worldwide. Propolis has been used to enhance Human’s health and to protect from diseases such as cancer, diabetes, inflammation and heart diseases. Propolis has been focussed by researchers due to its natural origin and various biological activities including anti-cancer activity (Banskota et al. 2000; Oršolić et al. 2004), antimicrobial (Lu et al. 2005; Marcucci et al. 2001), antiviral (Kujumgiev et al. 1999) anti-inflammatory (Cardile et al. 2003), antioxidant activity (Trusheva et al. 2006; Wang et al. 2004), and parasiticidal (Wagh 2013).

Bees produce propolis for many reasons including its role as an antibacterial to prevent microbial growth in honey stores, larvae and combs. How the whole process occurs from the bee on flower nectar/glucose, process inside bee gut to complete the energy demand and processed propolis is shown in Fig. 5.1. Propolis is applied by bees to areas with which combs are to be attached that help in the creation of germ-free and smooth surfaces. Populations of honeybee found in close contact, therefore, the disease in single bee may be spread easily into whole hive. Nonetheless, the hives remain healthy due to the antibiotic activity of propolis that decreases microbial growth on walls of the hive. Moreover, propolis guards the hive against external moisture and air-flow. Propolis offers an impervious lining that reduces water escape and keeps internal uniform hive’s humidity (Seeley and Morse 1976; Visscher 1980).

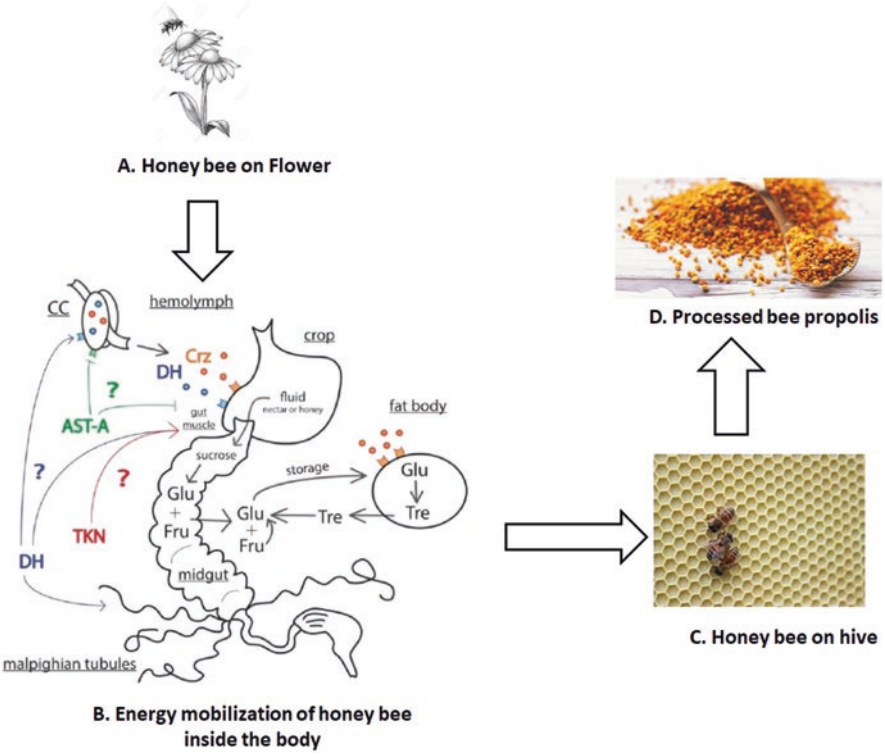

Fig. 5.1 Bee Propolis preparation process. (**A**) Honey bee or flower. (**B**) Glucose (Glu), trehalose (Tre) tachykinin-related peptides (TRPs), diuretic hormone-I (DH-I), corazonin (Crz) and adipokinetic hormone (AKH)). (**C**) Honey bee working in hive. (**D**) Processed propolis ready to use

2 Chemistry of Bee Propolis

Propolis consists of about 50% resin, 30% of wax, 10% of essential oils, 5% of pollen and 5% of organic compounds (Gómez-Caravaca et al. 2006). This composition varies due to two important factors i.e. the type of propolis and collection time. The main organic components in propolis are; phenol containing compounds, flavonoids, esters, beta-steroids, terpenes, aldehyde sand aromatic alcohols. A dozens of different flavonoids have been found in propolis which include chrysin (4.8%), rutin, quercetin (2.2%), pinocembrin (21.4%), apigenin, kaempferol, naringenin, luteolin, acacetin, catechin, myricetin, galangin (5%), and; phenolic acids like caffeic acid and cinnamic acid; and a derivative of stilbene like resveratrol have been detected in propolis (Huang et al. 2014; Volpi 2004).

The most significant phenolic compound present in propolis is caffeic-acid-phenethyl ester (CAPE) which is about fifty percent of the total composite. CAPE is chemically an active flavonoid which maximizes the free radicals scavenging activity by reducing hydroxyl radicals activity and becomes less reactive (Packer

2001). It has been revealed that propolis exhibits antioxidant activity due to phenol content (Kumazawa et al. 2004).

Besides phenols and flavonoids, it also consist of important nutrients like minerals and vitamins especially vitamin C, Vitamin E, Vitamin B (B1 (0.025–0.16 mg/100 g), B2 (0.304–0.777 mg/100 g), and B_6). About 16 essential amino acids are also present in propolis that is important for cell regeneration. Proline and arginine constitute 45.8% of all amino acids present in propolis. All minerals, except sulfur, are found in propolis. Iron (162μg/g) and zinc (23μg/g) are the most abundant minerals (Bankova 2009).

3 Cape Bioavailability and Metabolism

IC50 of 1–2 nM concentration of CAPE must be achieved at the target site to elicit its pharmacological activities. Being a constituent of propolis, CAPE is usually administered orally; therefore, it is important to determine its oral bioavailability. The bioavailability of CAPE and its fluorinated derivative (FCAPE) was determined intravenously in Sprague-Dawley rats. CAPE was administered at 5, 10, and 10 mg while FCAPE was administered at 20 mg per kg body weight. Higher increase in the area under the plasma-concentration-time curve was observed showing saturation of CAPE metabolism. Total clearance and volume of distribution declined with raising the dose. The elimination half-life was independent of dose. It can be concluded from these results that CAPE is extensively disseminated in tissues and eliminated very rapidly due to short elimination $t_{1/2}$ and the high volume of distribution (Wang et al. 2009). Another study was conducted that demonstrated that CAPE is hydrolyzed after 6 hours to its major metabolite, caffeic acid in rat plasma. In humans' plasma, such hydrolysis does not occur due to lack of carboxylesterase (Celli et al. 2007; Li et al. 2005). Moreover, it was also found that CAPE can cross the blood-brain barrier (Silva et al. 2013).

4 Biological Activities of Bee Propolis

4.1 Antibacterial Activity

Several researchers have screened propolis and the extracts of propolis against Gram + and Gram − bacteria as shown in Fig. 5.2. Different studies observed that propolis exhibits anti-bacterial activity against various strains of Gram + rods, but the activity against Gram − bacteria was limited (Akopyan et al. 1970; Grecianu and Enciu 1976; Vokhomina et al. 1969).

Propolis obtained from Turkey (Mugla) was tested against different bacterial strains. It was observed that *Shigella sonnei* in the Gram − and *Streptococcus*

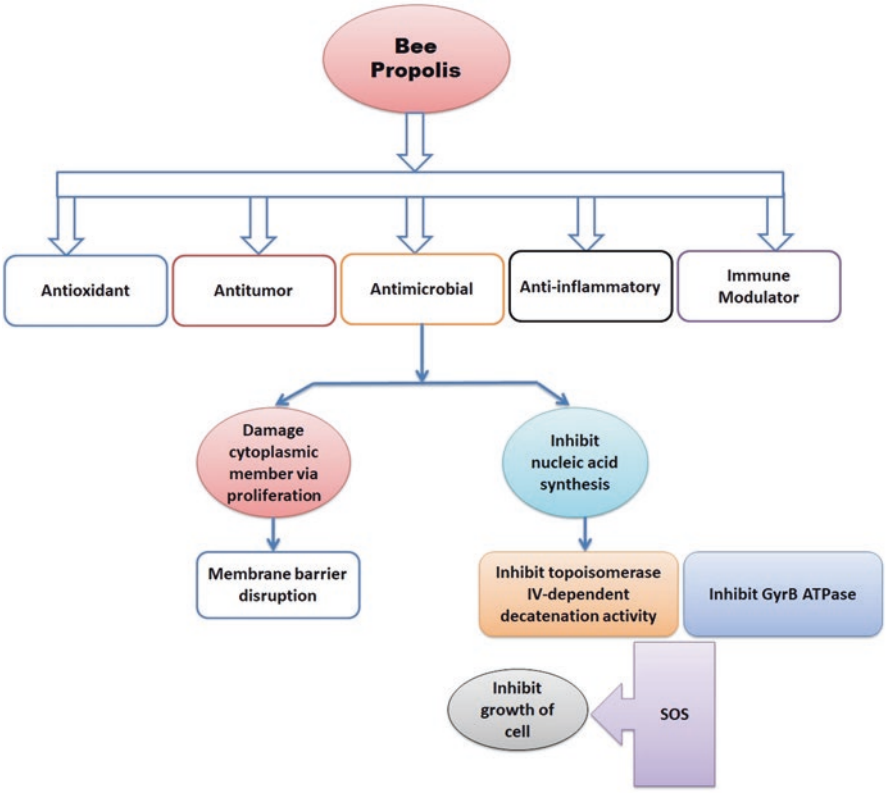

Fig. 5.2 Flow chart illustrating biological activities of CAPE

mutans (*S. mutans*) in the Gram + group were the most sensitive microorganisms to propolis. Furthermore, propolis samples demonstrated greater or similar inhibitory effect against *Salmonella typhi, S. mutans*, *S. sonnei,* and *Pseudomonas aeruginosa* in comparison to standard antibiotics (Ugur and Arslan 2004).

It was also demonstrated that propolis exhibits anti-bacterial action against *Actinobacillus actinomycetem comitans, Fusobacterium nucleatum, Campylobacter rectus, Tannerella forsythia, Porphyromonas gingivalis,* and *Prevotella intermedia*. Moreover, it has been found that propolis extract exhibits antibacterial properties against periodontitis causing pathogens. Some authors are of the view that the anti-bacterial effects are due to phenol acids, their esters, and flavonoids (Koo et al. 2000). Flavonoids like pinostrobin, pinocembrin and galangin, and the caffeic and ferulic acids are the most effective antibacterial compounds in propolis (Marcucci 1995).

The mechanism for anti-bacterial action of propolis is the growth inhibition of bacteria by thwarting cell division, which results in the development of pseudo mul-ticellular strepto-cocci. Moreover, it caused disorganization of cytoplasmic membrane (Fig. 5.2), cell wall and even cytoplasm, resulting in incomplete

bacteriolysis and the prevention of the synthesis of protein (Takaisi-Kikuni and Schilcher 1994).

4.2 Antiviral Activity

One of the constituents of bee propolis, methyl-but-2-enyl-caffeate, was found to decrease the synthesis of DNA of type-1 virus of *Herpes simplex* (HS-I) and viral titer (Amoros et al. 1994). Similarly, another constituent isolated from propolis (Isopentyl ferulate) was found to significantly inhibit the pathological action of influenza-virus A-1 Honey-Kong (H3N2) *in-vitro* (Serkedjieva et al. 1992). Moronic acid (a triterpenoid) isolated from propolis (Brazilian propolis) exhibited significant anti-HIV action in H-9 lymphocytes (Ito et al. 2001).

4.3 Antifungal Activity

A dozens of chronic sinusitis patients were investigated. The fungus (*Candida albicans)* was found sensitive to propolis *in-vitro* in 8 patients, weakly sensitive in 2 and resistant in other 2 cases (Kovalik 1979). The patients had been treated with 2–4 mL of an alcohol-oil emulsion of propolis after irrigation with isotonic saline. The condition of the patients after 1–2 treatments with propolis was improved. While after 5–8 administrations, clinical recovery was found in 9 patients and some improvement in remaining 3 patients. Complete recovery was observed after 10–17 days (Kovalik 1979).

Paracoccidoides brasiliensis (*P. brasiliensis*) cause paracoccidioidomycosis in Latin America. The mice (BALB/c mice) peritoneal macrophages were stimulated by using propolis and then challenged with *P. brasiliensis*. It was observed that antifungal activity of macrophages was enhanced by propolis stimulation (Murad et al. 2002).

4.4 Antiprotozoal and Antiparasitic Activity

Different extracts of propolis are effective against leishmaniasis. Propolis in combination with nitric oxide has been found to accelerate repair of tissue by moderating cell migration, collagen deposition, and production of cytokine (Cao et al. 2017; Miranda et al. 2015).

In-vitro effect of extract (ethanolic) of propolis for the growth and adherence of *Giardia duodenalis* trophozoites was evaluated. It was observed that growth of trophozoites was inhibited by propolis and the inhibition level varied according to the incubation time and concentration of extract. A maximum decrease in growth was

seen in cultures exposed to 125 mg/mL, 250 mg/mL and 500 mg/mL of propolis. About 50% reduction in growth was shown at 125 mg/mL propolis-treated cultures, while more than 60% inhibition of growth was observed at concentrations of 250 and 500 mg/mL (Negri et al. 1998). The dimethyl sulfoxide and ethanolic extract of propolis were found to be fatal to *Trichomonas vaginalis* (Starzyk et al. 1977) and active against *Trichomonas cruzi* (De Castro and Higashi 1995).

4.5 Anti-inflammatory Activity

Propolis exhibits promising anti-inflammatory activity in sinusitis, pharyngitis, and lung conditions. The anti-inflammatory potential of propolis is comparable to dexamethasone in the chemical induced corneal lesion in animal models (Niazi and Niazi 2005). Propolis has exhibited anti-inflammatory potential against ornithine decarboxylase, NADPH oxidase, and MPO. This activity has been attributed to cinnamic acid derivatives and flavonoids (De Almeida and Menezes 2002). Out of these flavonoids, galangin is capable of LOX and COX inhibition, thus reducing the activity of polygalacturonase and decreasing the expression of inducible COX-2 (Raso et al. 2001; Rossi et al. 2007).

One of the constituents of propolis is phenethyl caffeic acid ester which exhibits anti-inflammatory activity (Fig. 5.2). It inhibits arachidonic acid release from the cell membrane, causing the suppression of COX-1 and COX-2 actions and inhibits the activation of gene expression of COX-2. (Lee et al. 2004; Mirzoeva and Calder 1996).

Another flavonoid present in propolis is chrysin that has exhibited anti-inflammatory potential via inhibition of the pro-inflammatory activity of inducible nitric oxide synthase and COX-2 (Cho et al. 2004).

5 Neuroprotective and Hepatoprotective Properties of Bee Propolis

As oxidative stress and mitochondrial damage are the key factors in neurodegeneration, therefore, the antioxidant constituents of propolis may exert a neuroprotective role. Aqueous extract of propolis exerted neuroprotective effect against cerebral ischemia-induced oxidative injury in a mouse model of stroke (Bazmandegan et al. 2017). Improvement in the activity of antioxidant enzymes, decrease in lipid peroxidation and infarct volume was observed in comparison to control. Furthermore, the neurological deficits were also ameliorated by the propolis extract (Bazmandegan et al. 2017). Likewise, it was observed in another study conducted on SH-SY5Y cells that propolis pretreatment decreased both H_2O_2 induced ROS production and 8-oxo-2′-deoxyguanosine (Oxidative damage marker of DNA)

immunofluorescence signal intensity. It was concluded by authors that because of its antioxidant activity propolis shows protection against neuro-degenerative damage related to cognitive impairment due to aging or even by Alzheimer's disease (Ni et al. 2017).

Another profuse flavonoid in propolis is pinocembrin. It inhibited oxidative stress induced by hydroxy dopamine. The translocation of nuclear factor erythroid-2 (Nrf-2) is induced by pinocembrin resulting expression of antioxidant response of element that refereed antioxidant genes coding γ-glutamyl-cysteine synthetase and heme-oxygenase-1. Nrf2 is a main player in the adaptive response to oxidative stress and in the maintenance of cellular self-defence. Pinocembrin also declined the hydroxy dopamine-induced loss of cell viability, apoptotic-rate and somewhat inhibited the decrease of Bcl-2, an apoptosis inhibitor to Bax, an apoptosis promoter ratio after hydroxy dopamine treatment (Jin et al. 2015). The main component of propolis is caffeic-acid-phenethyl ester which exert neuroprotective effect against hydroxy dopamine-induced dopaminergic neuronal loss (in rats) (Silva et al. 2013).

Concurrent administration with caffeic-acid-phenethyl ester reduced the H_2O_2 formation in brain striatum homogenates. ROS scavenging in brain-affected areas was also made by CAPE via defusing the unpaired electrons of DPPH. Moreover, CAPE protected 6- hydroxy dopamine-induced elevation of metal levels (Mn, Zn, Cu, and Fe) in addition to the inhibition of mitochondrial permeability transition which act as mediator of neuronal death. This generates the cytochrome-c release and caspase-3 activation while this effect was not related with dysfunction of mitochondria. Based on its ability to cross the blood-brain barrier and on the obtained findings, it was concluded by the authors that CAPE could be a promising agent in the treatment of Parkinson's and other neurodegenerative diseases (Silva et al. 2013).

Hepatoprotective activity and mechanism of propolis against acetaminophen-induced toxicity was studied on the culture of rat hepatocytes (Won Seo et al. 2003). The authors concluded that propolis employed a protective response on the hepatic damage which may be due to phase-I enzymes inhibition and phase-II enzymes induction (Won Seo et al. 2003).

The mechanism of hepatoprotection is exerted at multiple points including the elevation of tissue catalase activity (Esrefoglu et al. 2012). CAPE also demonstrates protection against lipid peroxidation, aberrant cell proliferation, p65 activation and hepatocellular necrosis (Macias-Perez et al. 2013). It has been found that CAPE successfully reduced the chances of hepatic tumors induced by diethylnitrosamine (DEN)-by 43% via inhibition of CYP2B1/2 enzymes that are responsible for the bioactivation of DEN (Beltran-Ramirez et al. 2012). CAPE alleviates the systemic inflammatory response, hepatic and neuronal cell damage induced by LPS and galactosamine in rats. This was attributed to correcting the imbalance between the proinflammatory (TNF-a, IL-1a, IL-1b and IL-6) and anti-inflammatory (IL-4 and IL-10) cytokines that may contribute to inhibiting the expression of adhesion molecules (sICAM-1) (Korish and Arafa 2011).

In another study, CAPE attenuated bile duct ligation-induced cholestatic liver fibrosis as indicated by diminishing hydroxyproline content. The hepatoprotective effect of CAPE was found to be associated with its antioxidative potential through

reducing malondialdehyde (MDA) levels and elevating the reduced activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzymes in liver tissue (Tomur et al. 2011).

6 Anti-tumour Properties of Bee Propolis

Caffeic-acid-phenethyl ester is one of the important components of propolis which is responsible for the anticancer activity that is mediated via activation of DNA damage signalling in cancer cells. Moreover, the CAPE which is the ester of caffeic acid and phenethyl alcohol (Fig. 5.3) resulted in the arrest of the growth of tumours due to the downregulation of mortalin and activation of p-53 (tumour suppressor protein) (Ishida et al. 2018) (Fig. 5.4).

CAPE exerts potent anticancer effect against different cell lines including ovarian cancer (SK-OV-3), man leukemia (HL-60, CI41, U937), man hepatocellular carcinoma (HepG2), man lung carcinoma(NCI-H358), man pancreatic cancer (BxPC-3, PANC-1), human cervical cancer(ME180) and colon cancer (HCT116) (Wu et al. 2011). It has been found that CAPE causes cell cycle arrest, modulates NF-kB signals, induces apoptosis, and inhibits angiogenesis in cancerous cells without having considerable effect on cells of mammals (Wu et al. 2011).

In one study, the anticancer activity of CAPE was associated to the proapoptotic potential and the inhibition of NF-kB signals. The apoptotic inducing activity is due to the downregulation of Bcl-2, activation of caspase-3, and up-regulation of Bax in man leukemia cells (HL-60) (Chen et al. 2001).

6.1 Effects of Propolis on Angiogenesis, Metastasis and Tumour Invasion

The development of new blood vessels from existing endothelium is known as angiogenesis, which is an important feature of cancer. Angiogenesis contributes to the growth of tumour and metastasis development. Continuous tumour growth needs persistent development of blood vessel and inhibition of angiogenesis which leads to tumour dormancy. CAPE is a specific and potent inhibitor of the nuclear transcription factor (NF-κB) activation (Natarajan et al. 1996). CAPE has

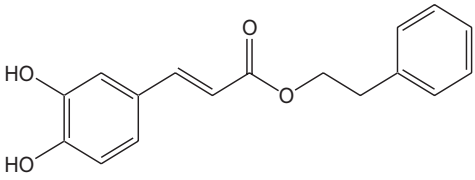

Fig. 5.3 CAPE chemical structure

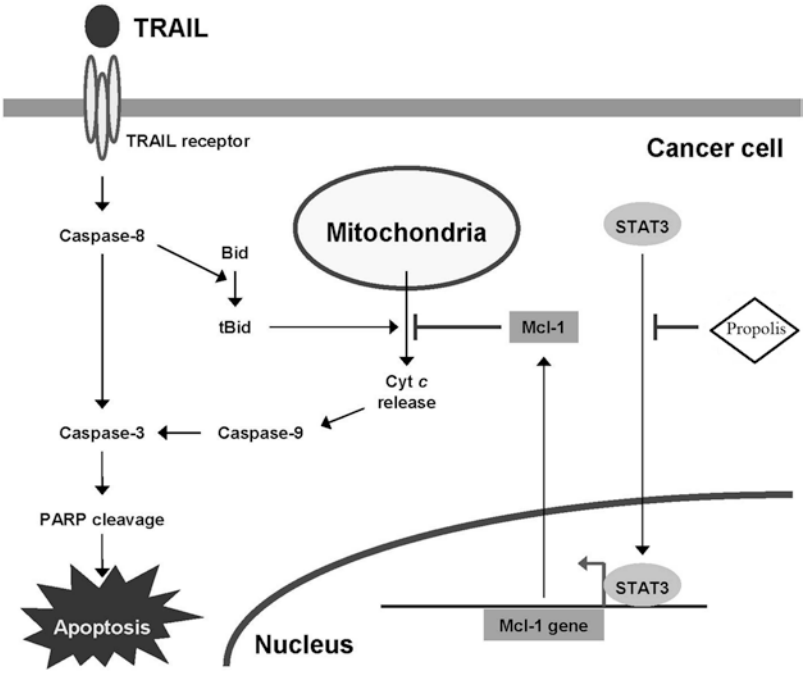

Fig. 5.4 Targeting the apoptosis pathways in cancer cells stimulated by chrysin

demonstrated to stop the expression of VEGF in CT26 colon adenocarcinoma cells in angiogenesis (Liao et al. 2003). CAPE has also been known to suppress the process of prostaglandin E2 synthesis which is mediated by 12-O-tetradecanoylphorbol-13-acetate and calcium ionophores. Therefore, caffeic-acid-phenethyl ester may be used as a potential antiangiogenic substance that may decrease neovascularisation (Michaluart et al. 1999).

6.2 Effect of Propolis Against the Toxicity of Drugs

Chemotherapeutic agents are useful drugs in the treatment of cancer but at the same time, they cause serious adverse effects such as hepatotoxicity, nephrotoxicity, bone marrow depression, gastrointestinal disorders, etc. The reduction of severity and incidence of adverse reactions caused by chemotherapy is important to enhance the life quality of cancer patients. Therefore, scientists are working hard to develop such agents that can serve the purpose. Cyclophosphamide alone or in concomitant use with other drugs can be used in the treatment of different types of malignancies in humans (Bass and Mastrangelo 1998). However, it can lead to several adverse reactions including hepatotoxicity due to oxidative stress (Selvakumar et al. 2005).

Propolis has been used as an adjuvant to reduce hepatotoxicity caused by cyclophosphamide (Padmavathi et al. 2006).

Similarly, irinotecan, an anticancer similar in structure to camptothecin, cause adverse effects including hepatotoxicity and toxicity to the cholinergic system in GIT. Administration of propolis alongwith irinotecan enhanced antitumour activity of irinotecan and resulted in prolongation of the life span of mice (Kopjar et al. 2007).

7 Nutraceutical Usage of Bee Propolis

CAPE has been used from last few decades in many pharmaceutical industries as anti-cancer, anti-inflammatory and due to its immunomodulatory properties. It has significant effect in skin papilloma patients whose are intentionally or accidently exposed to 12-O-Tetradecanoylphorbol-13-acetate (TPA). CAPE or bee propolis has some nutraceutical applications including

1. Can be used in cancer (Kurt et al. 2010; Yucel et al. 2017).
2. To inhibit propagation of viruses such as HSV-1, HSV-2, poliovirus type 2, adenovirus (Viuda-Martos et al. 2008).
3. Dentistry, dermatology, gynaecology, ophthalmology, otorhinolaryngology, pulmonary, and digestive system diseases (Stangaciu 1999).
4. As a preservative in food technology to increase the shelf life of meat in combination with potassium sorbate (Ferraz et al. 2010).
5. It can be used as antiseptic and antifungal due to the presence of caffeic acid, galangin, and ferulic acid (Ferraz et al. 2010).

8 Conclusion

It has been concluded that CAPE or bee propolis can be used as a promising anti-inflammatory, anticarcinogenic and immunomodulatory substance that induce cellular antioxidant activities along with neural cell protection against oxidative stress caused by ROS. It's time to understand that such neuroprotective and hepatoprotective properties of CAPE maintain the cell integrity and viability. Therefore, further research is required to explore the possible molecular mechanisms of CAPE in neuron and brain cells which can be fruitful for neurodegenerative disorders.

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Chapter 6
Brown Algae (Fucoxanthin) Against Cancer

Umair Younas, Sana Tehseen, Fazlullah Khan, and Kamal Niaz

Abstract Fucoxanthin is recognized well all over the world due to its beneficial health effects. It is widely available in nature as a carotenoid and accounts for more than 10% of total carotenoids production in the world. Since, it disguises the green chlorophyll a and c therefore; it gives yellow to brown color to diatoms and seaweed (Brown macroalgae). The season and geographic distribution accounts for the large variation in the contents of fucoxanthin. In fact, brown seaweeds contain a large amount of fucoxanthin from March to September (sporophyte mature stage). The potential health benefits of fucoxanthin, reported by various researchers include antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective properties, among others. Recently much attention was given to it by consumers and at a commercial level to use it as a part of functional foods as fucoxanthin is Halal, Kosher as well as suitable for vegetarians. The biological activities, anticancer effects, molecular mechanisms of G1/G2 arrest including apoptosis, strategies to cope with unfavorable reactions in functional foods with fucoxanthin has been addressed in this chapter.

Keywords Fucoxanthin · Brown algae · Functional food · Fucoxanthinol

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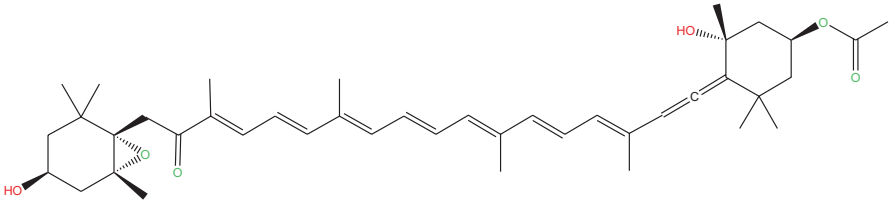
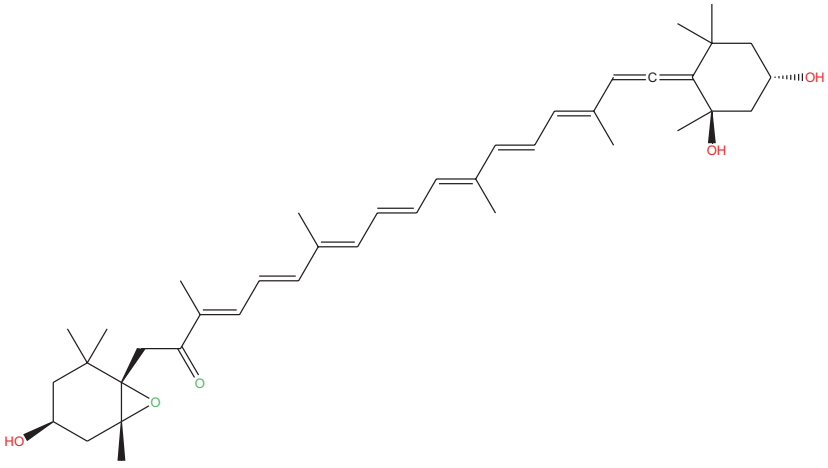
1 Introduction

Fucoxanthin is a carotenoid firstly isolated from brown seaweeds in Germany (Abu-Ghannam and Shannon 2017) and it is widely available in nature being as a carotenoid compound (Matsuno 2001).It is an orange color pigment and mainly present in brown algae and diatoms (Karpinski and Adamczak 2019). This may be also found in unicellular microalgae and chloroplasts of some eukaryotic cells (Abu-Ghannam and Shannon 2017). From total carotenoid production in the world, fucoxanthin is estimated to higher that 10% (Viera et al. 2018). Since, it disguises the green chlorophyll a and c therefore; it gives yellow to brown color to diatoms and seaweeds (brown macroalgae; Hurd et al. 2014; Peng et al. 2011). The epoxide group and hydroxyl group are present in the chemical structure of both fucoxanthin and fucoxanthinol that is believed to be strong antioxidants (Fig. 6.1) (Hu et al. 2010). The molecular formula of fucoxanthin is $C_{42}H_{58}O_6$ whereas; the molecular weight is 658.906 g/mol (Karpinski and Adamczak 2019).



Fucoxanthin



Fucoxanthinol

Fig. 6.1 Chemical structure of fucoxanthin and fucoxanthinol

In brown seaweeds, the fucoxanthin concentration range is 172 to 720 mg/kg. The concentration of fucoxanthin in turner (*Sargassum horneri*) is approximately 3700 mg/kg (Tsukui et al. 2009). Kombu is basically the eighteen edible species of Laminariaceae family and is also believed to be a good source of fucoxanthin. Almost 178–196 mg/kg fresh weight fucoxanthin content may be obtained from the stipe, blade, and holdfast material (discards). Similarly, the recovery ratio of fucoxanthin is 82% and from 10 t of Kombu waste, 1490 g of fucoxanthin has been obtained (Kraan 2013). The stable form of fucoxanthin was acquired and within 6 months of storage, it was reduced by 2% at 4 °C (Kanazawa et al. 2008).

For the extraction of fucoxanthin at a large scale, Japanese wakame was used at the industrial level due to an abundant level of fucoxanthin in this seaweed (Billakanti et al. 2013). The higher seaweed production in technically advanced industries for fucoxanthin extraction includes countries like China, Japan, Korea, etc. (Ryan 2014). Similarly, coastlines of Ireland also favorable for the growth and production of brown seaweeds containing fucoxanthin like sugar kelp (*Saccharina latissima*), sea spaghetti (*Himanthalia elongata*), channeled wrack (*Pelvetia canaliculata*), sea rod (*Laminaria hyperborea*), serrated wrack (*Fucus serratus*) and knotted wrack (*Ascophyllum nodosum*), among others. (Dominguez 2013; Morrissey et al. 2001).

Algae, plants, and photosynthetic bacteria contain carotenoids as tetraterpene pigments that have their chemical structure composed of hydrocarbon having no oxygenknown as carotenes. Whereas, some of the tetraterpene structures may include oxygen and classified as xanthophylls. Like plants xanthophylls, fucoxanthin is a seaweed xanthophyll (Kotake-Nara and Nagao 2011). Physico-chemical properties like antioxidation and lipophilicity are shared among xanthophylls and carotenes due to their characteristics of quenching the nitrogen and reactive oxygen species (Kim and Chojnaka 2015).

Thylakoids are compartments enclosed within a membrane where that carry fucoxanthin in algal cells. Through binding of fucoxanthin with apoprotein, chlorophyll A, and C, a complex is formed that tends to absorb light, and later the energy is passed to alga chlorophyll. The absorption of light happens in the blue-green range of the spectrum. This is the only wavelength of the spectrum which is available at several meters' depth (Kita et al. 2015).

As compared to chlorophyll a and c, a wide spectrum of light is captured by fucoxanthin i.e. 449–540 nm (Kim and Pangestuti 2011; Kim et al. 2011). The reactive species of oxygen may damage algal cells and this is prevented by fucoxanthin. These reactive oxygen species give damage due to long exposure of light and a high level of oxygen in the ocean. A significant amount of fucoxanthin is available in the seaweed thallus blade region thus; exposure to the light is more as compared to holdfast and stipe (Abu-Ghannam and Shannon 2017).

Both configurations i.e. *cis* and *trans* may be found in fucoxanthin. Compared with *cis* transformation, the trans isomer is more stable made up to 9% of fucoxanthin present in nature and also potentially antioxidant (Hold and Kraan 2011; Nakazawa et al.2009). Most diatoms and other microalgae have greater fucoxanthin content than brown seaweeds (Kawee-ai et al. 2013). Fucoxanthin content varies widely amongst macro- and microalgae.

Xia et al. (2013) reported different levels of fucoxanthin contents among eight different species of diatoms. It was reported that due to geographic and season variation, the fucoxanthin contents may vary greatly and between september to march, brown seaweeds contains a high level of fucoxanthin (Fung et al. 2013; Terasaki et al. 2009). Due to its potential antioxidant properties, the fucoxanthin is considered to be an important part of functional foods nowadays. The main underlying reason behind the worth of fucoxanthin is its mechanism of antioxidation that makes it suitable for use in diet and the pharmaceutical industry. In biological systems, the main physiological role of fucoxanthin is its effect on protein expression and specific genes (Kazuo et al. 2012). It was reported that the development of hypertension may be prevented or reduced by the action of fucoxanthin extracted from *Undaria pinnatifida* (Kim and Wijesekara 2017).

The anti-obesity effect of fucoxanthin is well known (Gammone and D'Orazio 2015). The fucoxanthin can produce DHA (docosahexaenoic acid; Maeda et al. 2008; Tsukui et al. 2009) which helps in lowering the cholesterol level in the body. Also, the activation of UCP1 (uncoupling protein 1; Maeda et al. 2007) is induced by fucoxanthin that plays a vital role in lipolysis. The antioxidant property of fucoxanthin was observed on BV-2 microglia by Zhao et al. (2016) and it was reported that the production of nitric oxide and reactive oxygen species (ROS) may be inhibited by fucoxanthin. Thus, cells may be protected from the oxidative stress and damage. It was reported by various researchers that alteration in the lipid metabolism and insulin resistance may be ameliorated by the action of fucoxanthin. Hence the antidiabetic role of fucoxanthin may be understood (Sun et al. 2018). The main objective of this chapter is to summarize all the data regarding biological activities, anticancer effects, molecular mechanisms of G1/G2 arrest including apoptosis, and strategies to cope with unfavorable reactions in functional foods with fucoxanthin.

2 Biological Activities of Fucoxanthin

Various edible seaweeds, green, brown and red marine macro-algae, are considered to be a good source of different bioactive components including sulfated polysaccharides, sulfo-lipids, PUFA, vitamins, carotenoids, and minerals (Mohamed et al. 2012). However, seaweed carotenoids include fucoxanthin, lutein, zeaxanthin, violaxanthin, and β-carotene. It was reported that Brown algae and diatoms produce a carotenoid named as fucoxanthin (Takaichi 2011). Various biological characteristics are attributed to this compound; the importance of which presents anti-diabetic, anti-cancer, antioxidant, anti-obesity, and antimicrobial properties.

Recently, it was noted that a wide range of biological activities are exhibited by fucoxanthin that also includes protection against oxidative stress. The cytotoxic effects exerted by an oxidative agent in a dose-dependent manner were also shown to ameliorate by the counteraction of fucoxanthin. Some other researchers reported the defensive action of fucoxanthin against DNA damaging factors as well as UV-B radiations (Chen et al. 2019; Maeda et al. 2018; Galasso et al. 2017; Heo and Jeon

2009). Different researchers reported the additional effect on lipid metabolism as well as anti-diabetic and anti-obesity activities (Koo et al. 2019; Muradian et al. 2015; Gammone and D'Orazio 2015). Similarly, Miyashita (2009) confirmed the effects of fucoxanthin in lowering the blood glucose level, resistance to high insulin level in the blood, and weight loss.

The beneficial effect of fucoxanthin on the cardiovascular system was reported by D'Orazio et al. (2012) which lead to a reduction in triacylglycerol and cholesterol level of blood thus helps in lowering the blood pressure. Also, the reduction in the inflammatory process was noted. The broad-spectrum anti-cancers activity of fucoxanthin was observed. Similarly, the anti-proliferative effect was reported *in vitro* against different cell lines including osteo-carcinoma (Rokkaku et al. 2013), gastric adenocarcinoma (Yu et al. 2011), non-small-cell lung cancer (NSCLC; Mei et al. 2017), leukemic (HD-60) (Hosokawa et al. 1999; Kim et al. 2010b), prostate cancer (Satomi 2012; Kotake-Nara et al. 2005b; Kotake-Nara et al. 2001), epithelial colorectal adenocarcinoma (Hosokawa et al. 2004), breast cancer (Wang et al. 2019), and urinary bladder cancer (Zhang et al. 2008). Some other researchers reported the biological activity of fucoxanthin as a preventive agent against cancer supposed to exert anti-angiogenic, anti-metastatic, and anti-lymphangiogenic effects (Garg et al. 2019; Wang et al. 2019; Ganesan et al. 2013).

2.1 Cardiovascular Protection

High lipid profile is associated with an increased risk of cardiovascular diseases. The lipid profile may be improved with fucoxanthin which prevents cardiovascular damages by stimulating the proportion of DHA in the liver (Park et al. 2011). It is observed that the prevalence of death rate during pregnancy and vascular cognitive impairment is higher due to high blood pressure or hypertension (Wang et al. 2016). The inhibition of angiotensin-1 Converting (ACE-1) enzyme could be a beneficial therapeutic strategy in the treatment of hypertension (He et al. 2013). In this regard, the marine bio-sources including fucoxanthin provide base as a rich source of bioactive compounds that are novel for ACE-1 inhibition (Fig. 6.2).

From seaweed and microalgae, various antihypertensive peptides have been extracted that act as competitive inhibitors. Their function is to bind the active sites of ACE-1, therefore blocking its action (Kim and Wijesekara 2017).

Ikeda et al. (2003) studied the outcomes of Wakame, as a good source of fucoxanthin, in the development of stroke in spontaneously hypertensive stroke-prone (SHRSP) and observed significantly delay in the stroke signs development whereas survival in SHRSP was markedly improved by the action of Wakame. Though no difference was noted for blood pressure among treatment groups and it may be concluded as improvement in cardiovascular diseases of SHRSP without the dependency of hypertension. Preventive effect of fucoxanthin present in Wakame on ischemic cultured neural cell death was found (Zhang et al. 2015). Also, neural cell injury in reoxygenation and hypoxia may be lessened by fucoxanthin.

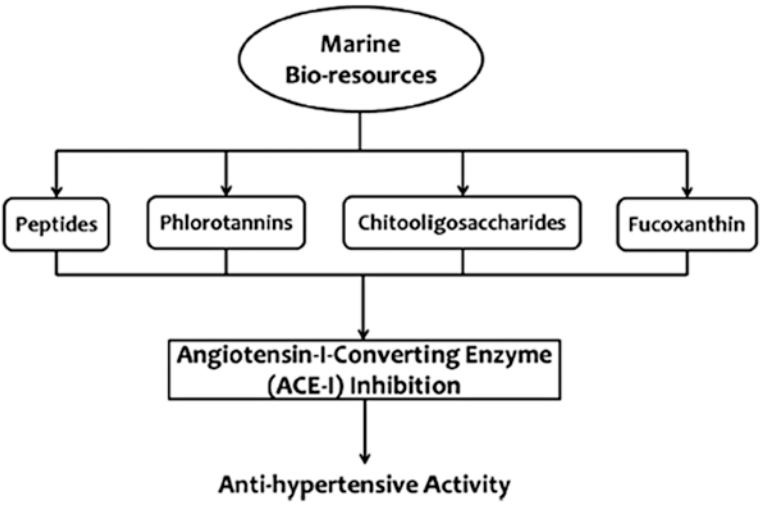


Fig. 6.2 Marine-derived ACE-1 inhibitors (Kim and Wijesekara 2017)

2.2 Anti-obesity Effect

The search for an efficient tactical approach to prevent obesity is a challenge. However, the consumption of fat-rich diets over a long period of time may affect the metabolism of lipids. Due to lipid metabolism that lead to the accumulation of visceral fat in the body and obesity may appear along with other disorders including hypertension, diabetes mellitus as well as dyslipidemia and cardiovascular diseases (Zhang et al. 2015). The supplementation of fucoxanthin in the diet may exert a valuable role in reducing the obesity.

The most promising sign of metabolic syndrome is abdominal obesity and various disease conditions are associated with it like atherosclerosis, chronic inflammation, cancer, type-2 diabetes, among others (Gade et al. 2010). There is a rising prevalence of diabetes type-2 with obesity all over the world (Reaven 2010). Most of the diabetes cases could be ameliorated just by getting no obese or overweight status (Bruno and Landi 2011).

Zhang et al. (2015) reported that a significant reduction in the hepatic and plasma triglycerides concentration may be associated with the action of fucoxanthin. Similarly, fecal TG cholesterol and enzymatic activities for cholesterol regulation may also be affected by fucoxanthin. The enzymes responsible for this enzymatic activity include acyl-coenzyme A and 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase. Gene expression may be affected by fucoxanthin for the lipid metabolism to reduce the concentration of potential lipids.

The anti-obesity effect of fucoxanthin has been observed by Maeda et al. (2007) and it revealed that UCP1expression was induced by fucoxanthin in the mitochondria of white adipose tissue (WAT) that results in oxidation heat production in WAT

as well as fatty acid oxidation. Ha and Kim (Ha et al. 2013) reported that mRNA expression of various enzymes may be reduced in rats subjected to the supplementation of fucoxanthin. These enzymes include fatty acid synthase, acyl-CoA cholesterol acyltransferase, hepatic acetyl-CoA carboxylase, glucose 6-phosphate dehydrogenase, hydroxy-3-methyl-glutaryl-coenzyme A.

By action of enzymes (cholesterol esterase and lipase; Miyashita et al. 2012) in the gastrointestinal tract, the fucoxanthin is hydrolyzed to its metabolite i.e. fucoxanthinol and later in the liver, it is converted to amarouciaxanthin A (Sugawara et al. 2002).

Both metabolites have same structure of one side of the ring containing two hydroxyl groups and one allenic bond. It was reported that metabolites of fucoxanthin accumulate in WAT (Hashimoto et al. 2009) in metabolite form i.e. amarouciaxithin A (Fig. 6.3). Fucoxanthinol was detected in all tissues of mice including plasma (Miyashita et al. 2012).

It was reported that significant attenuation of white adipose tissue (WAT) weight by the administration of 0.2% fucoxanthin in the diet of mice with increase uncoupling protein-1 (UCP-1) expression. The fucoxanthin may trigger the activity of beta-oxidation and counteracting the activity of phosphatidate phosphohydrolase that may progress to the lowered accumulation of hepatic lipid droplet (Park et al. 2011). The secretion level of leptin may get high due to the accumulation of fat in adipocytes whereas, fucoxanthin may change the level of leptin level in the plasma. Reduction in the acetyl-CoA carboxylase and AMPK enzyme is accelerated by high fat supplemented diet and this reduction may be restored by the counteractive measure of fucoxanthin (Kang et al. 2012).

Fucoxanthin was reported to affect the three differentiation stages of 3T3-L1 cells (Maeda et al. 2006). These differentiation stages are divided as early

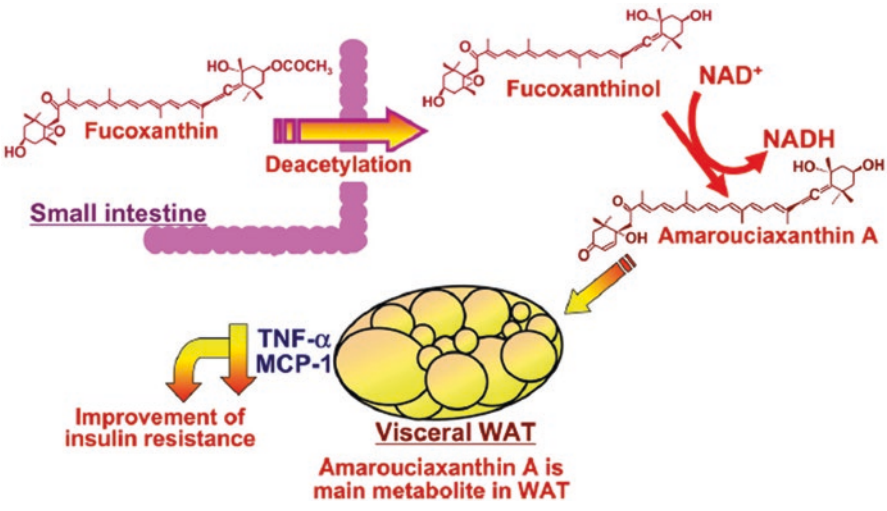


Fig. 6.3 Metabolism of Fucoxanthin and Metabolites target in abdominal WAT

(day0-day2), intermediate (day 2-day 4), and late-stage (from day 4 onwards) (Ntambi and Kim 2000). It was reported that fucoxanthin not only increased the protein expression of adiponectin, sterol regulatory element-binding protein, enhancer-binding protein α (C/EBP α) and peroxisome proliferator-activated receptor γ (PPAR γ) but also promoted the 3T3-Li adipocyte differentiation during an early stage of differentiation (day0-day2) (Maeda et al. 2006). However, by reducing expression of SREBP1c, C/EBPα and PPARγ, the fucoxanthin exhibits the counteraction to the accumulation of intercellular lipids during intermediate and late stages of differentiation (Kang et al. 2011).

In rats, the expression of monocyte chemoattractant protein-1 mRNA reported by Maeda et al. (2009) due to the administration of a high-fat diet, however, the expression of monocyte chemoattractant protein-1 mRNA was counterbalanced with use of fucoxanthin rich Wakame lipids (FWL). The results of the study conclude that the FWLs diet could be useful in ameliorating the high-fat diet that induced disorders related to lipid metabolism. The 3-hydroxy-3-methyl-glutaryl--coenzyme A reductase and acyl-coenzyme A cholesterol acyltransferase are two important cholesterol regulating enzymes and their metabolic activities were significantly counteracted by the action of fucoxanthin in rats Woo et al. (2010) Similarly, the mRNA expressions of peroxisome proliferator-activated receptor α (PPAR α) and γ (PPAR γ) was reported to change in the liver by fucoxanthin. The acyl-coA oxidase-1 mRNA expressions were altered as well.

It was reported the metabolite of fucoxanthin i.e. fucoxanthinol showed comparatively more suppressive action on the differentiation of adipocyte in 3T3-Lq 1 cells as compared to fucoxanthin. Another metabolite from fucoxanthin is amarouciaxanthin A, which presents a vital role in the suppression during adipocyte differentiation on C/EBPα and PPARγ. While comparing the two of these metabolites of fucoxanthin, amarouciaxanthin A exhibited a stronger counteractive effect on glycerol-3-phosphate dehydrogenase. Additionally, in 3T3-L1 cells, the downregulation of mRNA expression of lipoprotein lipase, adipocytes fatty acids binding protein, and glucose transporter 4 (Glut-4) was effectively observed by amarouciaxanthin A (Yim et al. 2011).

Many researchers reported that in white adipose tissue, the expression of uncoupling protein 1 is stimulated as an action of fucoxanthin thereby beneficial effect against obesity may be achieved. The uncoupling protein is not expressed in white adipose tissue (WAT) rather than brown adipose tissue (BAT) unless stimulated. Maeda et al. (2005) stated that uncoupling protein (UCP) and mRNA signals were detected in WAT when fucoxanthin supplementation was done to mice.

Zhang et al. (2015) reported that leptin plays an important role in controlling the adipose fat pad and body weight through energy expenditure regulation. Park et al. (2011) stated that the valuable effect of fucoxanthin that could reduce the leptin level in plasma related to the decrement of the weight of epididymal adipose tissue. A study was conducted on humans and it was noted body weight was reduced when 300 mg of brown seaweed extract and 300 mg of pomegranate seed oil holding 2.4 mg fucoxanthin were administered. Also, in obese females, the liver fat content was reduced when treated for 16 days (Abidov et al. 2010).

2.3 Hepatoprotective Effect

Improvement in the fatty liver could be achieved by lowering the level of fatty acids as concentrates and raising the fatty acid oxidation. It was reported that the activity of phosphatidate phosphohydrolase is reduced however, β -oxidation activity was stimulated by administration of fucoxanthin that leads to the reduction in lipid content of the liver (Park et al. 2011). The proportion of amino acids and docosahexaenoic acid (n-3 functional poly-unsaturated fatty acid) was reported to get higher by fucoxanthin supplementation (Maeda et al. 2008; Tsukui et al. 2007; Tsukui et al. 2009).

The effects of β-carotene and fucoxanthin were compared on oxidative stress indicators and results showed that as compared to β-carotene, fucoxanthin expressed more potential effects. Fucoxanthin was highly effective in reducing lipid peroxidation in liver and plasma (Sangeetha et al). Woo et al. (2010) and Park et al. (2011) reported that stimulation to the β -oxidation activity and controlling the activities of lipid metabolic enzymes of the liver, the lipid content may be lowered. Also, the oxidation of fatty acids in the liver may be increased by fucoxanthin. Woo et al. (2010) also reported that supplementation of fucoxanthin was able to reduce the concentration of plasma triglycerides as well as lowering the hepatic lipid content in mice. Activities of some of the lipogenic enzymes, phosphatidate phosphohydrolase, malic enzyme, glucose 6-phosphate dehydrogenase, and fatty acid synthase were inhibited by fucoxanthin. The rising activity of β-oxidation was counteracted by fucoxanthin.

Peng et al. (2011) reported that the metabolite of fucoxanthin, fucoxanthinol, was biologically converted to amarouciaxanthin A in the liver by isomerization/dehydrogenation. Also, the supplementation of fucoxanthinol via HepG2 cells to the culture medium was done and transformed to amarouciaxanthin A. Therefore, it may be concluded that conversion of fucoxanthinol from fucoxanthin has happened in GIT where it was further metabolized in the liver to form amarouciaxanthin A.

Tsukui et al. (2007) reported that the use of fucoxanthin enhances amino acid and docosahexaenoic acid contents in the liver. Liu et al. (2011) reported that ferric nitriloacetate induced damage to hepatic cells of rats as a result of oxidative damage that could be protected by pretreatment of fucoxanthin (1–20 μM) for 24 h. Decrease proliferation of cells was noted 30 minutes after fucoxanthin treatment. However, recovery in cell proliferation was achieved in a dose-dependent manner. The level of glutathione peroxide was increased by fucoxanthin and the proteins carbonyl contents along with thiobarbituric acid reactive substance were lowered as the counteraction of fucoxanthin. Various researchers reported that cytotoxicity in hepatics cells that was induced by ferric nitriloacetate may be inhibited by fucoxanthin (Zhang et al. 2015). All the results indicated that fucoxanthin could inhibit cytotoxicity in hepatic BNL CL.2 cells induced by Fe-NTA (Liu et al. 2011).

2.4 Anti-diabetic Effect

It is a well-known fact that abrupt change, high sugar and high-fat diets may lead to diabetes mellitus and obesity. Obesity could be a predisposing factor for diabetes mellitus as insulin resistance get high due to lipid accumulation and unnecessary energy intake (Campfield and Smith 1999).

It was observed that increasing cases of type 2 diabetes are directly correlated with obesity (Teixeira and Budd 2010). Harding et al. (2001) demonstrated that Hb A1c level could be increased due to the intake of saturated fats which linked with insulin resistance whereas, fucoxanthin may play a vital role in reducing insulin resistance and subsequently lowering the blood glucose level. For diabetic complications, the HbA1c work as a risk indicator (Sherwani et al. 2016; Goldstein et al. 1995). Similarly, Woo et al. (2010) reported that plasma insulin concentration and HbA1c levels in the blood could be significantly reduced by the administration of 0.2% fucoxanthin.

It was found that daily intake of fucoxanthin (2.4 mg) significantly resulted in reduction of body weight, lipid contents of the liver, plasma triglycerides, and body fat along with high energy consumption in human females under obese category with 100 kg average weight. It was concluded that the use of brown seaweeds containing fucoxanthin supplemented as general food would be enough to cope with the obese condition (Abidov et al. 2010).

Maeda et al. (2009) also observed that administration of 0.2% fucoxanthin may lead to a reduction in insulin resistance and blood glucose get markedly lower. They also investigated that Wakame lipids associated with fucoxanthin diet may help to counteract the insulin resistance. In this way the activity of glucose transporter 4 in skeletal muscle tissue becomes increased.

The results of supplementing fucoxanthin to diabetic or obese mice showed sufficient attenuation of white adipose tissue gain with more expression of UCP1 as compared to control mice (Pelleymounter et al. 1995; Miyashita et al. 2012). The antidiabetic effect observed in mice fed with a high-fat diet and diabetic mice were due to regulatory effect by fucoxanthin, fucoxanthinol, and other metabolites that gather in white adipose tissue on releasing adipokines. The TNF-α (tumor necrosis factor) is reported to be involved in type-2 diabetes development and its level gets elevated in obese conditions. It is positively correlated with insulin resistance (Zhang et al. 2015). The gluconeogenic enzyme activities in the liver are positively correlated with blood glucose level however; correspond to the activity of hepatic glucokinase by increasing the ratio of glycogen content and glucokinase, the insulin resistance could be decreased by fucoxanthin (Park et al. 2011).

2.5 Anti-inflammatory Effect

The process of inflammation involves a series of events in which a large number of monocyte-macrophages, neutrophils, and MAST cells (leukocytes) get attracted to inflamed tissues or organs and is a kind of body self-defense action. However, different inflammation mediators are activated by the action of inflammatory cells. As a result, nitric oxide radicals and superoxide anions are generated leading to harmful effects and self-damaging (Zaragoza et al. 2008).

Lee et al. (2003) reported that various inflammatory mediators should be suppressed by anti-inflammatory agents so that the overall inflammatory response may be reduced. Such inflammatory mediators include prostaglandin E2(PGE2), TNF-α (tumor necrosis factor-α), iNOS (inducible nitric oxide synthase; inflammatory cytokine), NO (nitric oxide), IL-1 β(interleukin) and COX (cyclooxygenase; inflammatory cytokine).

The production of TNF-α, inflammatory cytokines, IL-6 (interleukin-6), IL-1β (interleukin-1β) must be counteracted to decrease the inflammatory response by the help of anti-inflammatory agents. Also, the inflammatory mediators like prostaglandin E2 and nitric oxide production should be suppressed to cope with harmful and self-damaging effect (Peng et al. 2011).

The research observed that murine macrophages cells were activated by the action of fucoxanthin in lipopolysaccharide (LPS) so it was concluded that fucoxanthin may contribute to decreasing the severity of pro-inflammatory mediators by counteracting effect on MAPK phosphorylation and NF-B activation (Kim et al. 2010b). Decrease level of COX-2 and NOS was noted by fucoxanthin in a dose-dependent manner.

It was reported that inflammatory cytokines and macrophages were inhibited by the action of fucoxanthin. The effect of fucoxanthin is inhibition of expression of cyclooxygenase-2 and nitric oxide synthase. Through the suppression of protein kinase and NF-kB activation, the level of TNF- α, PGE2, nitric oxide, interleukin-6 (IL-6), interleukin-1β (IL-1β) were reduced (Heo et al. 2008; Kim et al. 2010b).

Sakai et al. (2009) also confirmed the anti-allergic and anti-inflammatory effects of fucoxanthin *in vivo*. The inhibition of the degranulation of MAST cells was observed by counteracting the accumulation of Fc ∈ RI due to the fucoxanthin effect.

2.6 Antioxidative Effect

During the metabolism of oxygen in aerobic species, the generation of free radicals leads to various pathological issues like diabetes mellitus, aging, cancer, and Alzheimer's disease (Kong et al. 2010). The pharmaceutical and food industry have higher concern for ROS like hydroxyl radicals and superoxide anions that are responsible for lipids oxidation thereby reducing the shelf life of food therefore use of synthetic antioxidants is common (Je et al. 2005).

The exclusive chemical structure of fucoxanthin make it likely a good antioxidant due to the epoxide group, allelic bond and hydroxyl group in its structure (Sangeetha et al. 2009) The potential radial scavenging property is possessed by fucoxanthin (Nomura et al. 1997; Kawee-ai et al. 2013). Researchers noted that fucoxanthin possesses a strong action against BchE and mixed inhibition type though, weak action was observed for AChE. The scavenging ability of fucoxanthin was reported to reduce DPPH reducing power, hydrogen peroxide, and superoxide anion by 21.0%, 19.7%, 16.0%, and 10.3% respectively when the increase of 2% in percent of cis-isomers was followed (Kawee-ai et al. 2013).

The safe substitution of synthetic antioxidants can be performed with natural antioxidants, especially for the food industry (Kim et al. 2011). The best source of natural antioxidants is marine algae (Cornish and Garbary 2010). The fucoxanthin has been identified as the main antioxidant abundantly available in nature and its C-70 position for double allelic bond (fucoxanthin and fucoxanthinol) enables their involvement in radical scavenging activity (Sasaki et al. 2008).

The plasma level of antioxidants was due to conversion of fucoxanthin to fucoxanthinol in the chicken plasma while broiler chicken meat color enhanced due to antioxidative potential of fucoxanthin as a result of fucoxanthin oral administration (Sangeetha et al. 2009). The inhibition of ROS generation may be correlated with the presence of hydroxyl groups in the structure of fucoxanthin (Kim et al. 2011). Ha et al. (2013) examined the effects of fucoxanthin used as an antioxidant. The plasma total antioxidant capacity (TAC) as a marker of antioxidant capacity and some enzyme-like super dioxide dismutase (SOD) and catalase (antioxidant enzymes) were observed in two groups of rats that were fed with a high-fat diet alone and high-fat diet along with fucoxanthin. The findings of their experiment were the significantly elevated level of glutathione peroxide in liver and plasma in a group of rats supplemented with fucoxanthin.

2.7 Anti-bacterial Effect

Karpinski and Adamczak (2019) evaluated the carotenoid effects against selected clinical strains of bacteria. However, it was confirmed that fucoxanthin shows stronger effect on the Gram-negative (G−) and Gram-positive (G+) bacteria as mean zones of inhibition was 7.2–10.2 mm and 9.0–12.2 mm respectively. The highest activity of fucoxanthin was exhibited against *Streptococcus agalactiae* followed by *Staphylococcus epidermidis* and *Staphylococcus aureus* with a mean ZOI 12.2 mm, 11.2 mm and 11.0 mm in agar disc-diffusion method.

3 Anticancer Effects

The higher incidence of mortality due to cancer is important to overcome with conventional way of chemotherapy. However, repeated chemotherapy play crucial role in the drug resistance and drug residue that lead to morbidity and even death. Therefore, searching for a suitable way to ameliorate the causes of cancer is an important task. Zhang et al. (2015) reported that fucoxanthin could play a positive role to control the malignancies by tempting the programmed cell death and cell cycle arrest.

Fucoxanthin is believed to be effective as an anti-cancer remedy due to its ability to induce apoptosis in tumor cells (Nakazawa et al. 2009). It was reported that 2 bioactive enzymes poly ADP ribose polymerase and procaspase-3 are cleaved to induce cell death in promyelocytic polymerase HL-60 cells of humans (Abu-Ghannam and Shannon 2017). Kim et al. (2010a) reported that the fucoxanthin play vital role in the inhibition of cell growth via inducing cell apoptosis and accumulate reactive oxygen species. In this process, the cleavage of poly ADP ribose polymerase, caspase-3,-7, and the inactivation of the Bcl-xL signaling pathway lead to HL-60 cells apoptosis. Fucoxanthin extracted from the *U. pinnatifida* was responsible for the growth inhibition of nine different tumor cell lines in humans (Wang et al. 2014a).

Similarly, enzymatic activity and expression of xenobiotic-metabolizing enzymes are significantly affected by fucoxanthin extract. Such enzymes (CYP3A4, CYP1A2, and CYP1A1) are responsible for triggering the pro-carcinogens. The concentration of fucoxanthin extract i.e. 45μM showed a counteractive effect on these enzymes in HepG2 cells (hepatocellular carcinoma) and recombinant CYPs in humans. So, in this way fucoxanthin can attenuate the activity of anti-cancer drugs which under normal circumstances may be triggered by CYP3A4 (Satomi and Nishino 2013).

4 Molecular Mechanisms of Fucoxanthin for G1/G2 Arrest, Apoptosis, Autophagy and Metastasis Inhibition

4.1 G_1 Cell Cycle Arrest in Tumor Cells

Induction of G_1 cell cycle arrest has been reported as a mechanism of action of fucoxanthin against tumor cell lines (Okuzumi et al. 1990). Fucoxanthin has shown to arrest G_0/G_1 stage at a concentration level of 5–10μg/ml. In GOTO cells of human neural blastoma, the expression of PD-L1 in stage-3 and -4 neuroblastoma that had inhibitory effect of other genes co-amplified with MYCN proto-oncogene (Satomi 2017). Fucoxanthin activity in a time-dependent (24 h and 48 h) and dose-dependent (25 and 50μM) manner was observed by Das et al. (2005) for the induction of cell cycle arrest. At 48 h, apoptosis was induced at a concentration level of 50μM in

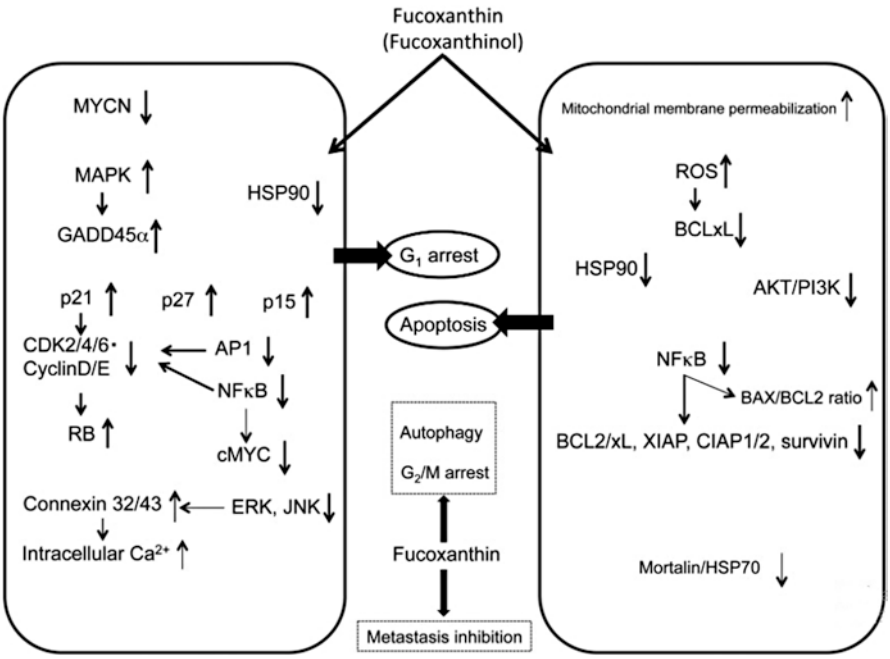


Fig. 6.4 Antitumor activities of Fucoxanthin and associated factors (Satomi 2017). ↑, Activation or Induction; ↓, Deactivation or suppression; Mitogen activated protein kinase,MAPK; DNA damage-inducible 45α, GADD45α; cyclin-dependent kinase, CDK; heat shock protein90, HSP90; nuclear-factor kappa B, NF-κB; AP1 transcription factor, AP1; RB transcriptional corepressor, RB; extracellular signal-regulated kinase, ERK; c-Jun N-terminal kinase, JNK; reactive oxygen species, ROS; B-cell lymphoma-extra-large, Bcl-xL; serine/threonine-specific protein kinase, AKT; phosphatidylinositol 3′-kinase, PI3K; BCL2- associated X, BAX; cellular inhibitor of apoptosis protein 2, CIAP2; X-linked inhibitor of apoptosis, XIAP; B-cell lymphoma2, BCL2

WiDr cells (colon carcinoma in humans). The lowered phosphorylation level of RB and the rise in CDK-inhibitory protein and P21WAF1/CIP1 were dose-dependent (Al Bitar and Gali-Muhtasib 2019).

Additionally, the increased the concentration of fucoxanthin (75μM and 50μM) resulted in a lower level of cyclin D and CDK4 along with the rise in P27KIP1 (Fig. 6.4) that phosphorylate RB protein (Das et al. 2008; Das et al. 2005). They considered p27KIP1 has a vital role regarding apoptosis by fucoxanthin and also hypothesized the important role of P21WAF1/CIP1 for cell cycle arrest during the G0/G1 stage (Rengarajan et al. 2013).

Das et al. (2008) reported that in HepG2 (human hepato-carcinoma cells), cell cycle arrest was induced by fucoxanthin at a concentration level of 25μM. The RB protein phosphorylated forms were decreased without reducing its level and accompanied the cell cycle arrest. During this, cyclin D_1 was not detected in quiescent cell, though, Cdk4 activity does correlate with changes in the level of the Cdk inhibitor

P21WAF1/CIP1. It was reported that for the proper action of fucoxanthin, the cyclin D downregulation may play a vital role.

Yu et al. (2011) stated the role of fucoxanthin for cell cycle arrest (M/G2 phase) and apoptosis in MGC-803 cells (human gastric adenocarcinoma) as these cells containing STAT3 (signal transducer and activator of transcription 3) and cyclinB1 protein whose expression is counteracted by fucoxanthin in a dose-dependent manner. The suppression of CyclinB1 expression may also be carried out through the JAK/STAT pathway by fucoxanthin. The mechanism of cell cycle arrest induced by fucoxanthin is described in a Fig. 6.4. It was reported that the cyclin D protein level was lowered by fucoxanthin and action of D/CDK4 was compacted. In humans' cells of MGC-803 (Gastric Adenocarcinoma), apoptosis and G2/M cell cycle arrest were noted as an action of fucoxanthin at the concentration level of 75μM and 50μM (Yu et al. 2011). It was reported that such factors like the lowered expression of surviving and cyclin B may have a synergistic effect on the activity of fucoxanthin.

There is an important role of GADD45 in suppressing the growth of cells. Proliferating cell nuclear antigen role in replication and repairing of DNA as well as part of the Cdk complex (cyclin-dependent Kinases). It was reported that bonding between PCNA and GADD45 leads to excision repair of DNA and restriction to the cells entering the S phase. Contact of the cell to cell is restricted by the induction of β-catenin translocation. This induction results from the interaction among Caveolin-1 and β-catenin which is promoted by GADD45 (Ji et al. 2007). It was observed that at a concentration level of 10μM and 5μM for fucoxanthin and its metabolites were more likely to cause caspase-dependent apoptosis and cell cycle arrest (G1 stage) in adult T-cells (Leukemia cells) Ishikawa et al. 2008). The reduction in the level of different proteins was noted like CDK4, CDK6, cyclinD1, and cyclinD2. Concurrently, the induction of GADD45α was noted. The surviving, XIAP, BCL2, and CIAP2 are apoptosis-related proteins for which reduction was reported (Bailon-Moscoso et al. 2017; Hydbring et al. 2016).

Researchers found that the GADD45 role in HepG2 cells is supported by fucoxanthin in G1 arrest and is positively carried out in HepG2 cells through the restriction of the p38 MAPK pathway (Satomi and Nishino 2009). It was observed that in DU145 (prostate cancer cells), HepG2, and LNCap cells, the MAPK pathways were activated associated with GADD45A gene induction and cell cycle arrest (G1) as an action of fucoxanthin at the concentration level of 3.8 to 5.5μM (Satomi and Nishino 2007, 2009, 2013). After cell cycle arrest (G_1) in hepG2 cells, a negative association was observed between GADD45A induction, mediated by fucoxanthin corresponding to a positive association between JNK and DU145 cells.

Furthermore, in LNCap cells, cell cycle arrest (G_1) by fucoxanthin and GADD45A induction is positively associated with JNK, and a negative association between ERK1/2 and p38 was reported. The counteraction of fucoxanthin against extracellular receptor kinase (ERK) increases the expression of GADD45 however, resulted in no action on G_1 arrest (HepG2 cells) that may be concluded as MAPK activity for the G_1 cell arrest and stimulation of GADD45 by fucoxanthin allied with the type of cells (Zhang et al. 2015).

The nuclear factor-kappa (NF-κB) and its inactivation is considered to be supportive for the activity of fucoxanthin and its metabolite fucoxanthinol although, this may also be mediated through transcriptional factor AP-1. In another study, it was noted that in Burkitts and Hodgkin lymphoma cells, the cell cycle arrest (G_1 stage) was caused by fucoxanthin and fucoxanthinol at the lower concentration level of 2.5 and 1.25μM, respectively (Takahashi et al. 2015). Similarly, the caspase-dependent apoptosis in same cells were noted to be induced by fucoxanthin and its metabolite at higher concentration level i.e. 5μM and 2.5μM respectively (Tafuku et al. 2012). There is a close association between the reductions BCL2, XIAP, cyclin D1and cyclin D2 with the regression of NF-κB action. It was hypothesized that vital role was carried out through the suppression of cell survival proteins that are dependent of NF-κB during apoptosis as well as a downfall in the action of cyclin D during cell cycle arrest induced by fucoxanthin and fucoxanthinol (metabolite).

It was studied that fucoxanthin help in lowering the spread of B16F10 cells along cell cycle arrest in $G(_1)/G(_0)$ stage (Kim et al. 2013). This cell cycle arrest fueled by the action of fucoxanthin has a close relation to the declined expression of phosphorylated-Rb which is a retinoblastoma protein. The CDK4 and cyclin D activity also lowered however, p27kip1 and p15INK4B proteins level were significantly increased. The lymphoma cells were observed for the apoptosis and cell cycle arrest (G_1) and this was induced by fucoxanthin and fucoxanthinol at a concentration level of 5, 10μM and 2.5, 5μM, respectively (Yamamoto et al. 2011). The expression of cyclinD2, XIAP, BVL-xL, CDK6, CDK4, and MYC protooncogene protein was lowered. Correspond to that, AP1, AKT, and NF-kB inactivation were reported in cells (HSP90). From the results, it was deduced that the action of fucoxanthin and fucoxanthinol might have a connection with the inhibition of HSP90 cells. It was reported that in mouse melanoma cells, apoptosis following G_0/G_1 cell arrest was noted at the various high concentration levels of fucoxanthin i.e. 50, 100, and 200μM (Kim et al. 2013).

It was noted that p27KIP1 and p15INK4B level was raised in the cells along with a decline in cyclinD1, CDK4, cyclinD2, and phosphorylated RB. Whereas, XIAP, CIAP2, CIAP1, and BCL-xL were lowered as well (Topacio et al. 2019). Cell cycle arrest (G_0/G_1) was noted at a concentration level of 5 and 10μM of fucoxanthin in T24 cells (human bladder cancer cells) accompanied by a decrease in cyclin D1, cyclin E, CDK4, and CDK2 and increase in p21WAF1/CIP1 (Wang et al. 2014b). Mortalin belongs to HSP70 which inhibits p53 functions treated with the 20 and 40μM of fucoxanthin leads to p53 recrudescence. Similarly, in human hepatoma cells (SK-Hep 1), the cell cycle arrest was caused by fucoxanthin at a dose rate of 1-20μM (Liu et al. 2009). These findings were correlated with the upregulation of the expression of connexin 32 and connexin 43 and GJIC (gap junctional intercellular communication) was also enhanced.

The JNK (c-Jun n terminal kinase) and ERK (extracellular signal-regulated kinase) were decreased in the cells. It was hypothesized that through enhancement in JGIC, the fucoxanthin raised the calcium level leading to cell cycle arrest and apoptosis. It was observed that in HeLa cells (cervical epithelial cells of the human)

lowered CDK2 and cyclinD1 and higher-level of CIP1/p21WAF1 lead to G0/G1 arrest by the action of fucoxanthin at a concentration of 10, 20 and 40μM (Hou et al. 2013). The rise in PTEN, phosphatase, and decrease in phosphorylated form of AKT along with p53 protein, p70S6K were reported by fucoxanthin and thus induced autophagy (De Amorim et al. 2010; Kharat and Gulwe 2016).

4.2 Apoptosis

Apoptosis is programmed cell death and activity of removing the physiological cell to maintain a balance between the proliferation of cells and their death (Rengarajan et al. 2013) The apoptosis could be an efficient way for cancer therapy (Zhang et al. 2015). Fragmentation of DNA was reported by Kotake-Nara et al. (2001) in DU145, LNCap, and PC-3 cells (human prostate cells) by the action of fucoxanthin (20μM). Similarly, at a concentration level of 10μM, apoptosis (caspase-dependent) was induced by fucoxanthin in HL-60 (promyelocytic leukemia cells) through the loss of mitochondrial membrane potential (Kotake-Nara et al. 2005a). Similarly, in another study the apoptosis induced by fucoxanthin (20μM) along with the downfall in the protein level of BCL2 and BAX in PC3 cells was reported (Kotake-Nara et al. 2005b).

Another promising and striking therapeutic strategy for cancer may be the inhibition of EGFR along with STATs (Quesnelle et al. 2007) as reported from the study that indicated that STATs possess an important role in EGFR mediated cancer cells proliferation (Song and Grandis 2000; Berclaz et al. 2001). Wang et al. (2012) reported the role of fucoxanthin in mice on xenografted sarcoma. The findings of this study were the inhibitory effect on STAT3, EGFR, STAT3 phosphorylated proteins, and Bcl-2 induced by fucoxanthin. Also, it was noted that the expression of caspase-3 was improved. It was reported that apoptosis may be induced by both fucoxanthin and metabolite of fucoxanthin i.e. fucoxanthinol. Through the down-regulation of EGFR/STAT3 signaling, apoptosis may be started by fucoxanthin in mice bearing xenografts (Zhang et al. 2015).

The modulation in the ration of BCL2/BAX is suggested to induce apoptosis by the action of fucoxanthin. The fragmentation of DNA as an indication of apoptosis may be induced by fucoxanthin at a minimum concentration of 7.6μM for about 48 h; however, the action was counteracted by the caspase enzyme inhibitor which plays its role in lowering the level of BCL2 protein in the colon cancer cells (Caco-2; humans). These consequences lead to a belief in the synergistic role of BCL2 proteins in apoptosis induced by fucoxanthin (Hosokawa et al. 2004). Similarly, in human breast cancer cells (MCF-7), apoptosis was induced by fucoxanthin, at the concentration level of 25μM (Konishi et al. 2006). Apoptosis in cancer cells of lungs (A549 and NSCLC-N6 cells) as indicated by morphological changes and DNA ladder, was reported due to fucoxanthin at the concentration between 7.6 and 60.7μM (Moreau et al. 2006).

Researchers reported that the cell viability of ATL cells and HTLV-1 infected T-cells could be obstructed by fucoxanthin and its metabolite fucoxanthinol. Also, the blood mononuclear cells and cell lines that were remained uninfected found to be resistant against fucoxanthinol and fucoxanthin (Ishikawa et al. 2008). It was noted that activation of caspase-3 along with fucoxanthin action help in inducing the apoptosis in cancer cells from the human bladder (EJ-1) at 72 h (Zhang et al. 2008). Following the decrease in the level of BCL-XL in HL-60 cells and generation of ROS (reactive oxygen species), the fucoxanthin brought apoptosis in a caspase-dependent reaction (Kim et al. 2010a). The caspase-dependent apoptosis and cell cycle arrest (G1 phase) could be induced by fucoxanthinol and fucoxanthin in lymphoma cells (Yamamoto et al. 2011). It has appeared that fucoxanthinol is more potent than fucoxanthin regarding the apoptosis-inducing activity.

There was a crucial role in the generation of ROS by fucoxanthin. The fucoxanthin at the concentration level of 60μM caused apoptosis in HL-60 cells along with the activation of caspase-3 (Ganesan et al. 2011). The caspase-dependent apoptosis was observed as an action of fucoxanthin in HeLa cells along with a decrease in Pl3K (phosphatidylinositol 3 -kinase), BCL2, AKT (phosphorylated form) and increase in the level of BAX (Ye et al. 2014). At a concentration level of 1–10μM of fucoxanthin, HepG2 cells showed an increase in BAX/BCL2 ratio (Liu et al. 2013). Cisplatin along fucoxanthin reduced the expression of thymidine phosphorylase and ERCC excision repair (DNA repair gene resulted in the improved activity of cisplatin).

It was also noted when treated with fucoxanthin that NF-κB was inactivated along with a reduction in translocation to the nucleus from the cytoplasm (Rolhion et al. 2016). The apoptosis was induced in MDA-MB-231 and MCF-7 (cancer cell lines in human breasts) by fucoxanthin and fucoxanthinol at a concentration range of 10–40μM (Rwigemera et al. 2014, 2015). The expression of p52, p62, and RELB proto-oncogene, which affiliates to the pathway of NF-kB, was reduced by fucoxanthinol alone, in estrogen resistant MDAMB 231 cells (Oeckinghaus and Ghosh 2009).

5 Fucoxanthin Application as a Functional Food

It is reported that the beneficial health effects of fucoxanthin are beyond ordinary nutrition (Shannon and Abu-Ghannam 2019). To date, there is no report of toxicity of fucoxanthin; therefore, it is considered suitable for being used as functional food. However, as a functional food, fucoxanthin is easily get oxidized due to various factors like long storage periods, unsuitable pH, high temperature and UV light, therefore, fucoxanthin faces organoleptic, chemical as well as bioavailability constraints (Kawee-ai et al. 2013; Mise et al. 2011). Various organoleptic characteristics including smell, taste, texture, appearance may deteriorate due to enzymatic and chemical interactions with ingredients. Fucoxanthin is insoluble in water and its combination with sauces and beverages may need emulsification (Socaciu 2007).

The bioavailability of fucoxanthin in mammals is affected by its lipidic nature (Sangeetha et al. 2010). Fucoxanthin is converted into its metabolite fucoxanthinol in the intestine by the action of lipase and cholesterol esterase and thereafter converted to amarouciaxanthin A in the liver (Bagchi and Preuss 2012; Dominguez 2013).

During the freeze drying process and extraction method, high energy is required, which leads to the high cost of fucoxanthin as a functional food (Billakanti et al. 2013). The vitiation of the content of fucoxanthin due to seasonal variation and absence of a method for artificial synthesis are other issues thereby, nutritional efficacy and health claims may also be affected. Along with astaxanthin (carotenoid), fucoxanthin may act as a more powerful antioxidant than other synthetic and natural antioxidants (Miyashita and Hosokawa 2007).

It's been more than a hundred years since fucoxanthin discovery and its isolation from seaweeds however; its underutilization in the pharmaceutical and food industry is questionable. Until the 1990s, after research start to understand the potential of fucoxanthin as a functional food, studies focused mainly on its quantification and biosynthesis pathway. Since the concept of antioxidants' role in ameliorating the chronic diseases was emerging therefore, fucoxanthin caught attention. Currently, retail consumers may find fucoxanthin in the form of costly weight loss supplements while its quality and purity may vary. Hurst (2002) stated that pure fucoxanthin cannot be sold as a bulk food ingredient due to an increase in extraction cost and oxidation reaction over a period of time. Hence, it's an unstable product. The cost of ≥95% pure fucoxanthin (analytical grade) is 636 euro per 50 mg, made for laboratory use (Sigma-Aldrich 2020; https://www.sigmaaldrich.com/catalog/product/sigma/f6932?lang=pt&region=PT).

The nutritional characteristics and functional attributes of meat sausages were observed to improve by adding fucoxanthin. Keeping in view the consumer preference, the addition of 0.04% of fucoxanthin developed various beneficial changes concerning health like inhibition of angiotensin-1 converting enzyme and lipid peroxidation, color stability, improved antioxidant capacity for almost 2 weeks (Sellimi et al. 2017).

Various epidemiological studies were conducted and found dietary compounds like fucoxanthin can lower the incidence of disorders directly correlated with the cellular damage due to free radicals (Shannon and Abu-Ghannam 2019). In various countries like China, South Korea, and Japan, some of the marine-based functional food can be used for retail users in the market as a sustainable substitute to antioxidants (synthetic/natural). Seaweed is a common part of the diet in these countries. Similarly, fucoxanthin is Halal, Kosher as well as suitable for vegetarians. Since the 1990s, most of the studies are conducted on fucoxanthin, not as a functional food, but rather focused on its health impact and medical point of view. Prabhasankar et al. (2009) demonstrated the fruitful results of combining the fucosterol and fucoxanthin into the semolina wheat-based pasta. Fucosterol possesses anticancer, hepatoprotective, and anti-cancer properties (Jung et al. 2013). Similarly, wakame powder incorporation in pasta was studied by Prabhasankar et al. (2009) to understand the nutritional and bifunctional quality of pasta.

Several compounds like fucoxanthin and other antioxidants are present in seaweeds that help to induce programmed cell death in cancer cells and could be of great value regarding functional food items (Jiang and Shi 2018). The analysis of processed and cooked pasta (250 ml boiling water containing 25 g of raw pasta) was done through HPLC and it was reported that <10% fucosterol and fucoxanthin contents were lost. This happened in the gluten protein matrix due to the increased stability of fucosterol/ fucoxanthin. More studies are needed to understand the stability of the food system.

To observe the anti-obesity effects of fucoxanthin Shannon and Abu-Ghannam (2019) and Hitoe and Shimoda (2017) given fucoxanthin capsules with the dose rate of 1 mg and 3 mg per day for 28 days to 50 women and men having body mass index above recommended value i.e. 18–25 kg/m^2. All subjects were quite healthy. It was noted that subjects receiving 3 mg dose of fucoxanthin, there was a significant reduction in the visceral fat, body mass index, basal metabolic rate, neck circumference, and abdominal adipose tissue. There were no reports of blood pressure, pulse rate, urinalysis, and blood parameters. In another study, *Ascophyllum nodosuma* that contains 100% water and 80% ethanol extract and *Fucus vesiculous* containing 60% ethanol extract (Zaragoza et al. 2008) were incorporated to fluid milk and yogurt at concentration of 0.25% and 0.50% for antioxidant activity (O'Sullivan 2013). The overall acceptability of milk was observed for its taste (fishy flavor) and appearance (least yellow or green coloration) and milk was noted more acceptable when aqueous extract from *A. nodosum* was incorporated only at 0.50%. Similarly, it was reported that on the basis of flavor and least yellowish appearance, the yogurt with aqueous extract of *A. nodosum* only, was preferred. It was observed that other characteristics like shelf-life, and pH were stable of milk and yogurt. Also, oxidation was not found after the *in vitro* analysis for antioxidant activity.

Various wholesale companies of china are offering natural food extracts including fucoxanthin as dry seaweed extract. Fucoxanthin extracted generally from kombu and wakame is sold with purity % mention on the product and ranges from 10% to 98%. Based on purity, there is a huge variation for prices i.e. 1$ to 2000$ (Alibaba 2015; Kyndt and d'Silva 2013). In another study, 15 panelists were used for sensory evaluation of the combination of seaweed and semolina blends that were made through the replacement method. i.e. 0:100, 5:95, 10:90, 20:80, 30:70 for wakame/semolina (w/w). Regular use of *Wakame* was taken as panelists and they found insignificant ($P < 0.05$) difference for organoleptic characteristics control vs. *Wakame* pasta (10%). However, panelists reported a decrease in acceptance with wakame content between 10% and 20% and beyond 20% strong seaweed and salty taste was described. Whereas, 10% wakame was equivalent to 1.25 mg/g of fecosterol in dry ingredient part, whereas, 0.04 mg/g on a dry weight basis of fucoxanthin.

A human trial for blood sugar (postprandial) was conducted using diet along with *Wakame* as a source of fucoxanthin and sporophylls (Mekabu). The control group was not supplemented with these bioactive compounds. Almost half an hour later it was noticed that glucose level was decreased in the treatment group as compared to the control group. It was concluded that fucoxanthin in Mekabu containing polysaccharides-rich content which stimulate insulin receptor to secrete and

overcome hyperglycemia in men and women (Tanemura et al. 2014). A functional food product was produced using kombu with 1–5% fucoxanthin content in it. The product is certified by the government for its commercial availability to retail consumers (Abu-Ghannam and Shannon 2017). It is observed that the thermostability of fucoxanthin in this product is 1 h at 80 °C and similarly, pH stability ranges from 3.0 to 10.0 (Zhao et al. 2014). Two other components were also added keeping in view the further stability and providing a safety matrix for fucoxanthin i.e. cyclodextrin and triglyceride. The successful incorporation of oil and powder products in various bakery items like potato snacks, cakes, shortbread is reported however, sensory evaluation results were not reported as discussed by Oryza (2015).

In another study, fucoxanthin capsules were administered to women having obesity but non-diabetic conditions, whereas, more than 11% of liver fat contents were present in them. The 2.4 mg of fucoxanthin as a daily dose was administered to them. The result of the study was the reduction in the weight (4.9 Kg) with high energy expenditure at rest and the conclusion was that extracts of seaweed could be used as an aid to treat the obese condition (Abidov et al. 2010).

6 Strategies to Cope with Unfavorable Reactions in Functional Food

The major hurdle of using fucoxanthin as a functional food is insolubility in water, high extraction cost, pH instability, and sensitivity against oxidation. These issues may be removed by using some techniques and approaches so that carotenoids and polyphenolics may also be stabilized as food ingredients.

One of these techniques is microemulsion commonly used for making a combination of carotenoids as lipid solute in the hydrophilic matrix for the pharmaceutical and food industry (De Campos et al. 2012). Similarly, another researcher fruitfully formulated a microemulsion for fucoxanthin which is oil in water, stable and clear. The microemulsion is worthy of transporting the hydrophobic antioxidant in the aqueous form of the food system (Suhendra et al. 2012). Another technique for the safeguard of acid-labile biological active components from the acidifying environment of the stomach is the use of nanogel. In this way, fucoxanthin stability and biological availability may be significantly raised by encapsulation with chitosan-sodium tripolyphosphate glycolipid nanogel (Ravi and Baskaran 2015).

7 Conclusion

Fucoxanthin is a known xanthophyll which is abundantly found in the chloroplasts of brown algae and other heterokonts due to which gives brown olive-green color. It is concluded that various pharmaceutical applications and biological activities of

this carotenoid include antioxidant, anti-cancer, anti-diabetic, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective properties. Fucoxanthin works as anti-cancer due to the potential to induce apoptosis in tumor cells, growth inhibition of cancerous cells, apoptosis induction, G_1 and G_2 cell cycle arrest. Fucoxanthin is easily get oxidized due to various factors like long storage periods, unsuitable pH, high temperature, and UV light therefore fucoxanthin encounter with organoleptic, chemical as well as bioavailability constraints. Under the light of the significance of research done on fucoxanthin, it may be concluded that fucoxanthin is potential bioactive compound to be used as functional food however, there is still a gap exist regarding its beneficial health effects in pharmaceutical industries which are needed to fill through further research that could be done in future.

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Chapter 7
Cruciferous Vegetables (Indole-3-Carbinol, Isothiocyanates) Against Cancer

Giuseppe Annunziata, Angela Arnone, and Gian Carlo Tenore

Abstract During the years, numerous evidence reported the extraordinary power of nutrition in both prevention and management of several chronic diseases, including metabolic disorders, cardiovascular diseases and cancer. Indeed, epidemiological studies established the existence of strong relationships between the regular consumption of such foods and the risk of disease development, with both positive and negative meanings. In this sense, a particular interest has been focused on the role of nutrition on cancer risk and prevention. Besides the well-established negative impact of high consumption of red meat- or processed meat-based products, several evidence highlighted the preventive effect of fruits and vegetables, or more generally fibre-rich foods, on various types of cancer, mainly colorectal cancer. Among these kinds of foods, cruciferous vegetables gained great interest by the scientific research. More specifically, bioactive compounds contained in cruciferous vegetables, including indole-3-carbinol and isothiocyanates, have been demonstrated to exert a marked anticancer potential. Several studies, indeed, reported that these phytochemicals show a chemo-preventive activity influencing cancer development since the initial phases, acting through various mechanisms. The present chapter aims to summarise the available literature providing the evidence for the use of bioactive compounds from cruciferous vegetables as chemo-protective agents, focusing the attention on the main representative phytochemicals belonging to this group, such as indole-3-carbinol and isothiocyanates. Also, the main putative mechanisms of action and signalling pathways will be presented and discussed.

Keywords Isothiocyanates · Chemo-preventive activity · Cancer · Indole-3-carbinol · Cruciferous vegetables

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Abbreviations

AHR	Aryl hydrocarbon receptors
CDKs	Cyclin-dependent kinases
CYP450	Cytochrome p450
ER	Estrogen receptors
GTS	Glutathione S-transferase
I3C	Indole 3-carbinol
mTOR	Mammalian target of rapamycin
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NQO1	NAD(P)H quinone oxidoreductase-1
PI3K	Phosphatidylinositol-3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PIP3	Phosphatidylinositol 3,4,5-trisphosphate
QR	Quinone reductase

1 Introduction

"*Let food be your medicine and medicine be your food*". With this statement, almost 2500 years ago Hippocrates offered to human population a beautiful recommendation dressed up like a medical prescription that still appears contemporary in our present era. Without having to disturb the first physicians and scientists in the history which, however, understood the medicinal properties of foods (Fenwick and Heaney 1983), it should be noted that, in the last decades, the scientific research paid particular attention on the impact of diet, in general, or such foods, specifically, on both several pathological conditions or maintenance of the wellness. In this sense, we could mention the first studies on the Mediterranean diet and the prevention of cardiometabolic diseases, as well as the previous studies on the bioactive compounds contained in red wine that led to the well-known French paradox. All this is to say that, besides the extraordinary researches in the pharmaceutical field, a relevant piece of evidence supports the potential benefits of natural products, mainly food-deriving bioactive compounds, in both prevention and management of various pathological conditions.

Notably, in the 1989 Professor Stephen DeFelice finalized the concept of the strong interplay between diet and health coining the term "nutraceutical", as portmanteau of the words "nutrition" and "pharmaceutical", identifying the pharmacological potential of food-derived compounds (Santini and Novellino 2014). It should be stressed that this is a science studying the effects of bioactive compounds from animal or vegetal foods on human health, following a pharmacological approach. It means that nutraceutical should not be confused with further alternative or non-conventional practices, since the single components of foods are investigated with a meticulous scientific rigour, properly designing *in vitro* and *in vivo* studies and

providing strong evidence. Interestingly, nutraceuticals are considered as supplements containing food-derived extracts, concentrated and administered in a suitable pharmaceutical form, highlighting once more the pharmaceutical meaning of nutrition. Overall, thus, the careful analysis of the available literature in this field produced in the last 10–15 years, allows considering the nutraceutical as a useful tool to contrast the development of several diseases, acting "*Beyond the diet, before the drugs*" as stated by Professor Ettore Novellino in 2012 (Santini and Novellino 2014).

This current shift of the scientific interest from the mere study of the drugs to the evaluation of further and eventually natural therapeutic approaches should not be interpreted as a new direction operated by the researchers, but rather as a novel route that run alongside. And it should not scare off, since this just represents the natural progress of the science. We would like to give an example that might sounds like as an off-topic, but may help to understand the importance to keep an open mind when approaching the scientific research.

Until not many decades ago it was thought that Universe consisted solely of ordinary matter, which constitutes stars, planets, gasses and cosmic dust. During '70s, several studies and researches provided a novel interpretation of the Universe, proving the existence of both dark matter and dark energy. Interestingly, it was proved that dark matter and dark energy constitute the majority of the Universe, whereas ordinary matter only represent a limited part. These findings opened the way to new fields of application in theoretical physic and a novel comprehension of the complexity of the Universe. This fascinating travel into the galaxy perfectly suggests that static certainties are not admitted in sciences, and that research is a dynamic process that continually remodels the previous discoveries, providing novel interpretations. In this context, medical science is not exempt from this continuous progress.

Coming back to the focus of this chapter, also the research in the tumour field deeply investigated the protective role of such diets or foods in prevention of various cancers (Verhoeven et al. 1996), specifically focusing on vegetable consumption. Among these, cruciferous gained a particular interest since their regular consumption has been linked to reduced development of different kinds of cancers, including colorectal, breast, kidney, and upper digestive tract cancers (Bosetti et al. 2012). Although the exact mechanisms by which these vegetables are able to counteract the processes of carcinogenesis are still unclear, many studies clarified the possible signalling pathways in which the bioactive compounds of cruciferous vegetables may be involved.

In the following sections of the present chapter we will offer you an initial overview on the cruciferous vegetables, in terms of botanical and chemical features, with a focus on the human metabolism of their bioactive compounds. Subsequently, we will discuss the relationship occurring among these vegetables and cancer, reporting the main putative anticancer mechanisms of action and signalling pathways.

2 Cruciferous Vegetables

In this section we will explore the Cruciferous vegetable world, describing in detail the botanical features and their bioactive compounds, in terms of chemical features and human metabolism after ingestion.

2.1 Botanical Features

Cruciferous are vegetables belonging to the botanical family *Brassicaceae*, order Capparales, in which sixteen families are included (National Resource Conservation Services 2020). The *Brassicaceae* family is very large, containing about 3000 species in 350 genera, including a large number of edible plants, such as *B. oleracea* (i.e. cabbage, cauliflower, broccoli, Brussels sprouts), *B. rapa* and *B. napus* (including Chinese cabbage and rape), and other genera including radish and cress. The main cruciferous vegetables used in the human diet are listed in Table 7.1.

Generally, the terms *Cruciferae* or *Cruciferaceae* refer to the so-called "cabbage family" or "mustard family", as the latin term "*Brassica"* means cabbage. The petals of the *Brassicaceae* family present a peculiar cruciform arrangement, from which it derives its name. These plants can be annuals, biennals or perennials. Due to their good adaption to mild temperatures ranging 16–18 °C, these plants can grow in temperate areas, during the cool season. In particular, *Brassicaceae* are cultivated in the Mediterranean region, Europe, North America and Southwest and Central Asia (National Resource Conservation Services 2020).

Table 7.1 Main Cruciferous vegetables used in the human diet

Genus	Species	Common name
Brassica	*B. oleracea*	Cauliflower, cabbage, Portuguese cabbage, white cabbage, turnip cabbage, red cabbage, savoy cabbage, Brussels sprouts, broccoli, curly kale, kale, Chinese kale
Brassica	*B. napus*	Rape, Swedish turnip, swede, canola, rutabaga, colza
Brassica	*B. rapa*	Turpin rape, Chinese cabbage
Brassica	*B. alba* *B. juncea* *B. nigra*	White mustard Indian mustard, spinach mustard, brown mustard Black mustard
Crambe	*C. abyssinica*	Crambe
Raphanus	*R. sativus*	Radish
Beta	*B. vulgaris flavescens*	Swiss chard
Armoracia	*A. rusticana*	Horseradish
Wasabia	*W. japonica*	Wasabi
Nasturtium	*N. officinalis*	Watercress
Eruca	*E. vesicaria*	Arugula, rocket, Italian cress
Lepidium	*L. sativum*	Cress, garden cress

2.2 *Chemistry of Bioactive Compounds*

Similar to other vegetables, Cruciferous vegetables are a good nutrients and bioactive compounds source, including fibre, folate, carotenoids and chlorophyll, which chemopreventive properties have been demonstrated. Among these, glucosinolates deserve to be discussed more in detail.

2.2.1 Glucosinolates

Glucosinolates are the most abundant phytochemicals contained in Cruciferous vegetables, and they are responsible for their pungent aroma and spicy taste (Drewnowski and Gomez-Carneros 2000). In plants, they derive from valine, alanine, leucine, phenylalanine, isoleucine, methionine, tryptophan and tyrosin (IARC 2004) with a complex biosynthetic process involving the formation of aldoximes as intermediate, the oxidation of the N-hydroxy-amino acids and the subsequent decarboxylation and glucosidation reactions of the thiol function. The so-produced glucosinolates may be involved in further transamination, condensation, isomerisation and decarboxylation reactions, resulting in the synthesis of different structures (Popolo et al. 2017). The chemical structures of the main cruciferous vegetables are represented in Fig. 7.1.

Glucosinolates are also subject to hydrolytic processes operated by such enzymes, mainly myrosinases, leading to the formation of further bioactive compounds, including indoles and isothiocyanates (Holst and Williamson 2004). In particular, myrosinase is a β-thioglucosidase catalysing the hydrolysis of the S-glucoside giving an aglycone and the D-glucose; the aglycone, in turn, rearranges giving sufate and other compounds, such as nitriles, isothiocyanates, thiocyanates and

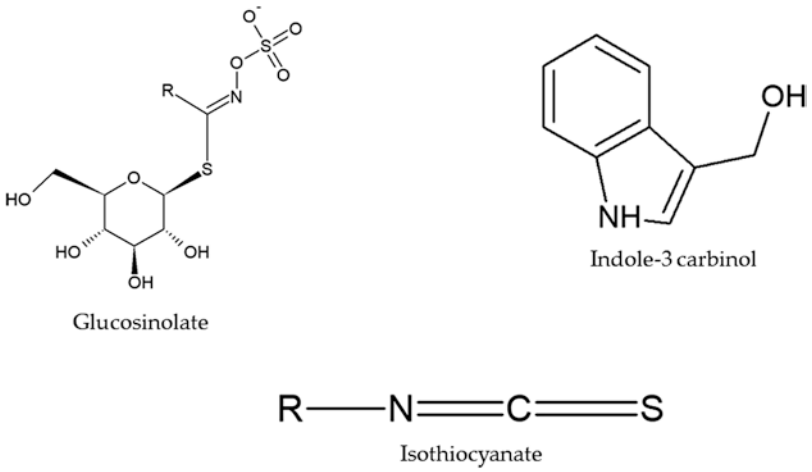

Fig. 7.1 Chemical structures of Cruciferous vegetables bioactive compounds

epithionitriles (Hayes et al. 2008). Among these, one of the most widely distributed glucosinolates is the indole-3-ylmethylglucosinolate, starting from which both indole-3-acetonitrile and indole-3-carbinol (I3C) are produced. Although these reactions occur spontaneously, glucosinolates and myrosinases are naturally stored in different compartments of the plant cell, and they can meet only when the cell structure is ruptured. This physical separation is an evolutionary adaptation aimed to preserve the plant integrity protecting from cell damages, since the glucosinolate breakdown is toxic to both herbivores and pathogens (Katz et al. 2018).

Interestingly, the glucosinolate profile may be severely affected by such cooking procedures (Palermo et al. 2014), including boiling that inactivates the myrosinases (Campbell and Slominski 1990). Moreover, since most glucosinolates are thermally stable and the myrosinases can be inactivated by high temperature, the formation of isothiocyanates and indoles might be avoided by such cooking procedures; however, both chopping and chewing cause a cell rupture leading glucosinolates and myrosinases come in contact (Higdon et al. 2007). In this context, it should be taken into account that glucosinolates are water-soluble, thus they may be lost into the cooking water. 9–15 min-boiling process of cruciferous vegetables, indeed, causes a total glucosinolates content decrease around the 18–59% (McNaughton and Marks 2003); in this sense, the use of such cooking methods including steaming or microwaving should be encouraged in order to reduce the glucosinolate losses (Higdon et al. 2007).

More than a hundred glucosinolates have been identified in plants, leading to unique hydrolysis products. As examples, broccoli is a rich source of glucoraphanin and glucobrassicin, which are precursors of sulforaphane and I3C, respectively. On the other hand, watercress is a source of gluconasturtiin, the precursor of phenethyl isothiocyanate (Higdon et al. 2007; Popolo et al. 2017).

2.3 Metabolism of Bioactive Compounds

Once ingested throughout the diet, cruciferous vegetable-derived bioactive compounds follow the physiological gastrointestinal digestion, thus they undergo several physic-chemical and biochemical conditions naturally occurring during these processes, which may severely affect both their chemical features and absorption rate. Overall, the metabolic fate of these compounds can be summarised as following described at different levels:

- Stomach: at the gastric acid pH, glucosinolate-derived products can combine with each other forming complexes of bioactive compounds, also known as acid condensation products (Shertzer and Senft 2000), including dimer 3,3′-diindolylmethane and a cyclic trimer, which biological activities differ from those of glucosinolate-derived products (Bjeldanes et al. 1991; Anderton et al. 2004a)
- Upper intestine: at the intestinal neural pH, the major glucosinolate hydrolysis products are stable isothiocyanates; in contrast, β-hydroxy-isothiocyanates are

unstable and undergo spontaneous processes leading to the formation of further compounds, including oxazolidine-2-thiones or alcohols (Higdon et al. 2007)
- Lower intestine: the enzymatic activities of the human microbiota may contribute to further metabolise glucosinolates, forming hydrolysis products (Shapiro et al. 1998).

Additionally, the main pharmacokinetic features may be summarised with the so-called ADMET scheme (abbreviation for absorption, distribution, metabolism, excretion and toxicity).

- A – absorption: glucosinolate-derived products are quickly absorbed; isothiocyanates can be absorbed by both upper and lower intestine (Popolo et al. 2017)
- D – distribution: after the absorption, glucosinolate-derived products are distributed in high-vascularised tissues, such as heart, kidney, lung, liver and brain (Anderton et al. 2004b)
- M – metabolism: after the intestinal absorption, glucosinolate-derived products undergo complex metabolic processes. Firstly, isothiocyanates are conjugated to glutathione, forming complexes that are further metabolized to mercapturic acid through the activities of various enzymes, including γ-glutamyltranspeptidase, cysteinylglycinase and N-acetyltransferase (Higdon et al. 2007)
- E – excretion: glucosinolates and their hydrolysis products have a low half-life, due to the quick renal clearance, the rapid inactivation, the low molecular weight and the low water solubility. However, glucosinolate-derived metabolites have been detected in urine (Popolo et al. 2017)
- T – toxicity: at high dose may be laxative. As a major concern, prolonged exposure to elevated amounts of glucosinolates may cause cytotoxicity and genotoxicity, resulting in dysfunctions at renal, thyroid gland, liver and pancreas level (Stoewsand 1995; Kassie et al. 1999; Holst and Williamson 2004). These toxic doses, however, are not reachable with the dietary consumption.

3 Cruciferous Vegetables and Cancer

Over the years, cruciferous vegetables gained great interest by the scientific research due to their potential chemopreventive effect. In this sense, one of the main challenges in investigating the relationship existing between the consumption of cruciferous vegetable and the risk of cancer in humans is related to the need to separate the benefits deriving by a general vegetable rich diet from those deriving directly by those rich in cruciferous vegetables (McNaughton and Marks 2003). However, evidence reports that the regular cruciferous vegetable consumption is linked to reduced rates of incidence of cancer (IARC 2004). In particular, epidemiologic studies published in the last century evidenced an inverse association between the consumption of some type of cruciferous vegetables and the risk of developing such types of cancer (Verhoeven et al. 1996), including lung, digestive tract, breast and prostate cancers (Higdon et al. 2007; Popolo et al. 2017). In general, these chemopreventive

Table 7.2 Evidence reporting the role of Cruciferous vegetable in cancer

Type of cancer	Main observations	References
Lung cancer	People with diagnosis of lung cancer consume significantly lower amounts of cruciferous vegetables than cancer-free people	Verhoeven et al. (1996)
	High intakes of cruciferous vegetables (> 3 servings weekly) is associated with reduced lung cancer risk	Feskanich et al. (2000), Voorrips et al. (2000a), Neuhouser et al. (2003)
Colorectal cancer	People with diagnosis of colorectal cancer consume significantly lower amounts of cruciferous vegetables than cancer-free people	Graham et al. (1978), Young and Wolf (1988), West et al. (1989), Benito et al. (1990)
	High consumption of cruciferous vegetables may decrease the colorectal cancer risk *via* increasing the urinary excretion of potential carcinogens, such as 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine and related dietary heterocyclic amines	Walters et al. (2004)
	People consuming on average 58 g of cruciferous vegetable daily are less prone to develop colorectal cancer compared to people consuming on average 11 g of these vegetables daily	Voorrips et al. (2000b)
Breast cancer	Women with diagnosis of breast cancer consume significantly lower amounts of cruciferous vegetables than cancer-free women	Terry et al. (2001); Fowke et al. (2003), Ambrosone et al. (2004)
	Four-week intervention with increased consumption of cruciferous vegetables in healthy postmenopausal women increased the urinary 2-hydroxyestrone/16α-hydroxyestrone ratio, suggesting the ability of these vegetables to promote the estrogen metabolism shift	Bradlow et al. (1996)
Prostate cancer	Men with diagnosis of prostate cancer consume significantly lower amounts of cruciferous vegetables than cancer-free men	Jain et al. (1999), Cohen et al. (2000), Kolonel et al. (2000), Joseph et al. (2004)
	Significant inverse association between the intake of cruciferous vegetables and the prostate cancer risk in men having a prostate specific antigen test	Giovannucci et al. (2003)
Pancreas cancer	Cabbage consumption (≥1 servings weekly) is associated with a significantly lower pancreatic cancer risk	Larsson et al. (2006)

effects are attributable to the phytochemicals contained in this class of vegetables, acting *via* different mechanism of action, as will be discussed in the next section. In Table 7.2 the main evidences reporting the association between the consumption of cruciferous vegetables and cancer risk are summarised.

In addition to these studies investigating the effect of cruciferous consumption on the cancer risk, interesting evidence reported the existence of such human genetic

differences that may play a role in modulating the chemopreventing potential of these vegetables (Lampe and Peterson 2002). In particular, genetic polymorphisms affecting the enzymatic activities of glutathione S-transferases (GST; a family of enzymes metabolizing various compounds, including isothiocyanates, resulting in enhancing their excretion) may severely affect the metabolism of glucosinolate-deriving products. More specifically, homozygous individuals for the null variants of the GSTM1 and GSTT1 genes are not able to produce the GSTs (Coles and Kadlubar 2003), resulting in reduced elimination and increased exposure to isothiocyanates following the consumption of cruciferous vegetables (Seow et al. 1998). In this context, studies reported that in GSTM1-null and/or GSTT1-null individuals the inverse association between cruciferous vegetable-derived isothiocyanate intake and the risk of lung (Lewis et al. 2001; Spitz et al. 2000; London et al. 2000; Zhao et al. 2001) or colorectal cancer (Slattery et al. 2000; Seow et al. 2002; Turner et al. 2004) was more pronounced, suggesting that these genetic polymorphisms may enhance the chemoprotective effects of cruciferous vegetables *via* slowing the elimination rate of the bioactive compounds.

4 Main Putative Anticancer Mechanisms of Action

As aforementioned, there is evidence suggesting the chemompreventive potential of cruciferous vegetable-deriving bioactive compounds. In the following section we will discuss the main putative mechanisms of action related to this effect. In general, the anticancer activity of this class of phytochemicals is exerted through various signalling pathways involving hormone regulation, DNA repairing, cell division and growth, angiogenesis, apoptosis and inflammation (Wang and Jiang 2012), that are altered in cancer cells (Popolo et al. 2017).

4.1 Cell Cycle Arrest

The cell cycle consists of a complex process in which cells go from a resting phase (G0) to a subsequent proliferation, following distinct phases named G1, S, G2 and M. Cyclins are key proteins regulating the progression toward the single phases of the cell cycle. Cyclins may form complexes with cyclin-dependent kinases (CDKs), that are inhibited by specific enzymes, including p21 and p27. This mechanism that control the cell proliferation is deregulated in cancer cells (Diaz-Moralli et al. 2013). It has been demonstrated that isothiocyanates may modulate the expression levels of both cyclins and CDKs and up-regulate the p21 and p27 expression, resulting in induced cell cycle arrest. This effect has been reported in various studies conducted on different cancer cell lines, including human colon carcinoma, cervical cancer cells, bladder cancer cells, oral carcinoma cells, prostate cancer cells and human pancreatic cancer cells (Watson et al. 2013; Mitsiogianni et al. 2019).

4.2 Apoptosis

Apoptosis is a process inducing a programmed cell death that, in physiological conditions, is finely regulated, contributing to the maintenance of the normal cell number. Differently, in cancer cells the ability to respond to apoptosis signals is lost and cells proliferate rapidly and constantly. The pro-apoptotic effect of cruciferous vegetable-deriving phytochemicals, mainly I3C, was investigated in different cancer cell lines, including prostate, cervical and breast cancer cells, reporting that these compounds act at different levels, including (a) shifting in the expression levels of Bax and Bcl2 toward a ratio promoting the cell death, (b) PARP cleavage, (c) DNA laddering and (d) decreasing the nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) activation (Higdon et al. 2007; Watson et al. 2013).

4.3 Signalling Pathways Involving Akt

One of the most frequent targets of sporadic cancers is the phosphatidylinositol 3-kinases (PI3K)/Akt/mammalian target of rapamycin (mTOR) signalling pathway (Ahmad et al. 2013). PI3Ks are lipid kinases that phophorylate various targets, including phosphatidylinositol 4,5-bisphosphate (PIP2), leading to the formation of phosphatidylinositol 3,4,5-trisphosphate (PIP3), a second messenger involved in crucial cell pathways (Zhao et al. 2017). PIP3 binds to further kinases, including Akt that in turn, once activated, regulates various cell processes, such as progression of the cell cycle, promotion of cell survival and cell growth by phosphorylating the mTOR complex 1 (Popolo et al. 2017). One of the crucial targets of Akt is NF-κB, which role in cancer development is well-established. In particular, NF-κB represents a link between inflammatory status and cancer, regulating the expression of genes involved in inhibition of the apoptotic processes and cell cycle progression, resulting in promoting angiogenesis and metastasis (Karin 2006; Hussain and Harris 2007). I3C was reported to be able to block the Akt/NF-κB signalling pathways, resulting in exerting an anticancer effect *via* inhibition of (a) progression of the cell cycle, (b) survival and (c) metastasis; these effects have been demonstrated in different cell lines, including B-cell precursor acute lymphoblastic leukemia and lung cancer cells (Popolo et al. 2017). In particular, the modulation of the NF-κB activation mediated by I3C results in the inhibition of the expression of both proliferative and anti-apoptotic genes, including c-Myc, IAP1, Bcl-xL, Bcl-2 and XIAP (Safa et al. 2015). Moreover, it has been reported that in Hep-2 cells, I3C affect the expression of key proteins involved in the PIP3/Akt signalling pathway, including PI3K p110α, PI3K p110β, PI3K class III, GSK3-β, p-PDK1, Akt, p-Akt and p-c-Raf (Popolo et al. 2017). Similarly, in breast and prostate cancer cells, the loss in the activity of Akt mediated by I3C resulted in alteration of various growth factors, including FOXO3a (Rahman et al. 2004). Furthermore, I3C was shown to inhibit the prostate cancer cell proliferation down-regulating the IGF1/Akt signalling pathway (Li et al. 2014).

4.4 AHR Signalling Pathway

The aryl hydrocarbon receptor (AHR) is a transcription factor regulating the expression of genes involved in various processes, including inflammation, detoxification and cancer. More specifically, acting as a xenobiotic chemical sensor, AHR binds to xenobiotic responsive elements in the promoters of targeted genes, regulating their expression. It has been demonstrated that indole derivatives are able to modulate the AHR activity (Popolo et al. 2017). In particular, I3C inhibits the binding between AHR and COX-2 promoter, which over-expression plays a crucial role in the development of various cancers, including mammary (Banerjee et al. 2002) and esophangeal cancers (Li et al. 2002). Moreover, the chemopreventive effects mediated by the interaction between I3C and AHR has been also reported in *in vitro* studies on colorectal cancer (Bonnesen et al. 2001; Frydoonfar et al. 2002; Hudson et al. 2002; Lee et al. 2005; Neave et al. 2005; Díaz-Díaz et al. 2016; Megna et al. 2016). Further evidence reported the capability of I3C to down-regulate the expression of the estrogen receptors (ER)-α (that is involved in promoting the cell proliferation) and activate the ER-β (that is associated with reduced cellular proliferation) (Firestone and Sundar 2009).

4.5 Carcinogen Metabolising Enzymes

Metabolising enzymes, including phase I and phase II enzymes, play a pivotal role in cancer development. Among the phase I enzymes, there is the cytochrome p450 (CYP450), while among the phase II enzymes there are GST, quinone reductase (QR), and NAD(P)H quinone oxidoreductase-1 (NQO1) (Leone et al. 2017). Phase I enzymes increase the hydrophilicity of carcinogenic compounds *via* various reactions (i.e. oxidation, reduction, hydrolysis), resulting in increasing their reactivity and the consequent DNA damage. On the other hand, phase II enzymes cause the reactive intermediates conjugation, promoting their urinary excretion through the mercapturic acid pathway (Talalay and Fahey 2001; Keum et al. 2009). It has been reported that cruciferous vegetable-deriving bioactive compounds are able to inhibit the activity of phase I enzymes and activate the phase II enzymes; in particular, this activation seems to be exerted through increased transcription of these enzymes (Abbaoui et al., 2018).

4.6 Epigenetic Effects

Besides the aforementioned effects of both indoles and isothiocyanates in signalling pathways directly involved in cancer development, evidence suggests a role of these phytochemicals in epigenetic processes, in particular, altering the epigenetic

modulators expression, such as enzymes controlling histone methylation and microRNAs. Human *in vivo* studies, indeed, demonstrated that the supplementation with idole-deriving products increased the expression of Let family microRNA and decreased the expression of the histone methyltransferase EZH2; moreover, these compounds may restore the expression levels of the silenced miR-34a, that is recognized as a regulator of cancer suppression (Watson et al. 2013).

5 Conclusion

Cruciferous vegetables may be considered as good sources of bioactive compounds which chemopreventive effect has been widely reported and the mechanisms of action well-described, as schematically summarised in Fig. 7.2. Overall, this evidence supports the observational data showing the direct association between reduced cancer risk and regular consumption of this kind of vegetables reported in various studies. Such promising, this evidence may serve to drive the scientific research in the nutraceutical field toward the further investigation of this beneficial potential of Cruciferous vegetable-derived bioactive compounds, formulating proper nutraceuticals aimed to prevent the risk to develop various cancers. This in turn, may be a start point for designing animal-based studies and clinical trials aimed to substantiate these effects *in vivo*.

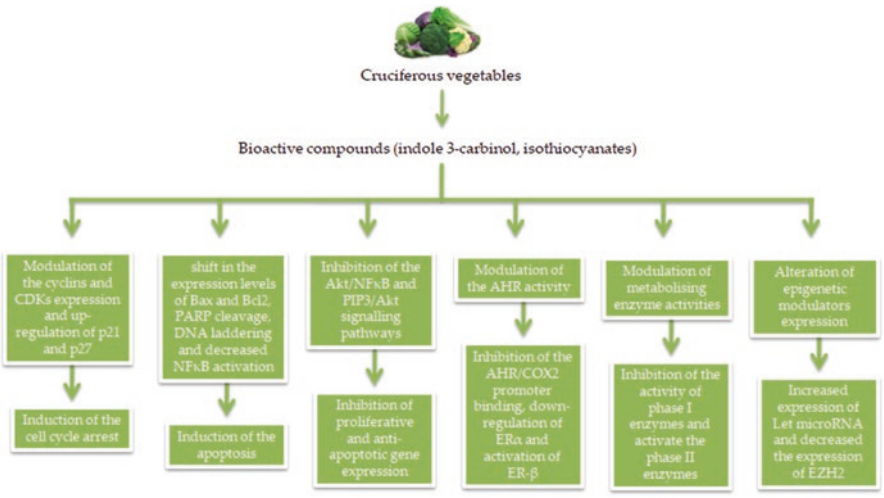

Fig. 7.2 Main mechanisms of action for the anticancer activity of Cruciferous phytochemicals

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Chapter 8
Crustacea (Carotenoids Namely Astaxanthins) Against Cancer

Renald Blundell, Jean Claude Grech, and Muhammad Ajmal Shah

Abstract In this chapter, we are putting our focus on biologically active compounds that are taken from natural resources, specifically compounds that act on molecular targets and are involved in several diseases in the human body. One of the most well-known biologically active compounds is astaxanthin, which is a xanthophyll carotenoid found in *Haematococcus pluvialis*, *Chlorella zofingiensis*, Chlorococcum and *Phaffia rhodozyma*. Astaxanthin has shown to provide a wide range of beneficial health benefits on the metabolism and in several organ systems of the human body including cardiovascular diseases, neurological disorders, endocrine diseases, ophthalmic diseases, rheumatological diseases, dermatological diseases, immunological diseases, nephrological disorders and obstetrics and gynaecological conditions, including pre-eclampsia and fertility. Additionally, astaxanthin has shown to provide a comprehensive set of activities which are beneficial to the human body such as anti-cancer activity, anti-inflammatory activity, anti-apoptotic activity, anti-oxidant activity and anti-cancer activity. Moreover, astaxanthin is found to be effective in enhancing sports performance during physical activity and therapeutic for the smoking population due to the high anti-oxidant activity found in astaxanthin.

Keywords Carotenoids · Astaxanthin · Cancer · Mechanisms · Apoptosis

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Abbreviations

$HgCl_2$	Mercuric chloride
ISP	Isoprostanes
MAPK	Mitogen Activated Protein Kinase
MDA	Malondialdehyde
MG	Milligrams
MMP	Matrix Metalloproteinases
NF-Kb	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric Oxide
PE	Preeclampsia
PI3K	Phosphoinositide 3-kinases
PI3K/AKT	Phosphoinositide 3-kinases/Protein kinase B
PPARγ	Peroxisome Proliferator-Activated Receptor Gamma
T2DM	Type 2 Diabetes mellitus
TAC	Tacrolimus
VLDL	Very low density lipoprotein
WHO	World Health Organization

1 Introduction

Dating back to the beginning of the twentieth century, astaxanthin was discovered in lobsters by Richard Kuhn (Kuhn and Soerensen 1938). Later in the twentieth century, many studies have been conducted and astaxanthin was approved to be a supplement for food ("CFR - Code of Federal Regulations Title 21"). In fact, in 1987, the United States Food and Drug Administration (US FDA) gave its approval to astaxanthin to be used as a feed additive in the aquaculture industry. In addition, in 1999, astaxanthin was further given the approval by US FDA to be used also in dietary supplementations (Guerin et al. 2003). The criteria for the approval was based on the properties that astaxanthin had exhibited throughout research. In fact, astaxanthin exhibited several anti-oxidative effects and a number of health benefits to human health especially in the skin, eye, cardiovascular and brain health and also in the prevention of cancer and diabetes mellitus (Fakhri et al. 2018).

Carotenoids are divided in two main categories including carotenes and xanthophylls. Xanthophylls are the derivatives of the hydrocarbons and are the oxygenated carotenoids while carotenes are the hydrocarbons carotenoids (Moran et al. 2018; Higuera-Ciapara et al. 2006) (Fig. 8.1). Astaxanthin is a xanthophyll and possesses an elongated molecular structure with a polar region at one or other ends of the ionone rings which gives astaxanthin the ability to neutralize free radicals. Additionally, in the middle of the molecular structure of astaxanthin, there is a non

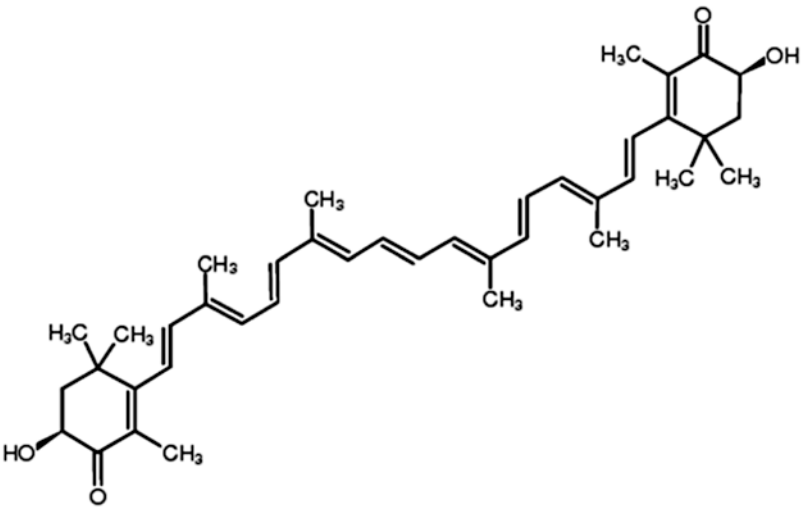

Fig. 8.1 Chemical Structure of Astaxanthin; (Taken from: Zhang, L., & Wang, H. (2015, July 1). Multiple mechanisms of anti-cancer effects exerted by astaxanthin. Marine Drugs, Vol. 13, pp. 4310–4330. https://doi.org/10.3390/md13074310)

polar zone made up of sequence of conjugated carbon-carbon double bonds. Astaxanthin possesses of a molecular structure that resemble that of a beta carotene. However, one of the major differences between them is that astaxanthin and beta carotene consists of 13 and 11 conjugated double polyunsaturated bonds respectively. This distinctive feature of astaxanthin gives it a unique molecular structure, chemical properties and light absorption properties (Higuera-Ciapara et al. 2006).

Astaxanthin is a lipid soluble pigment that is chemically synthesized by several microalgae, yeasts and bacteria. Evidence has shown that the microalga *Haematococcus pluvialis*, is the main source for the biological production of Astaxanthin (Davinelli et al. 2018). The bulk amount of Astaxanthin in microalgae is produced under stress conditions such as nitrogen deficiency, elevated temperatures and salinity. Astaxanthin is found everywhere in nature specifically in the marine environment such as in algae and cause the red or pink pigment to be present in many aquatic animals including trout, lobster, salmon, shrimp, asteroidean, crustacean and red sea bream (Davinelli et al. 2018). According to Bjerkeng et al. (2007), Ambati et al. (2014), Satoh et al. (2009), Astaxanthin is also found in yeasts such as *Xanthophyllomyces dendrorhous*, plants, few quantities in fungi, Chlorococcum species, *Chlorella zofingiensis* and in marine bacteria including *Agrobacterium aurantiacum* (Yuan et al. 2002, 2011).

2 Chemistry of Astaxanthin

Astaxanthins that are free are usually susceptible to oxidation (Hussein et al. 2006). This means that in nature, to contribute for the formation of monoester and diester forms, astaxanthin, can be either conjugated with proteins or esterified with one or two fatty acids (Hussein et al. 2006; Peng et al. 2008). According to Yuan and Chen (2000), astaxanthins are found as several astaxanthin esters in *Haematococcus pluvialis* and these are formed when fatty acids are combined with different isomers of astaxanthin. Hussein et al. (2006), established that several isomers of astaxanthin are characterized according to their configuration of the two hydroxyl groups on the molecule. The fact that each molecule consists of two chiral centers in C-3 and C-30, astaxanthin may have three configurational isomers, two enantiomers (3R, 30 R and 3S, 30 S) and a meso form (3R, 30 S). However, the main form present in *Haematococcus pluvialis* is the stereoisomer 3S and 30S.

According to Yuan and Chen (2000), the composition and profile of astaxanthin were different for different algal strains. However, Peng et al. (2008) found that the green microalga *Chlorella zofingiensis* had significantly higher astaxanthin diesters when compared to the *Haematococcus pluvialis* with a higher quantity of astaxanthin monoesters. In the alga Chlorococcum cells, the major carotenoids were astaxanthin, adonixanthin and free canthaxanthin (Yuan et al. 2002). However, in *Chlorella zofingiensis*, astaxanthin and canthaxanthin were the major carotenoids (Rise et al. 1994). In *Haematococcus pluvialis*, astaxanthin alone was the major carotenoid (Yuan and Chen 2000). According to Boussiba et al. (1999) it is found that esterified astaxanthin occupies more than 99% of the total carotenoids. The esterified hydroxyl groups of astaxanthin are found to increase its hydrophobicity and its solubility within the globules made from triacylglycerols. The fatty acid structure of the astaxanthin esters is relatively close to the fatty acid structure of triacylglycerols. They include mostly of oleic (C18:1), linoleic (C18:2) and palmitic acid (C16:0), in which oleic acid includes 51% of the fatty acids of the esters of astaxanthin (Zhekisheva et al. 2002). According to Miao et al. (2006), it is indicated that the main astaxanthin monoester and diester in *Haematococcus pluvialis* were the astaxanthin C18:1 and astaxanthin C16:1/C18:1 respectively. According to Zhekisheva et al. (2002), it is indicated that the accumulation of astaxanthin is accompanied by that of oleate-rich triacylglycerols. However, the capability to fit the structure of the esters of astaxanthin with that of triacylglycerols, is one of the main reasons why *Haematococcus pluvialis* is considered the most natural source of astaxanthin.

3 Isomerization of *Trans*-astaxanthin

The double bond from the polyene chain, within the astaxanthin molecule can be found in two configurations as geometric isomers, either *cis* or *trans* (Higuera-Ciapara et al. 2006). According to Higuera-Ciapara et al. (2006), in nature, most of the carotenoids found are mainly all *trans*-isomers. Despite that astaxanthin is usually present as trans-astaxanthin esters of several fatty acids, the esters of cis astaxanthin are also detected in the extraction of algal pigment. Having said that, Yuan and Chen (2000) found that an ester of astaxanthin, involved in the strain production of microalga *Haematococcus pluvialis*, is found to possess 13.5 mg/g of *cis*-astaxanthins (26.9%) and 36.7 mg/g of *trans*-astaxanthin. However, the isomerization of *trans*-astaxanthin to *cis*-isomers, is investigated in several different organic solvents (Yuan and Chen 1999). Additionally, the organic solvents are crucial in determining the isomerization rate of *trans*-astaxanthin. Despite the correlative contents of 9-*cis*- and 13-*cis* astaxanthins formed during isomerization in different solvents, the main cis-isomer from *trans*-astaxanthin is the 13 cis astaxanthin. Moreover, according to Yuan and Chen (1999), it is found that high temperatures can promote the isomerization rate of trans-astaxanthin. Yuan and Chen (2001) and Bohn (2008), found that the isomerization of trans-astaxanthin is a reversible reaction because Yuan and Chen (1999), Yuan and Chen (2001), found that the isomerization of *trans*-astaxanthin to *cis*-isomers cannot be isomerized completely. Moreover, several studies have shown that the isomerization of *cis*-astaxanthins can produce *trans*-astaxanthin (Yuan and Chen 2001).

4 Bioavailability and Safety of Astaxanthin

The structure is crucial for the bioavailability of the carotenoids because Bohn (2008) found that generally, polar carotenoids such as free astaxanthin possess a higher bioavailability than species that are apolar such as beta carotene and lycopene. However, according to Ranga Rao et al. (2010), the astaxanthin that is extracted from *Haematococcus pluvialis* is found to have better bioavailability when compared to beta carotene and lutein extracted from *Spirulina platensis* and *Botryococcus braunii* respectively. Additionally, according to Bohn (2008), it is found that the accumulation of *cis*-astaxanthin, usually occurs in the blood plasma because of the shorter chain lengths (Bohn 2008). Bohn (2008) found that the esters of xanthophylls seemed to have low levels of bioavailability. However, several studies have shown that the esters of xanthophylls, to be absorbed in humans, need to be hydrolyzed in the small intestine (Sugawara et al. 2009). On the other hand, according to Sugawara et al. (2009), it is found that within the intestinal cells, the esterification of xanthophylls, such as astaxanthin, mediated by the enzyme activity, occurs at lower rate thus it is suggested that the enzymatic esterification of xanthophylls occurs after the absorption in the intestines. However, the esterified xanthophylls

are absorbed within the chylomicron specifically within the lipid core and transferred to various tissues such as the skin. Additionally, it is found that the intestinal cells are protected from the cytotoxic effects of xanthophylls due to the esterification of xanthophylls into highly non-polar products.

Moreover, according to Sugawara et al. (2009), it is clarified that the esterification of xanthophylls is suitable within the intestinal cells thus one can understand the absorption, metabolism and biological activity of the carotenoids. However, according to Ranga Rao et al. (2010), it is found that the high bioavailability of astaxanthin is influenced by the esters of astaxanthin present in the *Haematococcus pluvialis.*

5 Potential Health-Promoting Effects of Astaxanthin

Several studies have shown that the biological activities of carotenoids are due to their previously conversion to vitamin A. Following several studies, it is shown that carotenoids in the absence of provitamin A activity are active the same or more than beta carotene (B. P. Chew and Park 2004). Studies have shown that astaxanthin without pro vitamin A activity, had several beneficial effects to human health including its anti-cancer, anti-diabetic, anti-inflammatory activities, gastro, hepato, skin, cardiovascular, ocular and neuro protective effects (Chew and Park 2004; Palozza et al. 2009).

5.1 Anti-inflammatory Activity

According to Turrin and Rivest (2006), inflammation is a series of immune responses that are initiated when the body suffers from an injury in order to begin the process of tissue repair. It is found that significant amount and not controlled inflammation can cause injury to the host and can damage cells and tissues of the host (G. C. Brown and Neher 2010). According to Lucas et al. (2006), the process of inflammation is significant to an acute and chronic neurodegenerative conditions. However the anti-inflammatory properties of astaxanthin, are able to prevent the progression of central nervous system disorders. According to Speranza et al. (2012) the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) dependent signaling pathway is blocked by astaxanthin because it works by anticipating the expression of genes of the inflammatory mediators of interleukin-1β, interleukin-6 and tumor necrosis factor-α. Under normal conditions, NF-κB dependent signaling pathway is not active in the cytosol because of the interaction with the inhibitory kappa b (Ghosh et al. 1998). However, phosphorylation of IκB by IκB kinase is precipitated by the stimulation of nuclear factor kappa-light-chain-enhancer of activated B cells. Moreover, according to Poyet et al. (2000), stimulation through the ubiquitin proteasome pathway, results in degradation of IκB and this causes the dissociation of

IκB from NF-κB while exposing the nuclear localization signal on the NF-kB. Evidence has shown that astaxanthin causes a significant reduction of the inflammation in tissues and organs (Ohgami et al. 2003). According to Wu et al. (2015) and Choi et al. (2008), inflammatory diseases such as arteriosclerosis, sepsis, rheumatoid arthritis, inflammatory bowel disease, gastric and brain inflammation are ameliorated by astaxanthin.

5.2 Anti-apoptotic Activity

Certain diseases such as neurodegenerative diseases such as ischemic stroke, heart disease, sepsis and multiple organ dysfunction syndromes are associated with excessive apoptosis. Depending on the pathological condition, it is found that Astaxanthin acquires both anti-apoptotic and pro-apoptotic effects (Kam and Ferch 2000). There are two major apoptotic pathways these are the intrinsic (mitochondrial pathway) and extrinsic (death receptor pathway) (Elmore 2007). However, according to Zhang and Wang (2015), Astaxanthin is found to modify apoptotic proteins thus preventing the diseases. Research has shown that astaxanthin stimulates the phosphorylation of BCL2 antagonist of cell death while also down regulating the activation of cytochrome c and caspase 3 and 9. However this is done through the regulation of mitogen-activated protein kinase 38 (MAPK p38). Moreover, astaxanthin is found to activate the Phosphoinositide 3-kinases/Protein kinase B (PI3K/AKT) survival pathway thus ameliorating the intrinsic mitochondrial pathway (H. Wu et al. 2015).

5.3 Antioxidant Activity

According to Ambati et al. (2014) and Edge et al. (1997), accumulation of oxidative molecules can cause a reaction with lipids, DNA and proteins and this can cause oxidation of lipids, proteins and damage to the DNA. The key mediator in the pathology of diseases is oxidative stress and is induced when there is disturbance in the equilibrium status of the pro-oxidant and anti-oxidant reactions in cells. The production of free radicals and reactive oxygen species is increased by oxidative stress (Valko et al. 2007; Wu et al. 2015). However, astaxanthin is found to work against oxidative damage by removing radicals to avert chain reactions, enhancing the immune system, neutralizing singlet oxygen, regulating expression of genes and preserving the structural membrane by inhibiting lipid peroxidation (Kamath et al. 2008; Rao et al. 2013). In fact, the polyene chain of Astaxanthin take the radicals from the cell membrane, while the terminal ring of Astaxanthin collect radicals inside and outside of the cell membrane (Augusti et al. 2012). This ensures that astaxanthin has a high antioxidant ability (Baralic et al. 2015). According to

Yamashita (2015), the antioxidant effect of astaxanthin is more than 100 times greater than vitamin E and 550 times more able to neutralize singlet oxygen than vitamin E (Shimidzu et al. 1996). Additionally, the antioxidant effects of astaxanthin are age depended. In fact, the youth expressed more when compared to older persons due to higher activity of antioxidant enzymes in the brain.

5.4 Neuroprotective Activity

Astaxanthin is a fat soluble compound and can pass easily through the blood brain barrier. In fact, according to Ying et al. (2015), astaxanthin can be used as a part of the treatment for patients suffering from an acute and chronic neurological diseases. According to Ambati et al. (2014), Astaxanthin is more vulnerable to oxidative stress, when there is an elevated levels of metabolic activity in the brain. However, in different areas of the central nervous system, Astaxanthin is found to reduce oxidative stress markers while increasing the effects of antioxidant enzymes. Additionally, astaxanthin is discovered to reduce nitric oxide (NO), interleukin-1β, IL-6 and tissue necrosis factor alpha. Moreover, astaxanthin has been found to exhibit neuroprotective properties because of its anti-inflammatory, antioxidant and antiapoptotic mechanisms specifically when used against neurological disorders such as in neurodegenerative diseases such as Huntington's disease, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis and injuries related to dementia (Kidd 2011). This has shown that astaxanthin is a broad spectrum pharmacological therapy in the treatment of neurological diseases indicates that astaxanthin is a multi-target pharmacological therapy (Zhang et al. 2014).

5.5 Effect on Bone

According to Rahmati et al. (2016), the inflammatory cytokines such as matrix metallopeptidase (MMP) and NO are significant in the pathogenesis of osteoarthritis. It is found that under pathological conditions such as rheumatoid arthritis, osteoarthritis and osteoporosis Chen et al. (2014), all bone cells express MMP 1,3 and 13, the phosphorylation of two MAPK p38 and ERK1/2 and IL-1β stimulated chondrocytes. However, astaxanthin has been found to reduce the expression of these thus preventing the risk for rheumatoid arthritis, osteoarthritis and osteoporosis. Additionally, astaxanthin has been found also to decrease arthritis inflammation of chondrosarcoma cells in humans by enhancing the proliferation and differentiation ability of osteogenic cells in neural stem cells. This is done by activating the downstream mediators including PI3K, p-MEK, p-ERK, and pStat3 (Kimble et al. 2013).

5.6 *Effect on Immune System*

The immune system is made up of cells such as T-cells, B-cells, natural killer cells, neutrophils and monocytes or macrophages. These are very sensitive to an injury caused by free radicals. However, Astaxanthin is found to provide restoration of the immune system specifically the defense mechanism and protect the free radicals from causing an injury. According to Jyonouchi et al. (1995), Jyonouchi et al. (1996), the production of secretory cells of antibodies, T helper cells and immunoglobulin M,G and A, could be increased by astaxanthin in response to a stimuli from T cells. Additionally, astaxanthin is found to use natural killer cells and T lymphocytes to elevate the immune response, to reduce C-reactive protein and damage of the DNA (Lin et al. 2015).

5.7 *Effect on Skin Health*

According to Choi et al. (2011), astaxanthin is found to be beneficial to the skin because of its lipid solubility properties. According to Yoon et al. (2014), regular intake of astaxanthin is found to improve the facial skin of humans. According to Tominaga et al. (2012), skin conditions of both male or female are found to benefit from astaxanthin. Additionally, astaxanthin is discovered to prevent thickening of the skin and induce reduction in collagen when there is injury to the skin caused by ultra-violet A (Hama et al. 2012; Rao et al. 2013). Moreover, evidence has shown that astaxanthin is discovered also to reduce NO synthase, COX2 and inhibit the apoptosis of keratinocytes and this makes astaxanthin capable of protecting the person from inflammation that is caused by ultraviolet radiation (Yoshihisa et al. 2014). However, the upregulation of MMP and skin fibroblast elastase induced by ultraviolet radiation A are reduced by astaxanthin (Suganuma et al. 2010).

5.8 *Effect on Cardiovascular Health*

The key factors associated with many symptoms in cardiovascular diseases are oxidative stress and inflammation. According to Kishimoto et al. (2016), astaxanthin is found to enhance, metabolism of lipids and glucose, oxidative stress and inflammation. According to Palozza et al. (2008), the antioxidant activity of astaxanthin is capable to inhibit free radicals and the formation of 7-ketocholesterol while also prevent the formation of atheromatous plaque. Astaxanthin is also known to prevent the development and progression of atherosclerosis by inhibiting the oxidation of low density lipoproteins (Higuera-Ciapara et al. 2006).

5.9 *Effect on Sports Performance*

In adults male volunteers, astaxanthin is known to decrease the concentration of lactic acid after running 1200 m for 2 min (Brown et al. 2017). In fact Astaxanthin is capable of improving muscle fatigue and increase the performance in sports. Additionally, astaxanthin is discovered to decrease the level of creatine kinase, elevate diffusion of lactic acid endurance and improve muscle fatigue (Sawaki et al. 2004). The production of free radicals and oxidative stress is positively correlated with exercise and football training thus the efficiency of the antioxidant system is reduced. However, dietary intake of astaxanthin is capable of preventing the production of free radicals caused by exercise (Djordjevic et al. 2012).

In the sports sector, astaxanthin was found to be of benefit for weight trained individuals with a high percentage area for fiber types IIA and IIAB/B due to being able to reduce the sensations of delayed-onset muscular soreness in muscle damage caused by exercise (Fry et al. 2004). Aoi et al. (2008), were able to show that astaxanthin helped during training by switching to lipid metabolism rather than glucose utilization; this is done through palmitoyltransferase I activation, leading to the improvement in endurance and a significant reduction in adipose tissue during exercise. The ability of astaxanthin to reduce glucose utilization was also studied by Ikeuchi et al. (2006) who also showed that it is able to increase fatty acid utilization to serve as an energy source during exercise. In doing so, the saved glycogen is available for usage in later stages of exercise; thus improving the endurance and exercise performance as well as delaying fatigue (Ikeuchi et al. 2006).

5.10 *Effect on Blood Glucose Control and Type 2 Diabetes Mellitus*

Diabetes mellitus is strongly related with oxidative stress. Consequently, there is an increase in the production of free radicals, reduction of antioxidant defenses or both (Leite et al. 2010). In patients diagnosed with diabetes mellitus, oxidative stress is found to cause dysfunction of pancreatic b cells and tissue damage as a result of high blood glucose levels (Uchiyama et al. 2002). According to Uchiyama et al. (2002), astaxanthin is found to reduce oxidative stress caused by hyperglycaemia in the pancreatic beta cells, improve tolerance of glucose, elevate serum insulin levels while decreasing blood glucose levels (Uchiyama et al. 2002). Moreover, astaxanthin is found to protect pancreatic b cell against glucose toxicity by preventing destruction of pancreatic b cells (Uchiyama et al. 2002).

Type 2 diabetes mellitus (T2DM) is an adult onset chronic condition caused by an imbalance between the insulin supply and demand as a result of glucose and lipid abnormalities. According to Weyer et al. (2001), it is found that the sensitivity of insulin, involved in the metabolism of glucose and lipids, is affected by the deregulation of adipocytokines such as adiponectin, secreted from the adipose tissue.

According to Rafraf et al. (2015), the blood glucose level of patients diagnosed with non-insulin dependent diabetes mellitus, is controlled by nutritional and pharmacological therapy because of the increased risk of microvascular and macrovascular complications. In fact, antioxidants are used to prevent the risk of diabetic complications and to detain the development of diabetic disorders (Ceriello et al. 2016). However, astaxanthin is a potent antioxidant found in several microorganisms and seafoods (Ambati et al. 2014). According to Mashhadi et al. (2018), it is found that the visceral fat is reduced while the adiponectin concentrations are increased with the administration of 8mg of astaxanthin supplement. However, the results of adiponectin being inversely proportional with the visceral adipose tissue induced by astaxanthin, were consistent with the previous studies (Park et al. 2010; Asayama et al. 2003). Through the results demonstrated in Mashhadi et al. (2018), an inverse relationship between adiponectin and diabetes was noted. As already found in previous studies of Weyer et al. (2001) and Kahn et al. (2006), this relationship was determined through a significant reduction in fructosamine concentrations and a marginal reduction in fasting plasma glucose concentrations. Furthermore, Kadowaki et al. (2006) and (Goldberg et al. 2016), have also reported that, through molecular mechanisms, adiponectin is able to increase fatty acid oxidation in the muscles and reduce plasma glucose concentrations; in return, these stimulate the AMP activated protein kinase and activate PPARα in the liver and muscles.

According to Mashhadi et al. (2018), astaxanthin is discovered to reduce the concentration of the low density lipoproteins (VLDL) and triglycerides in participants with diabetes. Additionally, it is noticed that adiponectin is inversely associated with triglycerides and VLDL cholesterol (Mashhadi et al. 2018). The reduction in the serum triglycerides concentration is enhanced by the breakdown of VLDL through lipoprotein lipase and by the VLDL receptor expression which is related to improve insulin resistance (Kishimoto et al. 2016; Yoshida et al. 2010). Finally, the 8 mg of astaxanthin given to the participants for a time period of 8 weeks, indicated a significant elevation of adiponectin concentrations, reduction of the visceral body fat mass, serum triglycerides, VLDL cholesterol, fructosamine concentrations and systolic blood pressure (Mashhadi et al. 2018).

5.11 Anti-cancer Activity

The chemoprophylaxis agents can be divided in two classes. These are retinoids or provitamin A carotenoids and the non-provitamin A carotenoids which are acting on separate mechanisms (Bertram and Vine 2005). According to Palozza et al. (2009), carotenoids possess strong properties related to chemoprevention in treating cancer, and these are independent of their antioxidant activity or their potential to convert to retinoids. However, Bertram (2004), found that beta carotene failed to provide protection to individuals who are smokers or those who are continuously exposed to asbestos with the risk of developing lung pathology. Otherwise, astaxanthin was found to demonstrate protection without causing toxicity related with retinoids

(Bertram and Vine 2005). According to Chew and Park (2004) established that astaxanthin is found to exhibit the greatest ability to act as an anti-tumor agent when compared to canthaxanthin and beta carotene. Additionally, astaxanthin is found to have growth inhibitory effects and these were demonstrated in several tumor cells such as oral fibrosarcoma, prostate cancer, breast and colon cancer and embryonic fibroblasts (Palozza et al. 2009). According to Palozza et al. (2009), revealed that astaxanthin which is the extract of *Haematococcus pluvialis*, is found to impede the growth of HCT-116, HT-29, LS-174 and SW-480 human colon cancer cells, by arresting the progression of the cell cycle and enhancing apoptosis. A fatal malignancy of T lymphocytes known as the adult T cell leukemia, is caused by human t cell leukemia virus type 1 and remains untreated (Ishikawa et al. 2008). However, it is found that astaxanthin is found to be beneficial in this case because it possesses a mild inhibitory effect on human T cell leukemia virus type 1 (Ishikawa et al. 2008).

5.11.1 Astaxanthin and Its Mechanisms of Anti-cancer Activity

Astaxanthin consists of potent anti-oxidant activity that can be used in several types of human cancers such as lung, breast, leukemia, colon and hepatocellular carcinoma (Kavitha et al. 2013; Nagendraprabhu and Sudhandiran 2011; Zhang et al. 2011; Song et al. 2011, 2012). The anti-cancer activities enhanced by astaxanthin are associated to its activity on the pathology of cancer cells through various pathways such as inflammation, apoptosis, cell junction (Zhang and Wang 2015).

5.11.2 Anti-proliferation of Cells

The formation of tumor is identified when there is fast proliferation of cancer cells. When cancer proliferate, they promote invasion, migration and adherence to the target tissue (Zhang and Wang 2015). In fact, following these steps, the tumor cell will get a metastatic phenotype. The signal transmissions by growth factors and adhesive proteins determine proliferation of cells and is regulated by mitogen-activated protein kinase (MAPK) and phosphatidylinositide 3-kinases (PI3K) cascades (Xu et al. 2015; Liu et al. 2015). The rearrangement of the actin cytoskeleton allows proliferation, invasion, migration and adhesion to take place. In fact, this requires the formation of pre-existence cell matrix and new integrin substratum contacts (Lauffenburger and Horwitz 1996).

However, many researchers have been exploring the effectiveness of astaxanthin on the proliferation of cancer cells. In fact, Song et al. (Song et al. 2011, noticed the anti-proliferation activity of astaxanthin against CBRH-7919 (human hepatoma) and strong relationship was noticed between the concentration of astaxanthin and the anti-proliferation effect on these cells within 24 h. Among the cells, the most sensitive cell to astaxanthin was CBRH-7919 with an IC50 value of 39μM (Song et al. 2011).

However, in another study, the growth inhibitory effect of astaxanthin was compared with other carotenoids including β-carotene, capsanthin and bixin, on K562 leukemia cells. In addition, it is found that astaxanthin is the most effective to induce cell growth inhibition on K562 leukemia cells among the other four carotenoids. Astaxanthin was followed by bixin, B-carotene and capsanthin (Zhang and Wang 2015).

Additionally, astaxanthin is found to impede the proliferation of cell nuclear antigen (PCNA) and reduce the viability of cells in human HCT-116 colon cancer cells in a time and dose dependent manners (Palozza et al. 2009).

Moreover, it is found that when astaxanthin is used, normal cells are not affected or less effected than cancer cells hence indicating that astaxanthin activity is focused and targeted to the cancer cell (Zhang and Wang 2015). In fact, the proliferation of CBRH-7919, SHZ-88 and Lewis cell lines were significantly inhibited by astaxanthin while little effect was noted on HL-7702 that is a normal human hepatocyte line (Song et al. 2011).

5.11.3 Apoptosis

According to Lo et al. (2011) and Elmore (2007), the process of programmed cell death known as apoptosis, occur in several cellular organisms and consists of many cellular events such as nuclear fragmentation, chromosomal DNA fragmentation, cellular blebbing and lastly death of the cell. According to Zhang et al. (2005), the tumor volume would reduce if apoptosis occurs within the tumor cells, hence decreasing the burden of the tumor and increase life expectancy. In addition, Song et al. (2011), noticed that when cells are treated with astaxanthin, apoptosis was detected by flow cytometry as indicated through a significant peak of hypodiploid.

Additionally, astaxanthin is capable of changing the morphology of the mitochondria, respiratory chain, transmembrane potential and mitochondrial apoptotic proteins including B-cell lymphoma 2 and B cell lymphoma associated X protein (Zhang and Wang 2015). According to Song et al. (2012), it is found that the expression of B cell lymphoma 2, b cell lymphoma extra large and c-myc are reduced by astaxanthin while the levels of bax and non-metastasis 23-1nm in hepatocellular carcinoma cell line are elevated by astaxanthin. This indicates that astaxanthin has the ability to induce mitochondrial apoptosis within the cancer cells (Song et al. 2012).

Moreover, astaxanthin is capable to suppress 6-hydroxydopamine induced apoptosis and is capable to inhibit 6-hydroxydopamine induced mitochondrial dysfunctions by lowering membrane potential and by cleaving caspase 9, 3 and poly ADP ribose polymerase in human neuroblastoma cell line SH-SY5Y (Ikeda et al. 2008).

5.12 Anti-oxidation

Oxidative stress occurs because of the formation of reactive oxygen species and free radicals (Zhang and Wang 2015). The hallmark of various types of cancer is when there is an imbalance of the irregular reactive oxygen species production and function that leads to the progression of the tumor (Cairns et al. 2011) and (Glasauer and Chandel 2014). According to Cairns et al. (2011), the activation and maintenance of specific signaling pathways occur by second messengers known as reactive oxygen species and these have a crucial role for the cancer to start, progress and spread. The endogenous and exogenous anti-oxidant such as astaxanthin is crucial for the inhibition of oxidative molecules. According to (Franceschelli et al. (2014), astaxanthin is found to reduce the intracellular oxygen production in order to restore the anti-oxidant activity of superoxide dismutase and catalase. This is crucial to reverse the induction of toxicity by lipopolysaccharide and the production of reactive oxygen species in U937 cells. Moreover, astaxanthin is found to inhibit the proliferation of cells, induction of cell apoptosis and interfere with the progression of the cell cycle in leukemia K562 cells by activating Nrf2 interceded anti-oxidant pathway (Zhang et al. 2011).

5.13 Anti-inflammation

According to Balkwill and Mantovani (2001), the role of inflammation is crucial for cancer development and it was initially outlined by Rudolf Virchow in 1863. When the body encounters harmful stimuli, the body responds by inflammatory process which is characterized by an elevation in plasma levels and cells' ability to form pro-inflammatory cytokines including tumor necrosis factor-α, interleukin-6 and interleukin-1 (Franceschi et al. 2000; Franceschi 2007). According to Franceschi et al. (2007) and Cevenini et al. (2013), the onset of cancer is triggered by the interaction of the genetic background, environmental factors with pro-inflammatory cytokines. In fact, according to Karin (2006) and Mantovani et al. (2008), inflammatory diseases are found to trigger several cancers and anti-inflammatory drugs including aspirin or cyclooxygenase-2 (COX-2) inhibitors could decrease the recurrence of the tumor (Chia et al. 2012) and (Giraldo et al. 2014). Furthermore, according to Speranza et al. (2012) and Franceschelli et al. (2014), astaxanthin is found to inhibit the reactive oxygen species activation of nuclear factor-kB transcription factor in U937 cell line. As a result the production of inflammatory cytokines such as IL-1β, IL-6 and TNF-α are suppressed by restoring the physiological levels of SHP-1 (Speranza et al. 2012).

5.14 Invasion and Migration

According to Friedl and Wolf (2003), cancer development compromises two crucial processes, the invasion and migration. For malignant cells to break away from the primary tumor, bind to and break down proteins that form the surrounding extracellular matrix, there should be invasion of the surrounding tissue and metastasis (Nguyen and Massagué 2007).

Migration to other areas of the human body occurs when cancer cells leave the original tumor site via the bloodstream, lymphatic system and by direction extension (Guo and Giancotti 2004). At this stage, MMP have an important role. In fact, according to Lu et al. (2011), MMP's are zinc binding endopeptidases, in which migration and invasion of tumor cells are promoted by breaking down extracellular matrix. In cancer cells, MMP's are overexpressed while in cancer invasion, extracellular matrix is broken down thus astaxanthin is useful to decrease mRNA and levels of proteins called MMP's 2 and MMP's 9 (L. Zhang and Wang 2015). Moreover, astaxanthin is found to have inhibition effects on invasion and metastasis by increasing the protein levels of TIMP-1 and reversion-inducing-cysteine-rich protein with kazal motifs (RECK), that are the endogenous inhibitors of MMP (Zhang and Wang 2015).

5.15 Gap Junctional Intracellular Communication (GJIC)

According to Castellano and Eugenin (2014), Gap Junctional Intracellular Communication are formed by intracellular channels that allow the diffusion of small hydrophilic molecules from cytoplasm to cytoplasm leading to a electrical and metabolic coordination. According to Evans and Martin (2002), to achieve a common and integrated metabolic activity, the communication between cells of the organ is regulated by allowing direct communication between the cytoplasm of cells without moving through the extracellular space. According to Willecke et al. (2002), loss of Gap Junctional Intracellular Communication is associated with cellular damage, inflammation and cancer thus astaxanthin is useful to enhance Gap Junctional Intracellular Communication.

Additionally, the expression of connexin 43 protein, that allows better gap junction intercellular communication among cells in order to regulate the proliferation, differentiation and death of cells, is increased by astaxanthin (Hix et al. 2004). As a result, the formation of connexin 43 immunoreactive plaques through the plasma membrane increased in proportional to the increased gap junction intracellular communication hence this can inhibit neoplastic transformation of 10T1/2 cells in vitro while reducing tumors of humans in xenograft (Hix et al. 2004). Moreover, according to Hix et al. (2005), astaxanthin is found to upregulate gap junction intercellular communication and elevate the expression of connexin 43 protein by inhibiting methylcholanthrene which is required to induce neoplastic transformation.

Furthermore, according to Daubrawa et al. (2005), the gap junction intracellular communication is increased through the primary human fibroblast by astaxanthin.

5.16 Molecular Targets of Astaxanthin

5.16.1 NF-kB

The expression of genes involved in inflammation are positively regulated by Nuclear factor kappa (Karin and Greten 2005; Courtois and Gilmore 2006). According to Karin and Lin (2002) and Beinke and Ley (2004) the proliferation of cells can be controlled via NF-kB by inducing growth factors. In addition, NF-Kb can activate cyclin D1 and C-myc by regulating the progression of the cell cycle (Zhang and Wang 2015). According to Christiaens et al. (2008), the upregulation of chemokines such as vascular endothelial growth factor, IL-8 and matrix metalloproteases by the action of NF-kB can lead to an increase of metastasis and angiogenesis hence NF-kB is an important target in the treatment of cancer. In fact, NF-kB and Wnt signaling are inhibited by astaxanthin by reducing the regulatory enzymes including IKKβ and GSK-3β leading to mitochondria-mediated caspase apoptosis (Kavitha et al. 2013). Despite there are no full clarification regarding the mediators between astaxanthin and NF-kB, research has shown that there is the involvement of reactive oxygen species during the inactivation of NF-Kb hence astaxanthin through the reactive oxygen species can regulate NF-κB (Zhang and Wang 2015). Furthermore, astaxanthin targets NF-kB in the treatment of cancer (Fig. 8.2).

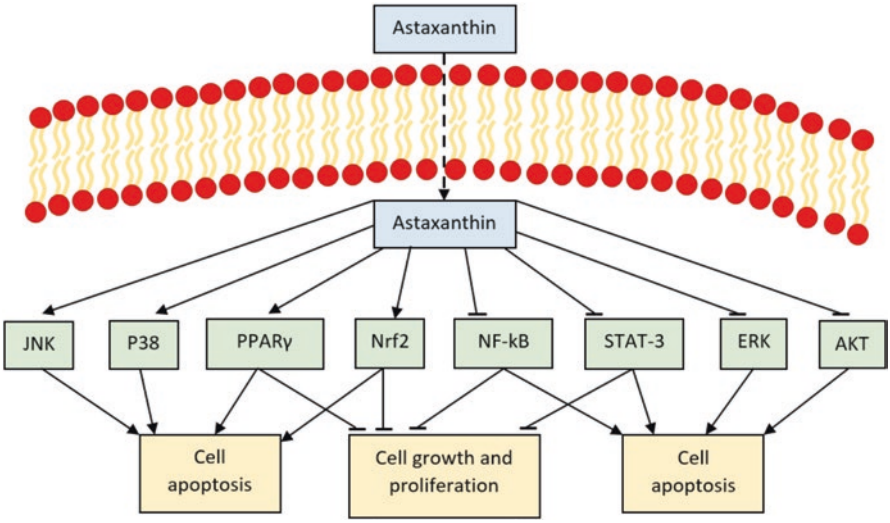

Fig. 8.2 Molecular targets of Astaxanthin

The target of astaxanthin to these molecular targets can induce apoptosis of cancer cells. Additionally, suppression of the proliferation and growth of cells occur by inhibiting NF-kB and STAT-3 and by activating PPAR and Nrf2 (Zhang and Wang 2015).

5.16.2 Janus Kinase/Signal Transducers and Activators of Transcription-3 (JAK/STAT-3)

According to Leonard and O'Shea (1998) and Schindler and Darnell (1995), the proliferation, invasion, differentiation of cells occurs by signaling through the JAK/STAT pathway. These can cause various pathological diseases including cancer O'Shea et al. (2013), renal diseases Chuang and He (2010), hepatic diseases (Mair et al. 2011). According to Xu et al. (2013), the family of STAT proteins consist of 7 members, these are STATs 1,2,3,4,5a,5b and 6. Among these, STAT-3 is usually activated in cancer cells such as multiple myeloma Zhu et al. (2015), prostate cancer Bosch-Barrera and Menendez (2015) and leukemia (Curran et al. 2015). The activation of STAT-3 occurs by phosphorylation through JAK, which is a crucial step within the apoptosis pathway.

Additionally, the crucial events during the signaling of JAK-2/STAT-3, particularly the phosphorylation and the nuclear translocation of STAT-3, are principally inhibited by astaxanthin (Zhang and Wang 2015). As a result, the development and progression of tumor is inhibited when there is down regulation of STAT-3 target genes, that are involved in the proliferation of cells (cyclin D1, PCNA), invasion and angiogenesis (MMP 2 and 9) and angiogenesis (VEGF, VEGFR2) (Zhang and Wang 2015). Moreover, according to Song et al. (2012), apoptotic proteins including b cell lymphoma 2, b cell lymphoma extra large, c-myc and Bax are regulated by astaxanthin by suppressing the signal of JAK-1/STAT-3 pathway.

5.16.3 PI3K/AKT

The most important signaling pathway to regulate the survival and death of cells is PI3K/AKT. The proliferation and apoptosis of cells is controlled by the signaling of this pathway (Owonikoko and Khuri 2013). The mammalian target of rapamycin (MTOR) can be activated by phosphorylation of AKT which in return enhances the transcription of mRNA's, upregulates the expression of proteins that are related to proliferation of cells and triggers the phosphorylation of the target p70S6K (Borders et al. 2010; Yap et al. 2008). According to Yothaisong et al. (2013), various types of cancers such as colorectal cancer, cholangiocarcinoma and breast cancer have been reported in response to deregulation the PI3K/AKT signaling pathway (Liao et al. 2013; Zheng et al. 2012; Yothaisong et al. 2013). In fact, astaxanthin is found to induce cell death by facilitating the PI3K/AKT signaling pathway. According to Kavitha et al. (2013), astaxanthin can be used for oral cancer, by reducing the phosphorylation of AKT, decreasing b cell lymphoma, p-bad and survivin while

increasing Bax, Bad and cleaved PARP hence causing significant apoptosis. Moreover, astaxanthin can be used for human colon cancer cells by inactivating AKT (Palozza et al. 2009).

5.16.4 MAPKs

According to Manning et al. (2002), MAPK are serine/threonine protein kinases and form part to the CDK/MAPK/GSK3/CLK (CMGC) kinase group. MAPK participate in the proliferation, apoptosis and differentiation of cells (Manning et al. 2002). In the MAPKs family there are three members, these are the c-Jun N-terminal kinase (JNK), extracellular regulated protein kinase (ERK) and p38, in which the ERK and JNK are the crucial to regulate death and survival of cells (Zhang and Wang 2015). However, astaxanthin is found to inhibit the growth of cells in human colorectal carcinoma in dose and time dependent manners by arresting the progression of the cell cycle while promoting apoptosis (Palozza et al. 2009). Furthermore, the phosphorylation of p38, JNK and ERK is increased by astaxanthin indicating that ERK can promote apoptosis (Palozza et al. 2009).

5.16.5 Peroxisome Proliferator-Activated Receptor Gamma (PPARγ)

Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) forms part of the nuclear hormone receptors family. According to Quintanilla et al. (2014), PPARγ is found within the vascular, brain and heart tissue hence is crucial in various types of cancers including colon, lung, breast, bladder, prostate and pancreas. Additionally, according to Sporn et al. (2001), the fact that the activation of PPARγ is found to inhibit the proliferation and growth of cells, makes PPARγ crucial target for prevention and treatment of cancer. Moreover, various vivo and vitro studies have shown actions of anti-proliferation and pro-apoptosis by PPARγ agonists including 15-deoxy-Δ-12,14-prostaglandin J2 (15dPG-J2) and thiazolidinediones (TZDs) Venkatachalam et al. (2011), Pignatelli et al. (2005), indicating that targeting PPARγ could be a solution in the treatment of cancer. In fact, according to Zhang et al. (2011), astaxanthin is found to inhibit proliferation, reduce viability, promote apoptosis and interfere the progression of leukemia K562 cells by elevating the expression of PPARγ.

5.16.6 NF-E2-Related Factor 2 (Nrf2)

Transcription factor Nrf2 is an essential vital regulator that regulates detoxifying enzymes in phase 2 and the expression of anti-oxidant gene within the cell. According to Dinkova-Kostova and Abramov (2015) and Sekhar and Freeman (2015) transcription factor Nrf2 is activated by oxidative and redox stress and consists of factors including heme oxygenase-1 (HO-1), NAD(P)H dehydrogenase

[quinone] 1 (NQO-1) and glutamate-cysteine ligase catalytic (GCLC), that leads to a reduction of reactive oxygen species. Various studies have demonstrated that cells can be protected from harmful stimulus, such as trauma, inflammation, haemorrhage and cancer in normal tissues by Nrf2 Dinkova-Kostova et al. (2005), Ramos-Gomez et al. (2000), Wang et al. (2007) thus when Nrf2 agent is given to humans it can alter the metabolism of the carcinogen (Osburn and Kensler 2008). In fact, according to Zhang et al. (2011), astaxanthin is found to increase the expression of Nrf2 which in return inhibits the proliferation of K562 leukemia cells, indicating that Nrf2 suppress the progression of cancer.

5.17 Effects on Kidney Function

Accumulated mainly in kidneys, inorganic mercury is known to induce acute renal failure by triggering reactive oxygen species which are the main cause of tissue damage. Augusti et al. (2008), studied the possible benefits of astaxanthin in preventing the negative effects caused by a certain compound of inorganic mercury known as mercuric chloride ($HgCl_2$); in their results, they found that astaxanthin may prevent lipid and protein oxidation, as well as change the activity of antioxidant enzymes and the histopathology of tissue; thus protecting against nephrotoxicity induced by $HgCl_2$ (Augusti et al. 2008).

5.18 Effects on Eye Health

According to Nakamura et al. (2004), the ingestion of 4 or 12 mg astaxanthin once a day for a period of 28 days, had significantly improved the visual acuity and shortened the accommodation time in healthy volunteers over the age of 40 years. However, the latter cannot be said for the pupillary reflex, refraction and the flicker fusion frequency. Additionally, it is found that extraction of astaxanthin from microalga *Haematococcus pluvialis*, had significantly improved the deep vision and the flicker fusion among healthy male adults (Sawaki et al. 2004). According to Nagaki et al. (2010), found that terminal workers with an eye fatigue in the visual display had improved after they are given 6mg of astaxanthin which is extracted from *Haematococcus pluvialis*. In addition, it is shown that astaxanthin had increased the blood flow in the capillaries of the retina in both eyes, but the latter cannot be said for intraocular pressure because remained unchanged (Hashimoto et al. 2016). According to Ohgami et al. (2003), it is found that astaxanthin demonstrated an ocular anti-inflammatory effect by preventing the endotoxin from causing uveitis. This is done by suppressing the production of nitric oxide, prostaglandin E2, and tumor necrosis factor a by directly blocking the activity of nitric oxide synthase (Ohgami et al. 2003). According to Suzuki et al. (2006), astaxanthin has promising effects in the treatment of ocular inflammation due to being able to downregulate

proinflammatory factors and inhibiting the nuclear factor-kB-dependent signaling pathway. It was also found to be capable of protecting vulnerable tryptophan residues and high-crystallins against oxidative stress, such as damage and degradation by calcium-induced calpain in porcine lens crystallins (Wu et al. 2006). In their experiments, Liao et al. (2009) reported that the interaction of astaxanthin and selenite, whose accumulation in the lens could be a cause of cataract formation, was found to delay selenite-induced cataractogenesis by delaying selenite-induced lens crystalline precipitation. In addition, Nakajima et al. (2008), also reported the neuroprotective effects of astaxanthin against retinal ganglion cell damage. In more recent study, conducted on rats with elevated intraocular pressure, Cort et al. (2010) showed that astaxanthin significantly decreased the percent of apoptotic cells on the retina, confirming the role of oxidative injury in elevated intraocular pressure and further highlighting the protective effect of astaxanthin in ocular hypertension (Cort et al. 2010).

5.19 Effects on Fertility

According to Eskenazi et al. (2005), it is found that the fertility and the quality of the semen are improved with a healthy diet consisting of high intake of antioxidants. In a pilot double blind randomized trial Comhaire et al. (2005) studied the effects of astaxanthin as a treatment to improve the outcome of the World Health Organization (WHO) treatment options for infertility in males. However, in the study of Comhaire et al. (2005) sixteen milligrams (mg) of astaxanthin was administered daily to male partners of 20 couples who are infertile in which their characteristics of the semen were beneath the recommended references values of the WHO. According to Comhaire et al. (2005), the results have concluded that the secretion of inhibin B by Sertoli cells and the reactive oxygen species are significantly reduced by astaxanthin thus the intake of astaxanthin is positively associated to the parameters of the sperm and fertility.

5.20 Effects on Smokers

Pryor et al. (1983) and Church and Pryor (1985), established that, significant quantities of reactive free radicals found in cigarette smoking, are found to cause extensive oxidative injury to macromolecules including proteins, DNA and lipids, which may also be involved in the pathology of various diseases including cancer and cardiovascular diseases. According to Kim and Lee (2001), it is found that people who smoke, had reduced the antioxidant enzyme activity, increased the peroxidation of lipids and the oxidation of DNA and proteins and depleted the antioxidants in the plasma. However, according to Cross and Halliwell (1993), it is found that smokers required more antioxidants when compared to the general population and those who

do not smoke. However, previous studies have shown that various antioxidants are found to be effective in decreasing oxidative injury while also possessing protective effects for those individuals who smoke (Kim and Lee 2001; Dietrich et al. 2002). However, the antioxidants effects of astaxanthin, are found to have a crucial role in protecting wide range of illnesses including cancer, immunological disorders, inflammation and cardiovascular diseases (Hussein et al. 2014; Higuera-Ciapara et al. 2006).

An elevated level of astaxanthin in the plasma with a significant reduction of Malondialdehyde (MDA) and isoprostanes (ISP) levels in the plasma are noticed after the intervention of astaxanthin. In the lipid membrane, the decomposed product of the polyunsaturated fatty acids is MDA while the eicosanoids that are formed by the free radicals that are accelerating oxidation of arachidonic acid are ISP's. The ISP's are usually used as biomarkers in the peroxidation of lipids and oxidative stress in various diseases (Michel et al. 2008). However, according to Naguib (2000) and Palozza and Krinsky (1992), it is found that astaxanthin possesses a crucial role because it protects the membrane of the phospholipids and peroxidation of lipids.

However, regarding the antioxidants biomarkers, it is noticed an elevation in the plasma levels of Superoxide dismutase (SOD) and tacrolimus (TAC) after an increased period of astaxanthin intake. In addition, the main antioxidant enzyme that extinguish superoxide anion is SOD and TAC against several free radicals, constitutes the full spectrum of the antioxidant activity. According to Yuan et al. (2011), (Higuera-Ciapara et al. 2006), it is found that astaxanthin possesses the capability of collecting and removing produced free radicals and reactive oxygen species, such as superoxide anions, which are found in cigarette smoking. However, it is found that the significant changes that have occurred in SOD and TAC, confirmed that the elevation of the antioxidant activity by astaxanthin occurred by increasing the activity of scavenging enzymes among those who smoke (Kim et al. 2011).

Furthermore, according to Spiller and Dewell (2003), it is found that among healthy adults, the world-wide recommended dose of astaxanthin is between 5 and 6 mg per day. Moreover, according to Naguib (2000) and Palozza and Krinsky (1992), astaxanthin is found to be a super vitamin E due to its effective effect of antioxidation especially when compared to other antioxidants. However, according to Karppi et al. (2007) it is found that the protective effect of astaxanthin is confirmed among healthy individuals who are non-smokers. Finally, this study concluded that the intake of astaxanthin, among healthy adults, is found to decrease the increased levels of oxidative stress that is found in smokers.

However, the increase of TAC by suppressing the peroxidation of lipids while also activating antioxidant enzymes is considered a possible mechanism for astaxanthin to have protective effects. In addition, the results shown that astaxanthin is the most powerful preventative agent to reduce oxidative stress caused by smoking (Kupcinskas et al. 2008). Furthermore, the supplementation of astaxanthin is effective to decrease the risk of pathological conditions that are associated with oxidative injury such oxidative stress induced by smoking.

5.21 Effect on Preeclampsia

The effects of astaxanthin have been studied in the context of women suffering from preeclampsia (PE) during their pregnancy. PE is a disorder associated with high blood pressure during gestation. Although its pathological mechanisms are quite intricate, causes of PE have long been linked to the damage of endothelial cells and inflammation in the placenta, all of which are caused by abnormal levels of oxidative stress (Redman and Sargent 2005; Biondi et al. 2005; Dekker and Sibai 1998). Studies of Vanderlelie et al. (2008) and Biondi et al. (2005) have found that the process starts through placental lesions, such as less placenta blood infusion. Affecting the maternal lipid metabolism, this results in an uncontrolled lipid peroxidation, providing excessive active oxygen and, in return, induce oxidative stress. This stress then results in damage to vessel endothelial cells found within the placenta, causing pathological damage and thus, inducing PE (Tsukimori et al. 2005; Bowen et al. 2001). Even in early stages of PE, excessive inflammation was found and inflammation-related chemokines and cytokines we found to be rapidly increasing in production (Redman and Sargent 2004). Furthermore, inflammation and oxidative stress to vessel endothelial cells have been previously linked to the development and progression of PE in previous studies (Bernardi et al. 2008). For this reason, antioxidative therapy is the method used to treat PE (Rumbold et al. 2008). This method was suggested by Serdar et al. (2003) after finding that the levels of vitamin E and carotene in patients with severe PE was significantly lower than those in pregnant women; this suggested that antioxidant supplements at the early stages of pregnancy could help in preventing PE. This was confirmed by Poston et al. (2006) who tested the incidence of PE in pregnant women consuming vitamin E and C supplements versus those in placebo groups and concluded that the occurrence of PE in the first group was lower than in those part of placebo group. Along the years, antioxidative therapy performed in various countries, such as the United States, Canada, Mexico and England, have been found to effectively help in treating PE (Sibai 2004).

Being the strongest known singlet-oxygen quencher, astaxanthin is known to possess antioxidant properties (Guerin et al. 2003). Furthermore, it is able to inhibit the expression of inflammation-associated genes and changing the ratio of Th1/Th2 cells, thus carrying also anti-inflammatory effects (Kidd 2011; Pashkow et al. 2008). It was further reported that astaxanthin is capable to decrease blood pressure and increase the utility of NO (Mortensen et al. 1997).

The polyene structure of astaxanthin has been found to result in low permeabilization due to low polarity and due to the fact, that it mainly exists in crystal form. For this reason, very few studies have been conducted to study its effects. However, Chew et al. (2013), were able to discover that astaxanthin slowly permeates into cells, peaking mostly between 24 and 48 h. In their study, hydrogen peroxide was used to induce oxidative damage and astaxanthin was used as an antioxidative treatment. In fact, it was shown that within 48 hours, hydrogen peroxide-induced endothelial cell death was significantly reduced while also reducing the production of

active oxygen and protecting the MMP. However, the treatment of astaxanthin is found to reduce the production of reactive oxygen species, protect mitochondrial function and rescue the active oxygen that is known to cause oxidative injury to the membrane. This is done by treating the human umbilical vein endothelial cell with hydrogen peroxide thus increasing the MMP (Chew et al. 2013).

Furthermore, according to Li and Verma (2002), it is established the involvement of nuclear factor kappa-light-chain-enhancer in the regulation of immunity and inflammation. However, in patients diagnosed with severe preeclampsia, elevation in the expression of nuclear factor kappa-light-chain-enhancer within the placental tissues, is found to affect the invasive capacity of the trophoblasts thus inducing damage within the endothelial cells and increasing the production of inflammatory cytokines. Additionally, astaxanthin is found to be effective to suppress the overexpression of nuclear factor kappa-light-chain-enhancer (Shah and Walsh 2007).

Finally, it is completely clear, that astaxanthin is found to have anti-oxidative effect within the endothelial cells. In fact, astaxanthin has the ability to reduce L-nitroargininemethylester, a nitric oxide synthase inhibitor, which is found to induce hypertension, proteinuria, inflammation, oxidative stress and apoptosis within the placenta. Thus, astaxanthin can decrease the damage to the endothelial cells while improving the symptoms of preeclampsia (Xuan et al. 2016).

5.22 Angiogenesis

Angiogenesis refers to the process where pre-existing vessels form new blood vessels, a process which causes tumors to grow Hayes et al. (1999) and, as a result, is a cruicial step in tumor invasion and metastasis. Research has been done to develop anti-tumor strategies through disrupting tumor angiogenesis, specifically studies on agents which can stop the tumor from developing further through inhibiting neovascularization (Zhang and Wang 2015). Multiple Mechanisms of Anti-Cancer Effects Exerted by Astaxanthin. Although the role of astaxanthin as a tumor angiogenesis disruptor has not yet been fully understood, a study by Kowshik et al. (2014) found that it helped to decrease a number of vessels in oral cancer by significantly modulating the expression of VEGF and VEGFR2 and by decreasing HIF-1a nuclear translocation

6 Conclusion

An increasing number of various studies have shown that astaxanthin is a crucial key player for the treatment of cancer because it can affect several molecular and cellular processes. The effects and molecular targets of astaxanthin on cancer were described including inflammation and apoptosis hence astaxanthin is useful therapeutic agent for the development of new treatment protocols especially for cancer therapy.

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Chapter 9
Curcuma and Breast Cancer: A Focus on Cell Signaling Pathways

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Abstract Cancer is a multifaceted disease characterized by deregulated epigenetic, genetic and metabolic signals which affect cellular metabolism and apoptosis. Breast cancer is regarded to be the most common malignancy in the women worldwide. The side effects of chemo-drugs such as non-selectivity, toxicity and resistance urge scientists to find more potent and safer drugs. Natural products from plants provide an extensive array of chemical scaffolds with biosafety profiles, and safer health effects. Curcuma, a genus of family *Zingiberaceae,* comprises of about 110 species natively distributed as well as cultivated in South Asia, China, Australia, Sri Lanka, West Indies, and Peru. This plant is used as a remarkable pharmacological remedy to prevent and cure various pathological disorders including cancer. The chemical constituents of this plant, terpenoids and betaketones, specifically act as the anti-breast cancer agents. Curcuma, the marvel of nature, can be regarded as panacea due to its versatile molecular targets and spacious therapeutic window.

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Various investigations on the essential oils and dry rhizome conclude that the chemical constituents of this plant e.g., curcumin, germacrone, furanodienone, bisdethoxycurcumin, demethoxycurcumin, curcumol, and aromatic turmerone are responsible for its anti-breast cancer potential. These compounds, either as single compound or in combinations with other drugs, are known to arrest cell cycle and induce apoptosis in breast cancer cells by modulating various signaling cascades including NF-κB, STAT3, PI3k/Akt/mTOR and MAPK. This book chapter intends to comprehend the biological and pharmacological mode of action of Curcuma-derived anti-breast cancer compounds in order to update the researchers and scientific community about the pharmaceutical potential of plants belonging to this genus.

Keywords Curcuma · Cancer · Cell cycle arrest · Apoptosis

1 Introduction

Cancer arises due to uncontrolled cellular proliferation (Sharma et al. 2017) caused by epigenetic and genetic mutations as well as environmental carcinogens (Perez-Herrero and Fernandez-Medarde 2015). Recent statistics about this disease acclaim it to be the second most fatal disease after cardiovascular disorders with 9.6 million deaths and 18.1 million newly reported cases in 2018 (Bray et al. 2018). Breast cancer (BC) is a multifarious disorder characterized by abrupt growth of breast cells in an uncontrolled manner ultimately leading to the formation of a lump or mass (Simos et al. 2014). It is most common malignancy in the women all over the world and is the major cause of mortality among women (Rojas and Stuckey 2016). Incidence and prevalence of BC is higher in developed countries as compared to underdeveloped countries (Ghoncheh et al. 2016). Statistical analysis provides us an insight about the facts and figures regarding increase in the incidence of breast cancer during recent years and the reasons behind this increase are reported to be the population growth (12.6%), aging (16.4%), and age related causes (4.1%) (Azamjah et al. 2019).

Despite of great advancements in the field of medicine and surgery, complete cure of cancer remains an unsolved mystery (Gupta et al. 2013). Drug discovery from natural products has emerged as a great area of research with more than 70% of anticancer drugs been isolated from natural resources (Newman and Cragg 2012). The quest for the discovery of anticancer drugs dates back to 1950s, when vinca alkaloids were first discovered from plants. Since then ~25,000 different phytochemicals have been isolated from fruits and vegetables which exert anticancer effects in humans (Sharma et al. 2017).

Curcuma, an auspicious genus of perennial rhizomatous herbs belongs to family Zingiberaceae which comprise of 110 species approximately (Rajkumari and Sanatombi 2018). There are more than 100 species which have been reported up till now. The word “Curcuma” has been originated from the Arabic word “Kurkum”,

meaning "yellow". The genus was firstly recognized by the Carl Linnaeus in 1753 (Sun et al. 2017). Most of the plants belonging to this genus are natively distributed in Southeast Asia and are cultivated on large scale in China, Indonesia, Sri Lanka, India, Peru, West Indies and Australia. Evidences show that many plants of this genus have long been employed in traditional medicines to cure various ailments (Sun et al. 2017) such as *C. longa, C. angustifolia, C. amada, C. aromatica, C. caesia,* and *C. zedoaria* (Chaturvedi et al. 2015; Sun et al. 2017). It has been reported that rhizomes are the most effective parts of these plants which exhibit a wide range of therapeutic properties to overcome the chemo-resistance against various types of cancers e.g., breast cancer, multiple myeloma, colorectal cancer, lung cancer, pancreatic cancer, oral cancer, and prostate cancer (Devassy et al. 2015; Zhong et al. 2018). This chapter focuses on the anti-breast cancer activity of plants belonging to genus Curcuma (the golden spice of South Asia) and the mechanisms lying behind their modes of action.

2 Phytoconstituents of Curcuma

Plants are the excellent reservoirs of potentially active natural compounds which endeavor them the multiple therapeutic effects against various diseases (Song et al. 2019). These natural products from plants are relatively safer than synthetic drugs in terms of efficacy, side effects and cost. The bioactive phytochemicals obtained from plants include flavonoids, alkaloids, phenolic compounds, and tannins (Edeoga et al. 2005).

Natural products are thought to be the foundation pillars regarding drug discovery since the times immemorial. In the modern times also, the therapeutic uses of traditional medicines can be never overwhelmed. The plants of genus Curcuma have also been utilized to target various diseases including breast cancer (Amalraj et al. 2017). Among all the known species of curcuma, *C. longa, C. amada, C. angustifolia, C. aromatica, C. caesia* and *C. zedoaria* are well documented in traditional system of medicines (Chaturvedi et al. 2015). Pharmacological evaluations of essential oils and pure extracts of curcuma species have recognized terpenoids and beta diketones as major bioactive classes of compounds which breast cancer by modulating different cell signaling pathways (Amalraj et al. 2017; Nair et al. 2019).

Researches to investigate the phytochemistry of 32 different plant species show that there were ~720 compounds derived from these species which include; terpenoids (529 compounds), diphenylalkanoids (102 compounds), phenylpropene derived compounds (19 compounds), flavonoids (15 compounds), steroids (7 compounds), alkaloids (3 compounds), and 44 compounds of miscellaneous origin (Sun et al. 2017). Different species of this genus have been reported to exhibit different phytochemical contents such as *Curcuma longa* contains highest phenols (curcuminoids) (Sarangthem and Haokip 2010), *Curcuma angustifolia* has highest alkaloid contents (Dutta 2015), while *C. karnatakensis* has lowest phenolic contents

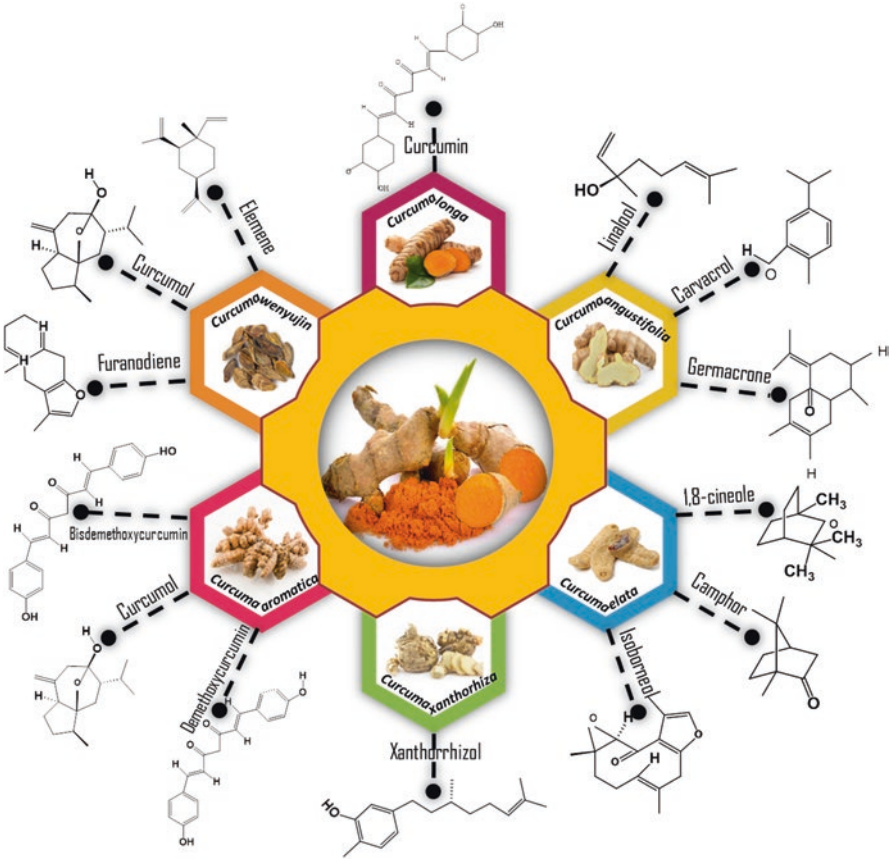

Fig. 9.1 Structural representation of anti-breast cancer compounds isolated from various species of genus Curcuma

(Tejavathi et al. 2017). Anti-breast cancer compounds isolated from various species of genus curcuma have been summarized in Fig. 9.1.

The brief account of potent compounds active against breast cancer is described here briefly:

2.1 Terpenoids

Terpenoids is a class of various non-aromatic as well as aromatic, volatile/non-volatile constituents which are pharmacologically active phytochemicals and play vital role in herbal medicine (Abdel-Lateef et al. 2016). According to an estimate, more than 30,000 terpenoids are known from plant sources exceeding over the number of alkaloids and other classes of compounds. Among the class of terpenoids in

genus curcuma, monoterpenes have been found to possess most significant anticancer activity (Pang et al. 2018). Generally, the term terpene tends to denote the compounds having integral number of C5 units. On the basis of number of C-atoms, terpenoids constitute mono-sesqui-di-tri- and isoprenoid units (de las Heras et al. 2003). Another class of compounds, beta diketones, is a main moiety of Curcuma family. It is not very common in nature but their excellent anticancer activity has made them to reach at preclinical stage of research (Kljun and Turel 2017).

2.1.1 Monoterpenes

Monoterpenes are categorized under the category of secondary metabolites. These compounds play role as a mediators among plants and their environment (Koziol et al. 2014). Several studies have documented the medicinal importance of natural and synthetic monoterpenes as antioxidant, antibacterial, anti-inflammatory and anti-cancer compounds (Moniczewski et al. 2011). Like all other terpenoids, monoterpenes are naturally derived from isopentyl-diphosphate and its allylic isomer dimethylallyl-diphosphate (Ramak et al. 2014).

There are several reported monoterpenes which are found potent against breast cancer. *Curcuma angustifolia* contain linalool and carvacrol, constituent of dried rhizome (Defilippi et al. 1991). Monoterpene constituent of *Curcuma elata* include camphene, camphor, 1,8 cineole, isoborneol and zederone (Syed Abdul Rahman et al. 2013; Ahmed Hamdi et al. 2014). Moreover, compounds such as linalool (Ravizza et al. 2008), carvacrol (monoterpenes) (Arunasree 2010), germacone (Zhong et al. 2011; Xie et al. 2014; Lim et al. 2016), and furanodiene (sesquiterpenes) (Li et al. 2011a, b) are reported to hold anti-breast cancer potential.

Linalool is an acrylic monoterpene alcohol extracted from essential oil of aromatic plants. It is reported to have antiproliferative and chemo sensitizing properties against breast cancer (Ravizza et al. 2008). Carvacrol recently captured attention of scientific community due to its high profile of biological activities. It is considered that hydrophobic interactions and hydrophilic properties of aromatic rings with OH group make it suitable antioxidant, antiproliferative and anticancer agent (Memar et al. 2017). Camphor is a transparent waxy solid with a stout aromatic odor and has a terpenoid heptanone origin (Chen et al. 2013).

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2.1.2 Sesqueterpenes

Sesqueterpenes, a sub-class comprised of three isoprene units whose formation is subjected to condensation of two precursor units, isopentenyl-diphosphate (IPP) and dimethylallyl-diphosphate (DMAPP) (Springob and Kutchan 2009; Jiang et al. 2016). Sesquiterpene lactones are the sesquiterpenes which hold a pentacyclic lactone moiety. Among these compounds, germacrone (Xie et al. 2014), furanodienone (Li et al. 2011b), zederone are cytotoxic compounds and carry potential against breast cancer.

Aromatic turmenone or 6S-2-methyl-6-(4-methylphenyl) hept-2-en-4-one is an antitumor bisabolane sesquiterpene (Afzal et al. 2013), that has been extracted from *C. longa, C. amada* and *C. wenyujin* (Park et al. 2012; Gao et al. 2014; Huang et al. 2017).

2.2 Beta-diketones

This subgroup of organic compounds possesses specific characteristics due its keto-enol tautomerism. Beta-diketone scaffold occurs naturally in curcumin and its derivatives. They are reported as ROS scavengers, anti-inflammatory, anticancer as well as chemopreventive agents with multitargeted molecular mechanisms. They influence on the signal transduction pathways (NF-κB and Nrf-2) and tumor suppressor gene p53 (Kljun and Turel 2017).

Curcumin, diferuloylmethane, has been described as chief constituent of *Curcuma longa* (Soleimani et al. 2018). It is the major anticancer compound and belongs to the polyphenols class of phytochemicals (Basnet and Skalko-Basnet 2011). Intensive investigations revealed the anticancer potential of curcumin that lies in inhibition/activation of transcription factors and tumor related proteins expression regulation (Ortega and Campos 2019). Other curcuminoids derived from *C. longa* include bisdemethoxycurcumin and demethoxycurcumin (Sasikumar 2001). Table 9.1 presents various classes of anti-breast cancer compounds isolated from genus Curcuma.

3 Anti-breast Cancer Activity of Genus Curcuma

Natural products have been utilized for the cure of various ailments and great source of drug discovery (Harvey et al. 2015). Many chemopreventive drugs are the molecules isolated from medicinal plants or their derivatives (Nageen et al. 2020). Nowaday's different chemotherapies and drugs have very confined success. These drugs are expensive and have toxic effects, so there is need of drugs that are inexpensive and less toxic (Wei et al. 2019). Cell signaling pathways are the leading pillars of cell communication as they are crucial for regulating cell proliferation and

Table 9.1 Curcuma derived compounds with their classes and natural sources

Class	Compound	Part of plant	Natural sources	Reference
Monoterpene	Linalool	Rhizome	*C. angustifolia*	Srivastava et al. (2006), Alinejad et al. (2013)
	Carvacrol	Essential oil		Sgorlon et al. (2016)
Sesquiterpene	Germacone	Dry rhizome	*C. angustifolia*	Kong et al. (2017)
	Furanodienone	Essential oil		
	Curcumol	Essential oil, dry rhizome		Ahmed Hamdi et al. (2014)
	Xanthorrhizol	Rhizome extract	*C. xanthorhiza*	Helen et al. (2012)
	Elemene	Rhizome	*C. wenjian*	Hughes et al. (2011), Liu (2013)
	Furanodiene	Rhizome		
	Zederone	Dry rhizome, essential oil, petroleum extract	*C. zedoaria*	Navarro Dde et al. (2002)
	Curdione		*C. elata*	Pimkaew et al. (2013)
	ar-turmerone	Rhizome	*C. longa*	Kocaadam and Sanlier (2017)
Beta-diketone	Curcumin	Rhizome	*C. longa*	Nabavi et al. (2018)
	Bisdemethoxycurcumin	Essential oil	*C. aromatic*	Dong et al. (2017), Liu et al. (2018)
	Demethoxycurcumin	Methanolic extract		

survival. Retrogression in these signaling pathways eventually leads to different pathological conditions incorporating cancer. Many chemopreventive and chemotherapeutic agents help to induce apoptosis in cancerous cells (Sarfraz et al. 2017).

Various studies reveal that different compounds isolated from *Curcuma*, are capable to retard the process of carcinogenesis by targeting different signaling network correlate with tumor cell proliferation. *In vitro* as well as *in vivo* studies of Curcuma derived compounds provide a clear image to researchers to investigate and summarize it deeply (Sun et al. 2017). By using high performance liquid chromatography different isolated bioactive compounds include flavonoids, terpenoids and diphenylalkanoids that were induced DNA fragmentation and leads towards the apoptosis in different cell lines of breast cancer (Zhou et al. 2016).

Carvacrol, an active constituent of *Curcuma angustifolia*, is known to exhibit cytotoxic potential against two breast cancer cell lines, MDA-MB 231 and MCF-7 (Zhou et al. 2016). Bismethoxycurcumin derived from the family *Curcuma aromatica* and it activates the p53 in MDA-MB 231 which is a tumor suppressor gene (Li et al. 2013). Curcumin isolate from fresh rhizome of *Curcuma* and it inhibits the p38MAPK and PI3K and downregulate the NF-κB (Chiu and Su 2009; Zhou et al. 2009; Palange et al. 2012). More experimentations and extensive investigations are the prerequisites to fill up the gaps regarding the molecular mechanisms by these

bioactive compounds in extrinsic as well as intrinsic mitochondrial pathways. The rhizome extracts of Curcuma (Rhizoma Curcumae) have been reported to enhance the doxorubicin activity in breast cancer (MCF-7) cells in by blocking the activity of P-gp and decreasing its expression (Zhong et al. 2018).

3.1 Curcuma and Cell Cycle Arrest

Cancer, uncontrolled cellular division, has been extensively investigated so that its cure could be found and the potential of conventional therapies could be enhanced. Natural compounds are crucial for increasing the potency of the targeted therapies. The inhibition of mitotic division declared to be promising target for chemotherapies and regulated through naturally occurring bioactive compounds (Wei et al. 2019). In cell cycle regulation different proteins and enzymes are involved such as cyclin dependent kinases (Asghar et al. 2015).

Various compounds derived from the different species of *Curcuma* have been known to arrest cell cycles at G2/G21 and M phase in breast cancer cell lines (Ravizza et al. 2008). The cell cycle arrest was found to be associated with the structural changes in tubulin proteins after which the chromosomal segregation occurred in an abnormal manner (Basile et al. 2009). In MCF-7 and BT-20 cancerous cell lines the cells are the number of cells decreased in G2/S phase after the treatment of the curcumin (Mehta et al. 1997).

With the treatment of xanthorrhizol to MCF-7 the growth was prohibited and caused the cell cycle arrest at G1 phase by increasing the level of p53 (Cheah et al. 2006). Furanodiene treatment leads the MCF-7 and MDA-MB231 towards G0/G1 phase by the hitting the molecular targets such as inhibition of the cycline D1 and CDK2 in does dependent manners (Zhong et al. 2012). The growth potential of breast cancer cell lines prohibited by the treatment of curcumol and cell cycle arrested at G1/sub G1 phase while enhanced the expression of p73, p-FAK, p-Akt and p-PI3K (Zhong et al. 2014; Huang et al. 2017).

3.2 Curcuma and Apoptosis

Apoptosis, programmed cell death, is an intricately fabricated event for cellular demise in human body which is regulated under the action of various signaling events to eliminate the harmful cells inside the body. It is essentially a vital feature of biological phenomena e.g., immune system functioning and embryonic development. Various disorders including neurodegenerative diseases and tumor formation can occur as a result of dysregulated apoptosis. Caspases are the cell death executioners which manipulate the process of apoptosis via intrinsic/extrinsic pathways (Wei et al. 2019). Chemopreventive agents which are isolated from mother nature

contribute towards the induction of apoptosis and activation of caspases (Ashkenazi 2015).

Compounds isolated from *Curcuma* exhibit great prevention against various cancer cell mainly against different cell lines of breast cancer. Cytotoxic activity of xanthorrhizol has been claimed to be responsible for causing cell death through the p53 regulation and by downregulation of Bcl-2 (Cheah et al. 2006), inhibiting the NF-κB (Palange et al. 2012), increasing proapoptic proteins, Bax (Xie et al. 2014), activation of caspase 9,3 (Zhong et al. 2011). According to the study curcumin have potential to decrease the level of c-jun/Ap-1 in MCF-7 cancerous cells (Mehta et al. 1997) and in MDA-MB 231 with the help of furanodienone HER2, Akt downregulates (Li et al. 2011a). Here further investigations are also required for the elucidation of apoptotic pathways different cancer cells via these bioactive biological compounds. Some of the anticancer agents from genus Curcuma and their molecular mechanisms have been discussed in Table 9.2.

The species belonging to genus Curcuma have been proclaimed as an attractive candidate with multitargeted chemotherapeutic effects. The anticancer property of Curcuma plants has been investigated to occur as a result of modulation of several cell signaling pathways (NF-κB, STAT3, Wnt, MAPK, and PI3K/Akt/mTOR) (Kunnumakkara et al. 2017a, b; Song et al. 2019), induction of tumor suppressor genes, alleviation of anti-apoptotic gene products (Bcl-2, XIAP, survivin, Bcl-xL), and activation of caspases (Cas-3, -7, -9) (Jiang et al. 1996; Bush et al. 2001; Chan and Wu 2004) in addition to suppression of MMPs (Fenton et al. 2002) and angiogenic cytokines (VEGF, TGF-β1) (Leyon and Kuttan 2003; Bobrovnikova-Marjon et al. 2004). Different types of cell signaling pathways, cell cycle regulators and cytokines, which are targeted by Curcuma-derived compounds have been elaborated in Fig. 9.2.

3.2.1 Curcuma and p53

Tumor suppressor gene, p53, has been reported to be involved in various cellular mechanisms e.g., repairing DNA, arresting cell cycle and inducing apoptosis. This particular gene becomes deregulated or non-functional in almost 50% of human cancers. In case of breast cancers, mutations in p53 gene are responsible for low survival rates and high resistance against conventional therapies. Hence we can conclude that targeting p53 activity is an important strategy in to treat cancer by the modulation of posttranslational modifications (ubiquitination, phosphorylation and acetylation) (Talib et al. 2018).

The phytochemical potential of medicinal herbs depends on the action mechanism of constituent molecules and moieties (Ooko et al. 2017). Curcumin induces the growth retarding effect on the breast cancer cells by acting as a proapoptotic agent (Talib et al. 2018). Linolool, a monoterpene, moderately inhibits the cancer cells proliferation by causing the programmed cell death and arresting cell cycle at increasing p53 levels and inducing cell cycle arrest at G2/M and G1 phase. An increase in the levels of p53 and p21was also noticed (Ravizza et al. 2008). In

Table 9.2 Curcuma-derived bioactive compounds with their mechanisms of action against breast cancer

Anticancer compounds	Cancer type/cell line used	IC_{50}/EC_{50}	Cell cycle arrest	Molecular targets	References
Linalool	MCF7 WT	200 μM	G2/M, G1	Bcl-xL↓, p53↑	Ravizza et al. (2008)
	MCF7 Adr^R	128 μM			
Carvacrol	MDA-MB 231	100 μM	S phase	MMP↓, PARP cleavage↑, $Caspases^{Act}$, Bcl2/Bax↓	Arunasree (2010)
Germacrone	MDA-MB-231	1.27 μM	G0/G1, G2/M, G1, G2	ERα↓, Bcl-2↓, p53↑, bax↑ PARP cleavage, Caspases 3, 7, 9^{Act}, LDH↑, Bok↑, ESR1↓, p-ATM↑, TFF1↓, GREB1↓, CCND1↓, PGR↓, CCND1 ⊥, MYC⊥, MDR1⊥, LDH release↑, p-ATR ↓, p-cdc2↓ p-Rb↓, cdc2↓, LDH1 release↑	Zhong et al. (2011), Xie et al. (2014), Lim et al. (2016), Kong et al. (2017), Al-Amin et al. (2019)
	MCF-7/ADR	180 μM			
	MCF-7	246.3 μM			
Furanodienone	BT474	---	G1	p-EGFR↓, EGFR/HER2⊥, HER2↓, Akt↓, Gsk3β↓, $p27^{kipl}$↑, ERα↓, c-Myc↓, Bcl-2↓, cyclin-D1↓	Li et al. (2011a, b)
	MDA-MB-231				
	SKBR3				
	MCF-7				
	T47D				
Bisdemethoxycurcumin	MCF-7	---	G2	p53 ^{Act}, p21 ^{Act}, p16 ^{Act}, Rb^{Act}, MMP↓	Li et al. (2013)
Demethoxycurcumin	MDA-MB-231	9 μM	---	ECM↓, MMP-9↓, AP-1↑, ICAM-1↓, CXCR4↓, NF-κB↓	Yodkeeree et al. (2010)
Curcumol	MDA-MB-231	---	G1, G1/subG1	MMP-9↓, JNK ½⊥, Akt⊥ eEF1A1↓, p73↑, $p53^{Act}$, Bak↑, PUMA↑, ABCC3↓, $NFAT^{Act}$, Cas-3 ↓, PARP cleavage↑, p-FAK, p-Akt↑, p-PI3K↑, p-p85↑, MMP-9↑, Cas-3, -7, -9^{Act}, ROS↑	Ning et al. (2016), Huang et al. (2017), Qi et al. (2017), Mbaveng et al. (2018), Zeng et al. (2020)
	4T1				
	MCF-7				

Curcumin	MDA-MB-231	20 μM	G0/G1, G2/S, G2/M	Bcl-2↓, ROS↓, Caspase-3, 9↑, MMP-3⊥, EGF↓, c-jun/Ap-1↓, Beclin1↓, PI3K⊥, uPA↓, p-ERK⊥, p-p38⊥, COX-2↓, p21/WAF/CIPI↑, p53↑, AP-1⊥, NFκB⊥, IL⊥, Cdk inhibitor↑, STAT-3⊥, p-PAR-γ⊥, NOS↓, TNFα↓, Tyr701↓, MMP-9↓, EGF↓, IL-8↓, PDGF↓, TGFα↓, VEGF↓, GH↓, pSTAT-3, Tyr694↓, JAK-2↓, caspase-3 ^{Act}, hTERT↓, p38-MAPK ^{Act}, GH↓, pSTAT-1, GCSF↓, JAK-2↓, pJAK-2↓, PIAS-3↑, SOCS-1↓, SOCSS-3↓, FAS⊥, Bax↑, Bcl-2↓, p-Akt↑, siRNA↑, Axl↓, Slug ↓, CD24 ↓, Rho-A↓, N-cadherin↓, β-catenin↓, Twistl↓ p21↑, BcL-xL↓, Bcl-2↓, p65↓, IKKα⊥, IKKβ⊥, ERK1/2^{p}↑, c-myc↓, Cyclin D1↓, bFGF↓, pSTAT-5↓, pSTAT-1	Mehta et al. (1997), Chiu and Su (2009), Zhou et al. (2009), Boonrao et al. (2010), Palange et al. (2012), Zong et al. (2012), Cine et al. (2013), Jiang et al. (2013), Kazemi-Lomedasht et al. (2013), Jain et al. (2015), Kumar et al. (2015), Kunnumakkara et al. (2017a), Coker-Gurkan et al. (2018), Gallardo et al. (2020)
	MDA-MB-453				
	MCF-10A	20 μM			
	NIH3T3				
	MCF-7	9.7 μM			
	T-47D	40 μM			
	BT-20				
	MDA-MB-468	22 μM			
	SK-BR-3				
	SK-BR-3	20 μM			
	MCF-7/LCC9	11.3 μM			
	MCF-7/LCC2	12.2 μM			
Xanthorrhizol	MCF-7	1.71 μg/mL	G1	pS2↑, PARP cleavage↑, p53↑, Bcl-2↓	Cheah et al. (2006), Anggakusuma et al. (2009)
Elemene	MCF-7			Heparanase↓, FDF-2↓, VEGF↓, p-ERK↓, p-AKT↓	Zhang et al. (2017)
	MDA-MB231				
	MDA-MB435S				
	4T1				
Furanodiene	MCF-7	75 μM	G0/G1	p-cyclin D1⊥, CDK2⊥, Rb⊥, p-Rb⊥, Bcl-xL⊥, Bad↑, Bax↑, Cas-9↑, PARP↑, I αV↓, β-catenin↓, p-Akt↓, p-PI3K/p85↓, p-FAK⊥, MMP-9↓, MMP-2↓, Cas-3^{Act}, Cas-7 ^{Act}, Bcl-xL↓, Bad↑, PARP ^{Act}, ROS↑, Cleavage of Cas-8↑, TNF-α↑, NF-κB↑, p-CDK2⊥, Akt ⊥	Yang et al. (2005), Zhong et al. (2012, 2014, 2017)
	MDA-MB231				
	MCF-7/DOX^{R}				

(continued)

Table 9.2 (continued)

Anticancer compounds	Cancer type/cell line used	IC_{50}/EC_{50}	Cell cycle arrest	Molecular targets	References
ar-turmerone	MDA-MB231		G0/G1	MMP-9 ⊥, COX-2 ⊥, p-PI3K/Akt↓, E-cadherin↑, CXCR4↑, CCR7↓, CXCR4↑, NF-κB⊥, p-ERK1/2↓	Park et al. (2012), Gao et al. (2014)
Zederone	MCF-7	>100.0 μg/mL			Syed Abdul Rahman et al. (2013), Ahmed Hamdi et al. (2014)
Curdione	MCF-7	125.6 μg/mL		Bax↑, cleaved caspase-3,-9↑, Bcl-2↓	Kong et al. (2013), Li et al. (2014)
	MDA-MB-231				
Terpecurcumin Q	MCF-7	3.9 μM	Sub G1↑	Caspases ^{Act}, Bcl-2↓, Bcl-xl↓	Lin et al. (2013), Essien et al. (2015)
	Hs578T	98.86 μg/mL			
Tetrahydrocurcumin		33.μM			Kang et al. (2014)
Curcumenol	MCF-7	9.3 μg/mL			Ahmed Hamdi et al. (2014)

Upregulation ↑, Down regulation ↓, Activation ^{Act}, Inhibition ⊥, transmembrane molecule B-cell lymphoma extra-large gene (Bcl-XL), apoptosis regulator gene (Bax), Bcl associated death protein (Bad), cyclin dependent kinase (Cdc2), poly ADP ribose polymerase (PARP), cysteine-aspartic proteases (Caspase3–9), tumor suppressor retinoblastoma protein (pRB), estrogen signaling receptor (ESR), tumor suppressor gene (p-53), serine threonine kinase protein (ATM), Tree foil factor family (TFF1), growth regulation by estrogen in breast cancer (GREB1), cyclin D1 gene for cyclin family (CCND), progesterone receptor (PGR), transcription factor encoding protoonco gene family (Myc), cyclin dependent kinase (CDK-1), intercellular adhesion molecule (ICAM)-1, epidermal growth factor receptor (EGFR), matrix metalloproteinases (MMPs), human epidermal growth factor receptor (EGFR), protein kinase B (Akt), glycogen synthetase kinase (GSK 3B), cyclin dependent kinase inhibitor-1 (P21), estrogen receptor α (ER α), tumor suppressor protein (P53), extracellular matrix (ECM), plasminogen activator inhibitor-1 (PAI-1), chemokine ligand-receptor (CXCR4), nuclear factor-κB (NF-κB), PUMA (p53 upregulated modulator of apoptosis), Jun N-terminal kinase (JNK), eukaryotic translation elongation factors 1 alpha (eEF1A1), tumor suppressor gene of P53 family (P73), cyclin dependent kinase inhibitor (P27), ATP binding cassette subfamily C member 3 (ABCC3), nuclear factor of activated T cells (NFAT), tissue inhibitor of matrix metalloproteinases (TIMP-1), reactive oxygen species (Ros), Jun Proto-Oncogene, AP-1 transcription factor subunit (c-jun/AP-1), autophagy related protein (Beclin-1), extracellular signal-regulated kinase (ERK), phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinases (P38), protein is a marker for hormone-dependent breast tumors (pS2), endo-glycosidase expressed in mammals, (Heparinase), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF-2), growth hormone (GH), protein inhibitor of activated STAT (PIAS), suppressor of cytokine signaling (SOCS-2), surface antigen receptor (Fas), small interfering RNA (siRNA), receptor tyrosine kinase (AXL), member of the SNAIL family of transcriptional repressors (Slug), family of GTPases is a family of small (Rho-A), cell–cell adhesion molecule (N-cadherin), embryonic transcription factors (*TWIST*), telomerase reverse transcriptase (hTERT), nuclear focal adhesion kinase (FAK), cyclooxygenase-2 (Cox-2), Janus kinase/signal transducer and activator of transcription (JAK/STAT)

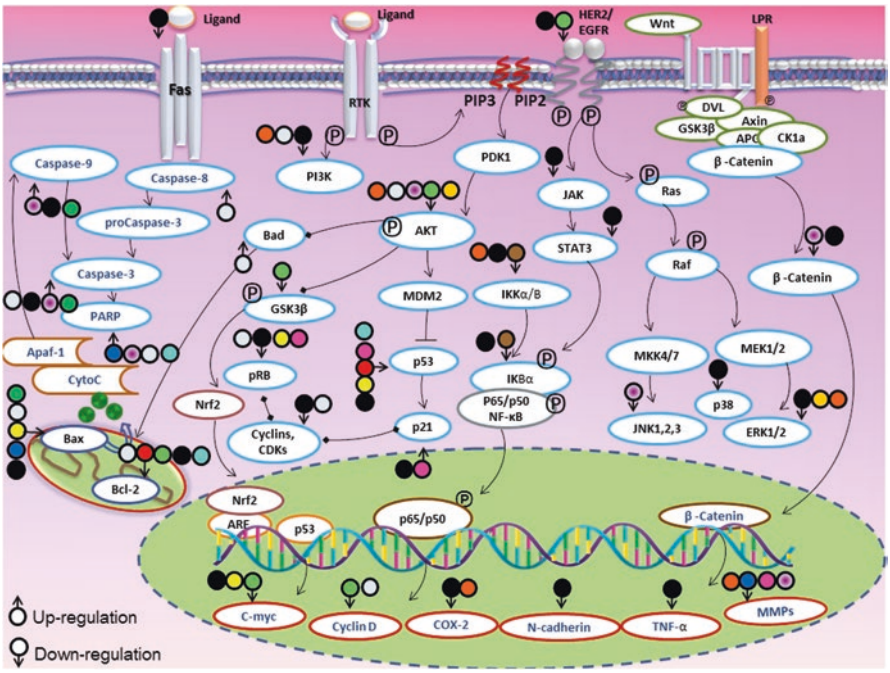

Fig. 9.2 An overview of the anti-breast cancer potential of different compounds extracted from plants of genus Curcuma. The molecular mechanism lying behind this activity involves the modulation of various cell cycle regulators, protein kinases, enzymes and hormones, and apoptosis related factors which are known to be involved in the invasion, proliferation, angiogenesis and metastasis of breast cancer. Dots of different colors symbolize the compounds given in the table below the figure

another experiment, the cytotoxic effect of germacrone was noticed on MCF-7/Adriamycin (multidrug drug resistant human breast cancer) cell line. The results elaborated that combined treatment of germacrone and adriamycin resulted in an increased cytotoxicity as compared with Adriamycin alone. The increased levels of proapoptotic proteins (p53 and bax) were also noticed (Zhou et al. 2009; Xie et al. 2014). Curcumol also activated the expression of p53 and induced apoptosis in human TNBC (triple negative breast cancer) cells (Huang et al. 2017) and is known to enhance doxorubicin sensitivity in vitro and in vivo (Zeng et al. 2020).

3.2.2 Curcuma and NF-κB

NF-κB, a proinflammatory transcription factor, is responsible for the regulation of ~500 genes which in turn control various inflammatory responses and tumor formation processes (Kunnumakkara et al. 2017a; Catanzaro et al. 2018). The alterations in the activation of NF-κB are involved in causing pathological abnormalities including cancer. Hence the modulation NF-κB is a major regime to control cancer

progression (Kunnumakkara et al. 2017a). Phytochemicals are the ray of light for the treatment of fatal calamities such as cancer.

The use of herbal medicines for the cure of cancer has been gaining a great attention due to the presence of bioactive constituents (Talib et al. 2018). Some bioactive compounds from genus *Curcuma* have been reached in the clinical trials, because of their chemotherapeutic potentials. Curcumin (diferuloylmethane) has been assumed to possess anticancer potential against breast cancer in a number of in vitro as well as in vivo experiments. Its treatments, either alone or in combination, are in Phase I/II trials against breast, colon, pancreatic, and prostate cancers targeting the molecular and transcription factors e.g., NF-κB (Zong et al. 2012; Coker-Gurkan et al. 2018). It is known to inhibit the migratory ability of human triple negative (MDA-MB231) cells by decreasing NF-κBp65 protein expression (Chiu and Su 2009). Another compound, Calebin A (4-[3 methoxy-4 hydroxyphenyl]-2-oxo-3-enebutanyl 3-[3-methoxy-4 hydroxyphenyl] propenoate), from *C. longa* is known to inhibit the activation of NF-κB by interacting with p65 protein ultimately leading to apoptosis induction (Tyagi et al. 2017). An experiment by Yodkeeree et al. (2010) stated that Curcuma derived compound, demethoxycurcumin, strongly inhibited NF-kB and expression of p65 in the nucleus of treated cells (Yodkeeree et al. 2010).

3.2.3 Curcuma and STAT3 Pathway

Signal Transducer and Activator of Transcription 3 is a transcription factor which has been reported to be involved in oncogenesis process. Its regulation is highly complex under different situations. It is involved in regulating the normal stem cells while on the other hand it is constitutively expressed in certain types of cancers. Hence its modulation can be regarded as an attractive target towards the control of cancer (Kunnumakkara et al. 2017a; Galoczova et al. 2018).

Various bioactive entities from natural plants have been proved their worth as effective anticancer agents against a variety of cancer types including breast cancer. Curcumin, a primary active ingredient from *C. longa* has been proved to be an effective modulator of JAK/STAT pathway by suppressing the phosphorylation of JAK 1, STAT 1 and STAT 3 (Li et al. 2018). Moreover, experimental evidences have stated that curcumol inhibited the Janus kinases activation and Akt signaling ultimately suppressing the breast cancer cell metastasis in triple negative breast cancer (MDA-MB231) cells (Ning et al. 2016).

3.2.4 Curcuma and Wnt Signaling Pathway

Another signal transduction pathway for the regulation of cellular development, death or demise is Wnt/β-catenin cascade, the dysregulation of which may contribute towards the spread of diseases e.g., cancer.

Curcumin, the important component of *Curcuma longa*, contributes towards the modulation of Wnt/β-catenin signaling in various types of cancers. Curcumin

treatment to MCF-7 as well as MDA-MB-231 cells, retarded Wnt/β-catenin signaling and changed the pattern of c-cyclin D1, Myc, E-cadherin, and GSK3β expression. The same bioactive compound was reported to stop the metastasis of CSCs of breast cancer by restoring E-cadherin expression, hence causing an increase in E-cadherin/β-catenin complex formation. Moreover, treatment with curcumin was investigated on ER-negative human breast cancer cells and the results elaborate that a transient increase in the level of β-catenin was noticed (Kunnumakkara et al. 2017a). Furanodiene decreased the expression of β-catenin, phosphorylation in FAK (Focal adhesion kinase), Akt and PI3Kp85, thus decreasing the tumor metastasis (Zhong et al. 2014).

3.2.5 Curcuma and MAPK Pathway

MAPK (Mitogen-activated protein kinase) pathway acts as a significant signal transduction cascade which constitutes the emerging point for other signaling pathways e.g., serine/threonine and tyrosine kinases, calcium signaling and G proteins. MAPK, the family of phosphoproteins, constitutes the signaling molecules which lead to the generation of proinflammatory mediators. Hence MAPK inhibition is thought to be an effective strategy for the control of cancer proliferation (Chauhan et al. 2018).

Curcuma longa, a widely utilized culinary spice, is utilized as anticancer agent against variety of cancers including breast cancer. Curcumin, an important constituent of *C. longa* exhibits robust potential against many diseases of malignant and non-malignant origin including cancer (Ooko et al. 2017). It modulates the MAPK pathway and induces apoptosis. Tetrahydrocurcumin, a polyphenolic compound from *Curcuma* species explicit antitumor potential in vivo by increasing Bax/ Bcl-2 ratio and activating caspase-2 as well as p38 MAPK in MCF-7 cells (Kang et al. 2014).

3.2.6 Curcuma and PI3K/Akt/mTOR Pathway

Protein kinases are involved in controlling the cellular functions e.g., RNA transcript formation, protein formation, and cellular growth by the process of phosphorylation. Their deregulations contribute towards the oncogenesis process. PI3K/Akt/mTOR signaling is stimulated to enhance the metabolism, survival and growth of cancerous cells (Asati et al. 2016). PI3K, Phosphatidylinositol-3 kinases, comprise of lipid kinase family which are able to catalyze the phosphorylation of inositol ring 3′-OH group in inositol phospholipids to generate the phosphatidylinositol-3,4,5-triphophate (Fresno Vara et al. 2004). Akt or protein kinase B is involved in various cellular events involved in the survival, progression and growth of the cell. The mutations as well as the amplifications in Akt result in the process of carcinogenesis (Fresno Vara et al. 2004; Kunnumakkara et al. 2017a).

Another kinase mTOR is also a part of cellular growth and progression machinery. The aberrations in its upstream activators as well as its downstream effectors are also found to be involved in malignant conformities (Kunnumakkara et al. 2017a).

Phosphorylation of Akt and its upstream targets EGFR, HER2 were decreased by furanodienone expose in case of HER2-overexpressing human breast cancer cells (Li et al. 2011a) while ERα negative MDA-MB-231 and MCF-7 cells responded less to furanodienone exposure (Li et al. 2011b; Zhong et al. 2012). Elemene extracted from Curcumae Rhizoma is reported to alleviate the phosphorylation of Akt and extracellular signal-regulated kinase (Zhang et al. 2017). Curcumin also inactivates the cell signaling pathways e.g., NF-kB, Src and Akt/mTOR pathways (Jiang et al. 2013). Likewise, aromatic turmerone also decreased the phosphorylation of PI3K/Akt signaling and ERK1/2 signaling (Park et al. 2012).

4 Conclusions

This book chapter aims to update the researchers and scientific community about genus Curcuma and its isolated compounds regarding their anti-breast cancer activity. The anticancer potential of this plant is attributed to the presence of bioactive terpenes and betadiketones such as curcumin, furanodienone and curcumol. These unique chemical structures have been reported as efficient modulators of several deregulated cancer signaling pathways (β-catenin, Wnt, and PI3K/Akt/mTOR signaling), apoptosis related factors (caspase-3, -7, 9, Bax, Bcl-2, and p-53), cell cycle regulators (cyclins and CDKs), protein kinases (ERK, JNK, MAPK) and transcriptional factors (NF-κB and STAT3). Being nutraceuticals, these compounds will emerge as biosafe lead candidates for cancer drug discovery. Various lines of evidences suggest that some compounds of this genus have entered into the preclinical trials which further ensure the curative potential of Curcuma derived compounds against many diseases including cancer.

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Chapter 10
Fruits and Vegetables in Cancer

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Abstract Cancer diseases have been widely recognized as a significant global public health problem and their incidence, morbidity and mortality are high in worldwide. There is a lot of evidence that fruit and vegetables could provide health benefits and reduce the risk of cancer. The anticancer properties of fruit and vegetables are attributed to their composition rich in phytochemicals or phytonutrients. This chapter will summarize the role of phytochemicals of fruit and vegetables namely quercetin, resveratrol, carotenoids and dietary fibers in cancer prevention. A large number of *in vivo* and *in vitro* experiments has demonstrated the beneficial effects of these compounds. These phytochemicals are also capable of increasing the effectiveness of drugs already established in treatments, reinforcing the importance of indicating the consumption of fruit and vegetables by the population.

Keywords Cancer prevention · Quercetin · Resveratrol · Carotenoids · Dietary fibers

Abbreviations

ABC transporter	ATP-binding cassette transporter
ADR	Adriamycin
AHR	Aryl hydrocarbon receptor (transcript factor)
Akt	Serine/threonine-specific protein kinase also known as protein kinase B
AMP	Adenosine mono phosphate
AMPK	Activated protein kinase
Bad gene	Inhibit the apoptosis-preventing activity of Bcl-2
Bax	BCL2 associated X, apoptosis regulator gene
Bcl-2	B-cell lymphoma 2 gene
BCRP	Breast cancer resistance protein
BET	Bromodomain extraterminal domain
CACO-2	Colon cancer cells lines
CDK	Cyclin-dependent kinases
CDK4	Cyclin-dependent kinase 4
cFLIPL	Cellular FLICE-like inhibitory protein
Chk2	Checkpoint kinase 2
cIAP-2	Cellular inhibitor of apoptosis protein 2
CK19	Citoqueratina 19
CK8/18	Cytokeratin 8/18
c-met	Tyrosine-protein kinase-met
COX-2	Cyclooxygenase-2
Cyclin D1	Regulator of cell cycle progression
DHT	Dihydrotestosterone
E-cadherin	Cell adhesion molecule
EGFR	Epidermal growth factor receptor

EGR1	Early growth response 1
ER1α	Estrogen receptor alpha (ERα)
ERK1/2	Extracellular signal-regulated protein kinases 1 and 2
FAK	Focal adhesion kinase
Fas-L/Fas	Fas ligand/first apoptosis signal (cell surface receptor induces apoptosis)
FOS	Fos proto-oncogene
FOSL1	FOS-related antigen 1 (oncogene)
Gefitinib	EGFR-specific tyrosine kinase inhibitor
HGPIN	High-grade prostatic intraepithelial neoplasia
hnRNPA1	Heterogeneous nuclear ribonucleoprotein A1
HOXA10	Homeobox A10
Hsp27	Heat shock protein 27
Hsp70	Heat shock protein 70
Hsp90	Heat shock protein 90
HUVECs	Human umbilical vein endothelial cells
ICAM-1	Cell adhesion molecule
IGF-1	Insulin-like growth factor-1
IGFBP3	Insulin-like growth factor binding protein-3
ILK	Integrin-linked kinase
ITGA5	Gene expression of integrin α5
ITGB1	Integrin β1
JAK2/STAT3	Janus kinase 2/signal transducer and activator of transcription 3 pathway
JNK	c-Jun N-terminal kinase
JUN	Proto-oncogene
MAPKs	Mitogen-activated protein kinases
Maspin	Mammary serine protease inhibitor
MCF-7/adr	Adriamycin-resistant human breast cancer cells
MDR1	Multi-drug resistance gene 1
miR-16	microRNA 16
MMP9	Matrix metalloproteinase 9
MOLT-4	Human T lymphoblast; acute lymphoblastic leukemia
MRP1	Multi-drug resistance associated protein
mTOR	Mechanistic target of rapamycin
N-cadherin	Cell surface protein related to cell adhesion
NF-κB	Factor nuclear kappa B
p53	Tumor suppressor gene protein 53
PDCD4	Programmed cell death 4
P-gp	P-glycoprotein
PLGA	Poly lactic-co-glycolic acid
PPARγ	Peroxisome proliferator-activated receptor gamma
RAR	Retinoic acid receptor
Ras/MEK/ERK	Signaling pathway involved in the proliferation of the cancer cells

RASSF-1α	Ras associated domain family-1α
RAW 264.7	Murine macrophage **cell** line
SIRT1	Sirtuin-1
Snall	EMT- inducible transcription factor
SOD	Superoxide dismutase
SRT501	Micronized resveratrol
STAT3	Signal transducer and activator of transcription 3
Survivin	Anti-apoptotic protein
TNF-α	Tumor necrosis factor alpha
Trans-RESV	Trans-resveratrol
VASP	Vasodilator-stimulated phosphoprotein
VCAM-1	Cell adhesion molecule
VEGF	Vascular endothelial growth factor
Vimentin	Characteristic mesenchymal marker of EMT (intermediate filament)
Wnt signaling pathway	Regulate cell proliferation and differentiation
α-V-β3	Integrin receptor

1 Introduction

Hippocrates, medicine father (460-377 BC) already said: "Let food be your medicine" (Arai 2005). Nowadays, several bioactive compounds from dietary sources promote potential health benefits and chronic disease prevention in addition to the nutrition function (Da Silveira et al. 2020). Therefore, the consumption of suitable foods ratifies Hippocrates prediction.

Over the last decades, cancer diseases have been widely recognized as a significant public health problem worldwide which incidence vary by age and race (Fitzmaurice et al. 2019; Force et al. 2019; Prager et al. 2018). The etiology of cancer is associated to intrinsic components (mutations in oncogenes and suppressor genes), and extrinsic causes including environment exposure and lifestyle such as smoking, diet, obesity, and physical inactivity (Islami et al. 2018; Siegel et al. 2019).

The intrinsic risk factors range to 10–30% to all cancer diseases while the extrinsic factors reach to 70–90% being potentially risk for carcinogenesis in most common cancer types (Wu et al. 2016). On the other hand, cancer is a preventable disease, which requires lifestyle changes mainly adoption of healthy dietary habits (Anand et al. 2008; Pedersen et al. 2016).

A global study analyzed the food consumption of populations in 195 countries, listing the main dietary factors that influence population health. This systematic analysis revealed that the diet is responsible for more deaths than other risks such as tobacco smoking. The non-optimal intake of whole grains, fruit and vegetables (F&V), in addition to other inappropriate eating habits was responsible for mortality

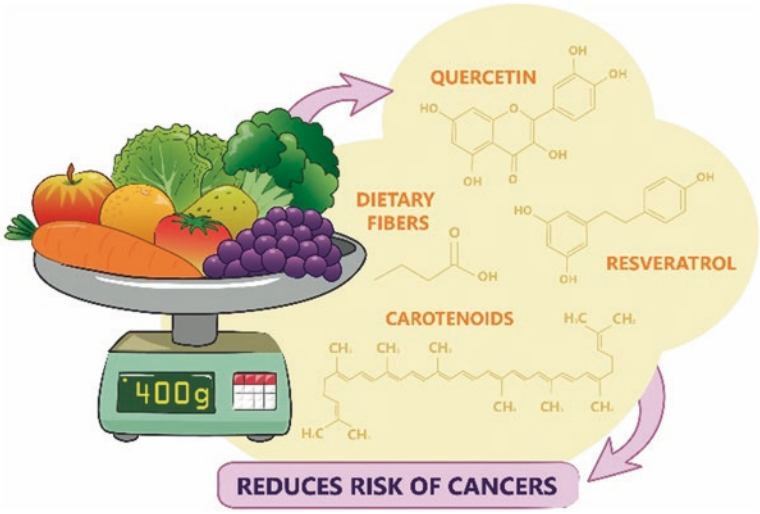

Fig. 10.1 Five servings per day of fruit and vegetables is beneficial to cancer prevention. A healthy diet for cancer prevention involves an adequate daily intake of fruit and vegetables (~400 g) to provide phytochemicals such as quercetin, resveratrol, carotenoids and dietary fibers with chemopreventive potential

by cancer across those countries (Afshin et al. 2019). Thus, the suitable diet with higher intakes of fruits and vegetables could be beneficial for cancer prevention (Stepien et al. 2016; Turner 2014).

In this way, the consumption at least five servings per day of fruit and vegetables (400 g/day) which provide a higher intake of phytonutrients and fiber (Fig. 10.1), was recommended as a healthy diet for cancer prevention (WCRF/AICR 2018). Moreover, epidemiological studies reported that a diet rich in fruits and vegetables was associated with a reduced risk of several types of cancer such as breast, prostate, colorectal, gastrointestinal, and other ones (Madigan and Karhu 2018; Ranjan et al. 2019).

It's known, that the anticancer properties of fruit and vegetables are attributed to their composition rich in phytochemicals or phytonutrients such as phenolic compounds (*e.g.,* flavonoids, stilbenes, lignans, and phenolic acids), terpenoids (*e.g.,* carotenoids), alkaloids, terpenes, organosulfur compounds and dietary fibers (De Silva and Alcorn 2019; Fraga et al. 2019; Meybodi et al. 2017; Tang et al. 2019).

Among the phytochemicals, the polyphenols are the most important bioactive compounds known as potential anticancer agents, and their biological effects depend on their bioavailability as well as biotransformation through gut microbiota (Poe 2017; Sajadimajd et al. 2020). Most of them have ability to inhibit cancer cell proliferation by cellular, molecular, and genetic levels through stimulating multiple cell-signaling pathways (Desai et al. 2018; Manayi et al. 2020).

This great deal of anticancer activity of substances derived from natural sources as dietary phytochemicals make them a potential target for use as new and more

effective strategy to minimize adverse impacts and resistance to conventional treatments or can be stablished as a complementary treatment in a safe way (Mitra and Dash 2018).

The aim of the present chapter is to review the role of phytochemicals from fruit and vegetables, namely quercetin, resveratrol, carotenoids and dietary fibers on cancer.

2 Quercetin

Quercetin (QE) is a biologically active polyphenolic flavonoid, belonging to flavanol subclass, widely distributed among fruit and vegetables such as apples, red grapes, raspberries, cherries, onions, broccoli, tomatoes, citrus fruits and green leafy vegetables (Brito et al. 2015; Hashemzaei et al. 2017). The beneficial effect of quercetin on health has been extensively studied due to perform several pharmacological effects such as antioxidant, anti-inflammatory and anticancer (David et al. 2016).

Plant quercetins are mainly found as glycosides but the aglycone also promotes biological effects (D'Andrea 2015). Intestinal β-glucosidases from gut microbiota catalyze the hydrolysis of glycosidic bonds, releasing quercetin conjugates prior to enterocytes absorption. So, the bioavailability could be affected, which could also reflect in systemic effects explaining the differences between the pharmacological activities *in vitro* and *in vivo* (Guo and Bruno 2015; Kawabata et al. 2015).

The anticancer effects of quercetin have been confirmed *in vitro* and *in vivo* assays that reveal anticancer activity against different tumors such as oral, breast, gastric, prostate and colon, among others (Kee et al. 2016; Li et al. 2019; Liu et al. 2017b; Shu et al. 2018; Srinivasan et al. 2015; Wu et al. 2019; Zeng et al. 2018; Zhao et al. 2019). It must be highlighted that quercetin may act as antioxidant promoting chemopreventive effects as well as pro-oxidant revealing chemotherapeutic effects. Indeed, it must be pointed that the anticancer effect of quercetin is exerted through multiple intracellular molecular targets (Fig. 10.2), that are involved in carcinogenesis (Neuwirthová et al. 2018).

Although there is a wide variety of quercetin preclinical trials concerning cancer disease, few reports assessing its clinical effects are available. Thereby, the steady increase in information about quercetin anticancer properties is sure to stimulate further research in humans.

2.1 Quercetin and Colorectal Cancer

The potential of flavonoids to modulate risk cancer comes from strong evidences of epidemiologic studies that recommend increasing flavonoid-rich foods consumption (Bondonno et al. 2019; He and Sun 2016). In this way, a long-follow up

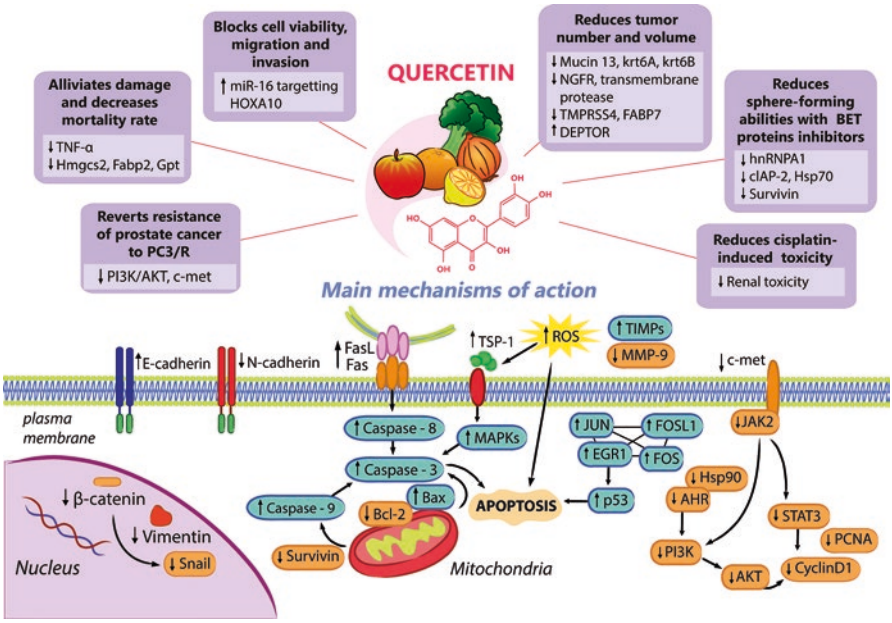

Fig. 10.2 Main molecular mechanisms triggered by quercetin to exert the anti-cancer effect. Quercetin has multiple intracellular targets in a cancer cell, triggering several cellular signals modulating carcinogenic genes and proteins. These anti-cancer effects include to promote the loss of cell viability with induction of caspases or ROS production, autophagy through MAPKs, PI3K/AKT, JAK2/STAT3 pathway or Fas activation, leading to apoptosis, and causing cell cycle arrest. Quercetin can regulate TSP-1, miR-16, matrix metalloproteinases, E-cadherin, N-cadherin, β-catenin and snail expression to reduce metastasis. The tumor growth can be delayed by modulation of PCNA, Bax, hnRNPA1, Hsp70 and Survivin expression by quercetin treatment

prospective cohort study with 56,048 participants revealed that a moderate and habitual intake of total flavonoids (~500 mg/day) and individual flavonoid subclasses including flavanols was associated with a lower risk of all-cause mortality, included cancer-related (Bondonno et al. 2019).

The colorectal cancer (CRC) is the fourth most diagnosed in the world (Lucente 2018). The quercetin protective effects in CRC were reported *in vitro*, *in vivo* and in human studies. These researches reveal that QE effects depend on the amount of quercetin dietary intake and involve mechanisms related to inflammatory pathways, inhibition of enzymes responsible for carcinogenesis as well as apoptosis induction and metastases inhibition (Bondonno et al. 2019; Djuric et al. 2012; Darband et al. 2018; Kee et al. 2016; Qi et al. 2019).

In vitro results showed QE potential to suppress colorectal metastasis (Kee et al. 2016). This flavanol inhibited the cell viability of colon 26 (CT26) and colon 38 (MC38) inducing apoptosis through mitogen-activated protein kinases (MAPKs) pathway in CT26 cells. Moreover, QE inhibited CT26 cells migration and invasion

through matrix expression metalloproteinases (MMPs) and tissue regulation inhibitor of metalloproteinases (TIMPs).

In addition, *in vivo* studies showed QE positive effects in CRC when mice were supplemented with alternated QE and β-glucan diet which alleviated colon damage and reduced the mortality rate in mice. This treatment significantly caused changes in the inflammatory pattern by decreasing TNF-α level and downregulating three kvv genes (Hmgcs2, Fabp2, and Gpt) associated to inflammation and the cancer (Qi et al. 2019).

A large case-control study included a total of 1163 cases and 1501 control participants, between 45 and 80 years, aimed to assess the QE consumption and the risk to both proximal and distal colon cancers. The authors found that the high intake of fruits rich in QE corresponded to a significant reduced risk for proximal colon cancer (Djuric et al. 2012).

In general, QE can display a wide variety of anticancer mechanisms in colon cancer, including cell cycle arrest, inhibition of cell proliferation, inflammation, angiogenesis, and metastasis as well as apoptosis and autophagy inductions which could be associated to positive results in humans' studies (Darband et al. 2018).

2.2 Quercetin and Breast Cancer

Breast cancer (BC) accounts for 30% of all new cancer diagnoses and is one of the tumors that causes more mortality in women (Siegel et al. 2019). Some observational studies have showed the association of lower incidence of breast cancer in population with a diet rich in flavonoid-rich foods (Desai et al. 2019; Hui et al. 2013).

In a meta-analysis conducted to examine the correlation between flavonoids intake and the breast cancer risk, it was verified that it significantly decreased in women with high intake of flavanols. Also, it was observed that flavanols, flavones or flavan-3-ols intake was correlated with a significant reduced risk of breast cancer in post-menopausal women (Hui et al. 2013).

Garlic and onion are consumed by Puerto Rican women which have a lower breast cancer rate compared to Europe and other countries. The combined effect of their intake was investigated in a population case-control study involving 314 breast cancer cases women and 346 controls from Puerto Rico. These vegetables were associated with a reduced risk of breast cancer, and the authors suggested that their moderate or high consumption are protective against breast cancer risk (Desai et al. 2019). These vegetables are rich in flavanols as quercetin as well as others natural compounds that could explain the referred results.

The lower risk of breast cancer with flavonoids intake could be correlated with pre-clinical experiments results which reiterate the great QE potential in breast cancer (Ezzati et al. 2020).

Triple Negative Breast Cancer (TNBC) do not express hormone receptivity or HER-2, tends to be more aggressive than others subtype of breast cancer and has a

high recurrence rates due metastatic lesions in distant sites (Watkins et al. 2019). The invasion ability is related to epithelial mesenchymal transition (EMT) that can be target of inhibition by flavonoids contributing to suppress cancer metastasis. The anti-tumor and anti-invasive ability of QE was investigated in TNBC cells *in vitro* (Srinivasan et al. 2015). QE induced the anti-tumor activity by inhibiting the migratory ability of TNBC cells as well as EMT markers modulation in a mesenchymal-to-epithelial transition. Furthermore, QE up-regulated E-cadherin and downregulated vimentin levels (Srinivasan et al. 2015).

The dose-dependent QE supplementation effects on mammary tumorigenesis were investigated in triple negative C3(1)/SV40Tag transgenic mouse model for 16 weeks to determine the optimal dose and to establish a novel mRNA expression profile (Steiner et al. 2014). This study demonstrated that the benefits in tumorigenesis capable to elicit an anti-neoplastic response can be reach with moderate doses of dietary quercetin (0.2%). The results showed tumor growth reduction (78%) and genes differential expression (31 down-regulated/9 up-regulated). The tumorigenesis reduction was mainly correlated with Mucin 13 downregulation, keratin 6A (krt6a) and keratin 6B (krt6b), nerve growth factor receptor (NGFR), transmembrane protease, serine 4 (TMPRSS4) and fatty acid binding protein-7 (FABP7) genes. In addition, the tumorigenesis reduction was also associated with DEP domain containing mTOR-interacting protein (DEPTOR) increased expression. Thus, the moderate QE treatment induced specific gene expression on mammary cells that could be responsible by anti-carcinogenic actions in breast cancer (Steiner et al. 2014).

QE associated to cisplatin can act synergistically to improve the efficacy against breast cancer cells. This effect is due to cancer growth inhibition and oxidative damage reduction, which leads to a decrease in the renal toxicity caused by cisplatin in EMT6 breast tumor-bearing mice (Liu et al. 2019b).

Additionally, QE has a great potential for use as natural compound in a complementary way or alternative treatment for breast cancer. This is confirmed by its anticancer effects that involves several anticancer mechanisms such as those described above and others including apoptosis induction, cell cycle progression inhibition, cell morphology alteration, inhibition of migration and as antiproliferative through the modulation of several metabolic pathways (Ezzati et al. 2020).

2.3 Quercetin and Prostate Cancer

Prostate cancer (PC) is a malignant disease within male population. The incidence worldwide is increasing, and the affected people can develop metastasis to bone or other organs.

QE was also assayed as prostate anticancer and successfully inhibited human prostate cancer cell xenograft tumor growth by preventing angiogenesis *in vitro* and *in vivo*. The QE treatment inhibited PC-3 and human umbilical vein endothelial

cells (HUVECs) metastasis, including proliferation, migration and invasion in a dose-dependent manner. Furthermore, QE inhibited PC-3 cell xenograft tumor growth in BALB/c mice without toxic reactions and reduced angiogenesis through TSP-1 upregulation (Yang et al. 2016).

A case-control study was developed in Western New York, involving 433 men with primary prostate cancer and 538 population-based controls correlating selected nutrients intake (including quercetin) to the risk of prostate cancer. The food frequency questionnaire analysis revealed that higher intakes of vitamin C, α- and β-carotene, lutein, lycopene, lignans, and quercetin significantly reduced PC risk. Since QE plays an important role in the antioxidant metabolism, which prevents carcinogenesis, QE intakes must be fundamental in reducing cancer risk (McCann et al. 2005).

The quercetin-doxorubicin combined treatment reversed the PC cell line resistant (PC3/R) to doxorubicin. Quercetin was able to downstream the phosphoinositide 3-kinase/protein kinase-B (PI3K/AKT) pathway, increasing the cell sensitivity to apoptosis *in vitro*, through tyrosine-protein kinase-met (c-met) downregulated expression (Shu et al. 2018).

2.4 Quercetin and Gastric Cancer

Gastric cancer (GC) remains one of the most common and deadly cancers in the world (Rawla and Barsouk 2019). Therefore, several studies were taken into account for quercetin potential in GC and several assays *in vitro*, *in vivo* and clinical trials were performed in order to establish an ideal protocol.

Human NCI-N87 gastric cancer cells were treated with QE and its DNA was isolated. Bioinformatic analysis revealed several target genes differentially expressed compared to control cells revealing four transcript factors upregulated (EGR1, FOSL1, FOS, and JUN) and one downregulated (AHR). These factors were involved with extracellular signal-regulated kinases 1/2-EGR1 pathway modulation and phosphatidylinositol-3-kinase/Akt signaling (Zeng et al. 2018).

QE inhibited cell growth and induced apoptosis, necrosis and autophagy in cancer cells (Haghi et al. 2017). In addition, *in vivo*, QE induced p53-dependent apoptosis by increasing expression of cleaved forms of caspase-3, -9 in xenograft mice models with human gastric carcinoma (Lee et al. 2016).

A study involving 505 Swedish patients, including men and women aged from 40 to 79 years, was carried out to assess the correlation between inclusion of QE in the diet and the risk of noncardia gastric adenocarcinoma. It was found that high dietary QE intake protected those from this adenocarcinoma and the effect was stronger for women exposed to oxidative stress, such as tobacco smoking (Ekström et al. 2011).

2.5 Quercetin and Oral Cancer

Oral cancer (OC) is one of the most frequent malignant diseases contributing for death and poor prognosis worldwide (Zhao et al. 2019). Since the conventional treatment for OC has several side effects, the search for new biomolecules and the use of new technology delivery system to improve the natural compounds effectiveness is fundamental. For this purpose, QE has been studied and has shown excellent results in several studies.

In OC cells from tumor tissues collected from patients, QE inhibit cell viability, cell migration as well as cell invasion by enhanced miR-16 expression. Indeed, the knockdown of miR-16 gene reversed the effect of QE on OC progression (Zhao et al. 2019). Further assays revealed that QE induced morphological cell changes and cell viability reduction (50%) in human OC cells. The last mechanisms occur by inducing apoptosis via cell surface receptor (Fas-L/Fas) and mitochondria-dependent pathways. The cell death mechanism also involves an early ROS production as well as increased levels of caspases 3, 9 and 8 in a time dependent way (Ma et al. 2018a).

QE performed a chemopreventive effect in oral squamous cell carcinoma in hamster's model. The QE treatment (12.5–50 mg/kg via oral gavage daily for 14 weeks), mainly in high doses, was able to induce tumor reduction as well as apoptosis through suppression of factor nuclear kappa-B (NF-κB) signaling and its target gene Bcl-2. Furthermore, the QE treatment induced an increase in the expression of the Bax gene. Other effects observed included significant reduction in the severity of hyperplasia, dysplasia and in the body-weight loss (Zhang et al. 2017a).

Currently, there are 15 studies listed in the ClinicalTrials.gov database (http://www.clinicaltrials.gov) that involve quercetin and cancer. The main studies include the following cancers: prostate, colorectal, pancreatic, lung, oral, renal, kidney, and lymphoma. Among these, one study characterized as phase 1 and phase 2, had a status of completed (Kooshyar et al. 2017). Since oral mucositis (OM) is one of the significant problems in chemotherapy, a protocol was designed to evaluate the quercetin role in this condition. Although the mucositis incidence was lower in the quercetin group (n = 10), further research must be recommended to support this result (Kooshyar et al. 2017).

2.6 Quercetin and Other Cancers

In vitro assays revealed the QE anticancer activity in several tumor cell lines as acute lymphoblastic leukemia MOLT-4T-cells, human myeloma U266B1 cells, human lymphoid Raji cells and ovarian cancer CHO cells, with various IC_{50} values (50–120 μM, 24 h) (Hashemzaei et al. 2017).

In human papillary thyroid cancer cells (B-CPAP) QE was able to induce cell death. The treatment resulted in cell proliferation decrease and apoptosis rate

increased by caspases-3 activation and arrested cells in S phase. These effects were triggered by Hsp90 (heat shock protein) downregulation that is involved in the decrease of chymotrypsin-like proteasome activity which causes the reduction of cell growth and induces cell death in thyroid cancer cells (Mutlu et al. 2016).

Indeed, *in vitro* and *in vivo* assays, with liver cancer cells, QE revealed tumor progression inhibition by apoptosis, metastasis, and autophagy (Wu et al. 2019). In hepatocellular carcinoma LM3 cells, both cell migration and cell invasion inhibition were associated with the regulation of N-cadherin, E-cadherin, vimentin, and matrix metalloproteinase-9 (MMP9) expression. *In vivo*, the treatment with QE affected the tumor growth significantly and showed proliferating cell nuclear antigen (PCNA) downregulation and Bax upregulation, both of them related to apoptosis induction and tumor cell proliferation (Wu et al. 2019).

As already well showed above, the anticancer effects of QE against different tumors such as colon, breast, prostate, gastric, oral, among others were confirmed *in vitro* and *in vivo* assays. There are several studies demonstrating the effect of QE treatment on cell lines, however, the corresponding effect in clinical trials in humans still is scarce.

3 Resveratrol

Resveratrol (RESV) is a polyphenol stilbene (3,5,40-trans-trihydroxystilbene) found in grapes, peanuts and berries (Xiao et al. 2019). The application of grape extracts for human health can be dated over 2000 years in Ayurvedic medicine (Paul et al. 1999). However, the benefits of RESV became famous from the observation of a reduced rate of coronary heart disease among followers of the Mediterranean diet who habitually consume red wine between meals (Renaud and Gueguen 1998). For investigate this issue, a large prospective epidemiologic study in Eastern France was carried out with 34,014 middle-aged men, showing that the moderate red wine intake was really associated with a reduced mortality rate including deaths from cancer (Renaud et al. 1998). These effects were attributed to high content of polyphenol antioxidants in red wine in which resveratrol is considered one of the main constituents. Nevertheless, one of the highest concentrations of resveratrol in nature is found at the *Polygonum cuspidatum* root, a plant used in traditional Chinese and Japanese medicine been referred as green anti-cancer drug (Guo et al. 2018).

The phytochemical RESV was isolated in 1939 and after its discovery RESV was object of several researches including its activities related to cancer chemoprevention and other applications to human health (Paul et al. 1999; Pezzuto 2019; Ramírez-Garza et al. 2018; Takaoka 1939). The anticancer potential of RESV was demonstrated on the cyclooxygenase-1 (COX-1) inhibition, human promyelocytic leukemia cell inhibition, phase II enzymes induction, preneoplastic lesions and tumorigenesis inhibition without cytotoxicity (Jang et al. 1997).

Nowadays, RESV exhibits extraordinary potential associated with life extension in addition to anticancer properties (Fig. 10.3), including anti-inflammatory (Hu

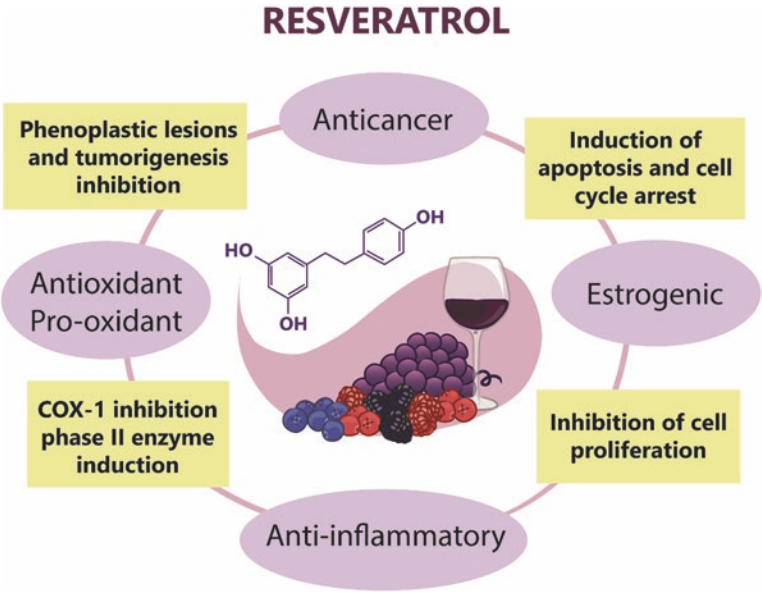

Fig. 10.3 Main biological activities of resveratrol involved on its anticancer effectiveness. The anticancer properties of resveratrol are associated with its anti-inflammatory and estrogenic attributes and it has a dual effect acting as an antioxidant or pro-oxidant in a dose-dependent manner. In antioxidant activity can decrease ROS and modulate antioxidant enzymes while in transient prooxidant effect it induces both ROS production and cytoprotective oxidative stress-activated enzyme NAD(P)H dehydrogenase expression. The anti-inflammatory properties have been demonstrated by its ability to downregulate COX-2 and prostaglandin E-2. Furthermore, resveratrol also has a dual estrogenic activity of block and activate estrogen receptors, such as α-V-β3 integrin receptor that induces pro-apoptotic genes and anti-proliferation

et al. 2010), and antioxidant activities, autophagy and apoptosis process (Sirerol et al. 2016), and others multiple intracellular pathways (Chottanapundet al. 2014; Honari et al. 2019; Liu et al. 2014; Moosavi et al. 2018; Zhou et al. 2018). Moreover, RESV has estrogenic effects which have been tested in several *in vitro* and *in vivo* carcinogenesis assays with different types of cancers (Chin et al. 2015; Hasan and Bae 2017; Martínez et al. 2014; Salehi et al. 2018; Zhao et al. 2018).

The autophagy has a dual role in cancer biology, however under stress conditions, this process can trigger the tumor cell death and therefore its regulation has significance in the treatment of cancer (Kocaturk et al. 2019). RESV can be autophagy an inducer of autophagy in different cancer cells (Andreadi et al. 2014; Russo and Russo 2018) by signaling pathways such as AMPK and SIRT1 activation and directly inhibiting the mechanistic target of rapamycin (mTOR) which contributes for reverse multidrug resistance in cancer cells (Tian et al. 2019).

Informationon the chemotherapeutic effect of RESV in humans is increasing due to the large number of anti-cancer activities demonstrated in preclinical evidences.

Thus, epidemiologic and clinical trials have been carried out in different cancer types to show additional evidence of resveratrol. Here we highlighted the main outcomes of resveratrol in different levels of evidence as well as its synergic, adverse, and controversial effects in some cancers.

3.1 Resveratrol and Colorectal Cancer

Regarding the anticancer properties of resveratrol on gastrointestinal system, there are promising results on the prevention of CRC. A study with colon cancer cell lines (CACO-2) revealed cells susceptibility to RESV, which in a dose-dependent manner increased apoptosis-promoting effects predominantly through cell cycle arrest by caspase-dependent and cyclin-CDK pathways (Liu et al. 2014). A phase I study in patients with CRC and hepatic metastases, analyzed the micronized RESV (SRT501) effects in hepatic tissue after after administration of 5.0 g daily, for 14 days. This concentration induced an increase in cleaved caspase 3 (Howells et al. 2011).

Wnt signaling pathway is associated with colon cancer initiation. The RESV-containing freeze-dried grape powder (GP) effects were studied through the administration of GP (80 g/day containing 0.07 mg of resveratrol) for a fortnight. The results showed that Wnt target gene expression was inhibited only in the normal colonic mucosa, with no effects on cancerous mucosa. The authors suggest that resveratrol may act preventing colon cancer through the inhibition of the Wnt pathway (Nguyen et al. 2009). Indeed, Patel et al. (2010) studied 20 patients with CRC treated with RESV (eight daily doses of resveratrol at 0.5 or 1.0 g before surgical resection). The RESV consumption reduced tumor cell proliferation suggesting anticarcinogenic effects.

As previously cited, RESV presents antioxidant and anti-inflammatory properties. In a study using CRC Apc^{Min} mice, low RESV dose reduced tumor growth. This corresponded to ROS *in vitro* increase detected in patients' samples with resectable CRC who received RESV capsules (5 mg or 1.0 g for 6 days). In this condition, RESV-prooxidant activity was related with its anticancer efficacy. In addition, low RESV dose supplementation increased the expression of cytoprotective oxidative stress-activated enzyme NAD(P)H dehydrogenase, quinone 1 (NQO1) regulated by Nrf-2 in human CRC cells (Cai et al. 2015).

As a matter of fact, in response to intestinal mucosa stress, there was an increase in adenosine mono phosphate activated protein kinase (AMPK) expression followed by autophagy which may contribute for tumor-suppressing mechanism. Thus, it was highlighted that a low RESV dose promoted anticancer effect mediated by transient prooxidant activity and autophagy induction (Cai et al. 2015).

Indeed, remembering the inflammatory process reported in intestinal cancers, RESV also revealed an anti-inflammatory effect in CACO-2 cells by downregulation of both COX-2 and prostaglandin E-2 (PGE-2) associated to NF-kB inhibition (Cianciulli et al. 2012) as well as COX-2 mRNA expression reduction due to superoxide and peroxide suppression in human CRC cells (Gong et al. 2017).

3.2 *Resveratrol and Breast Cancer*

Breast cancer has been intensively studied and there are some evidences that RESV might be an important ally in the treatment.

RESV can act as a phytoestrogen once it is capable of blocking and activate estrogen receptors (ER) and due to this reason, it is considered as potential anti-cancer adjuvant (BHAT et al. 2001). On the other hand, it is known that dihydrotestosterone (DHT) is a strong promoter of the breast cancer for binding in ER. Interestingly, the receptor for DHT also exists on α-V-β3 integrin receptor that also binds to RESV, inducing pro-apoptotic genes and anti-proliferation (Chin et al. 2015). Although both RESV and DHT signals are transduced by ERK1/2, the responses are different. Chin et al. (2015) studied the effects of both RESV and DHT in ER-α-positive and negative breast cancer cells. In both types of cells, RESV bound to integrin α-V-β3, promoting nuclear accumulation of COX-2 and p53-dependent action, inducing anti-proliferation. This inhibition of apoptosis by DHT in RESV-treated cells was showed to be due to the binding of DHT to the integrin receptor on the cell surface for the hormone. This fact promoted suppression of the nuclear interaction of COX-2 protein and activated ERK1/2, which are related with decrease in inflammatory molecules as well as is essential to the pro-apoptotic action of RESV.

Recently, the resistance to chemotherapy on the breast cancer treatment became an issue on the therapy effectiveness. To overcome this situation, many studies were developed with RESV in combination with other chemotherapeutic agents or drug-delivery systems to decrease resistance to treatment, improve its metabolization and bioavailability as well as to reduce the related side effects in anticancer therapy (Castillo-Pichardo and Dharmawardhane 2012; Kim et al. 2014a; Lee et al. 2019a, Redondo-Blanco et al. 2017; Sheu et al. 2015; Zhao et al. 2016).

RESV also showed promisors results on the reduction of multidrug resistance when co-encapsulated with paclitaxel (Meng et al. 2016). Furthermore, this interesting molecule plays a role in the sensibilization to gefitinib (an epidermal growth factor receptor specific tyrosine kinase inhibitor), reduce metastasis as well as tumor burden (Castillo-Pichardo and Dharmawardhane 2012). In addition, new drug-delivery systems increased the efficacy and delivery rate of RESV in human breast cancer (MCF-7) cell line when conjugated with gold nanoparticles (Lee et al. 2019a).

The treatment with both RESV and doxorubicin (DOX) combined notably increased the cellular accumulation of DOX. This action occurred due the down-regulation of the expression levels of ABC transporter genes, MDR1, and MRP1 and due the inhibition of drug-resistant in human breast cancer (MCF-7/adr) and MDA-MB-231 mice cell line. Thus, RESV was able to enhance the DOX-induced cytotoxicity (Kim et al. 2014a). Indeed, another study suggested the co-encapsulation of both RESV and DOX in a modified PLGA nanoparticle (NPS) to overcome the DOX-resistance *in vitro* and *in vivo* (Zhao et al. 2016). The complex DOX-RESV-NPS could overcome DOX resistance by inhibiting the expression of P-gp,

MRP-1 and BCRP, and induce apoptosis through down-regulating the expression of NF-κB and BCL-2. In tumor-bearing mice, the complex DOX-RES-NPS mainly delivered DOX and RES to tumor tissue. Compared with free DOX, the complex DOX-RESV-NPS inhibited the DOX-resistant tumor growth in mice, do not presenting expressive systemic toxicity.

In cancer chemotherapy, RESV reduced the intracellular ROS level and increased the SOD level in a co-treatment with doxorubicin in breast cancer cells demonstrating its efficacy in decrease the cardiotoxicity in doxorubicin-mediated chemotherapy (Sheu et al. 2015).

A case-control study carried out on 369 cases vs. 602 controls of Swiss women with follow up for 10 years demonstrated that dietary intake of RESV from grapes is inversely related to the risk of breast cancer (Levi et al. 2005). At the treatment with trans-RESV (5 or 50 mg twice a day for 12 weeks), 39 adult women with increased breast cancer risk presented a decrease in the fraction of methylated RASSF-1α DNA, a tumor suppressor gene associated with breast cancer. Furthermore, it was observed an increased level of trans-RESV in the circulation and a decreased (Zhu et al. 2012).

3.3 Resveratrol and Prostate Cancer

Regarding prostate carcinoma (PC), RESV inhibits cell proliferation and induces apoptosis of human (PC) DU-145 cell lines (Agarwal et al. 2000). This effect occurs due the induction of cell cycle arrest in PC3 and DU145 androgen-insensitive cells (Lin et al. 2002; Sgambato et al. 2001). MicroRNA-21 levels (miR-21) are frequently elevated in PC contributing for the invasiveness. Thus, the strategy to inhibit miR-21 by gene therapy could be useful on cancer treatment (Zennami et al. 2019). *In vitro*, RESV revealed miR-21 expression decrease in PC-3 M-MM2 cells and increased expression of PDCD4 and Maspin genes, which are negatively regulated by miR-21 (Sheth et al. 2012). The *in vitro* results were corroborated by *in vivo* model of PC since RESV reduced PC growth and metastasis. The authors pointed in evidence that the Akt/miR-21 pathway is responsible for anticancer actions of resveratrol in prostate cancer (Sheth et al. 2012).

In a phase I clinical study, 35 μg of resveratrol contained in 4000 mg of pulverized muscadine grape extract was daily administrated in patients with prostate cancer and delayed the recurrence by prolonging the prostate specific antigen doubling time by 5.3 months (Paller et al. 2015). RESV has also been shown to be effective in improving chemotherapy in prostate cancer cells. The association between docetaxel (DTX) and RESV induced upregulation on pro-apoptotic genes and downregulation on anti-apoptotic genes (Singh et al. 2017).

3.4 Resveratrol and Other Cancers

RESV has demonstrated its effectiveness also in lung cancer. Several studies have been performed to evaluate its effect and mechanism. The main findings are related to the induction of apoptosis (Thomas et al. 2016; Whyte et al. 2007; Zhang et al. 2015a, b), increasing in cell cycle arrest (Han et al. 2012; Whyte et al. 2007; Zhao et al. 2009), and reduction of tumor cells proliferation (Han et al. 2012; Thomas et al. 2016; Wu et al. 2010; Zhao et al. 2009). The mechanisms involved on the findings include mainly the induction of caspase-3 and caspase-9 (Lucas and Kolodziej 2015; Yousef et al. 2017; Zhao et al. 2009; Zhang et al. 2015a) and upregulation of p53 expression (Luo et al. 2013; Whyte et al. 2007; Yuan et al. 2015). The reduction of tumor cells was also verified *in vivo* studies (Savio et al. 2016; Wu et al. 2010; Zhao et al. 2009). Furthermore, the action against lung metastatic cancer may be maybe related to its anti-angiogenic activity (Savio et al. 2016) as well as the ability of reduce tumor suppressor genes levels (miR-21 and elevated PDCD4) as occurs in prostate cancer (Sheth et al. 2012).

A study with oral squamous cancer cell lines revealed a dose-dependent action of RESV on both inhibition of cell proliferation and cell cycle arrest in the G2/M phase. Furthermore, REVS induced an increase in phospho-CDC2 and cyclins A2 and B1 expression by oral squamous cell carcinoma (Yu et al. 2016). *In vitro* studies using human esophageal cancer cell line TE-1 demonstrated that high concentrations of RESV inhibited cell growth (Dun et al. 2015). In addition, the anti-angiogenic effects of RESV were evidenciated when it was used in combined treatment with 5-fluorouracil (5-FU) on B16 murine melanoma tumors models. The co-treatment induced downregulation of COX-2, VEGF and VASP levels while occurred an upregulation of AMPK. *In vivo*, this effects resulted in an antiproliferative activity. On the other hand, *in vivo* occurred a reduction in microvessel density, angiogenesis inhibition and consequently reduction in tumor size (Lee et al. 2015).

The dietary pattern rich in RESV and other phytochemicals was associated with the low risk of esophageal cancer development in a case–control study. In this investigation, 181 cases of esophageal adenocarcinoma, 158 cases of esophageal squamous-cell carcinoma, 255 cases of gastro-esophageal junctional adenocarcinoma and 806 controls were enrolled. The results demonstrated that RESV may act synergistically with dietary phytochemicals in prevention of all types of esophageal cancer evaluated (Lin et al. 2014). These results emphasize the idea that natural dietary agents are good options on ~~the~~ cancer prevention.

The effects of RESV and the mechanisms involved in pancreatic and ovarian cancer are similar to the others *in vitro* and *in vivo* models discussed above. The pretreatment with resveratrol showed an antineoplastic effect on ovarian cancer cells with tumor growth suppression after the treatment with cisplatin, suggesting a prolonged disease-free survival (Tan et al. 2016). A synthetic analog of resveratrol, DHS (Trans-4,4′-Dihydroxystilbene), enhances the sensitivity of pancreatic and ovarian cancer cells to chemotherapeutic agents *in vitro*. This action occurs via inhibition of ribonucleotide reductase regulatory subunit M2 (RRM2), a potent inhibitor

of DNA replication. In murine models of tumor xenografts, DHS was efficient against pancreatic, ovarian, and colorectal cancer cells (Cheng et al. 2018). In addition, a study on pancreatic cancer demonstrated the effect of resveratrol *in vitro* and *in vivo* on the downregulation of NF-kB and NF-kB dependent gene expression and suppression of proliferation, metastasis and angiogenesis markers (Harikumar et al. 2010).

4 Carotenoids

Carotenoids represent a diverse group of phytochemicals tetraterpenoids that include pigments such as lycopene, β-carotene, α-carotene, lutein, zeaxanthin, and cryptoxanthin (Rowles and Erdman 2020). In plants, these pigments contribute to the visible colors and vary in range from light yellow through orange to red (Krinsky and Johnson 2005). Fruit and vegetables are the primary sources of carotenoids, in which its concentration vary widely (Khoo et al. 2011). In human diet, they are primarily derived from crop plants, in edible leaves, flowers, and fruits such as banana, acerola, apple, mango, orange, tomato and papaya (Cardoso et al. 2017; Lemmens et al. 2014; Namitha and Negi 2010). In vegetables, they are found in carrots, pumpkins as well as spinach, broccoli, and green leafy vegetables (Namitha and Negi 2010).

Epidemiological studies showed a correlation between carotenoids rich diet and preventive actions such as low incidence of specific types of cancer, improved efficiency of treatment and decreased aggressiveness (Abar et al. 2016; Baglietto et al. 2011; Boggs et al. 2010; Chen et al. 2001, 2013; De et al. 2004; Giovannucci 2002; Kim et al. 2018; Liu et al. 2003; Rowles et al. 2017, 2018). The main mechanisms by which carotenoids are involved in anti-cancer action are associated with processes of cell growth and death as well as that related to the antioxidant effect (Liu et al. 2003; Namitha and Negi 2010; Niranjana et al. 2015; Rowles and Erdman 2020).

Studies *in vitro* and *in vivo* have reported several biological properties from carotenoids, among them chemopreventive in a wide range of cancer types (Fig. 10.4) (Milani et al. 2017; Saini and Keum 2018). Lycopene shows potential to interfere in the process of carcinogenesis in different cancer types such as (Rowles et al. 2017; Wang et al. 2015), breast (Aune et al. 2012), oral cavity (Leoncini et al. 2016), pharynx (Kubo and Corley 2007), digestive tract (Yang et al. 2013), and lung (Abar et al. 2016; Gallicchio et al. 2008). β-Carotene showed biological activities against several cancer types such as leukaemia (Upadhyaya et al. 2007), colon cancer (Palozza et al. 2002), gastric cancer (Jang et al. 2009), adenocarcinoma (Palozza et al. 2001), neuroblastoma (Kim et al. 2014b), hepatocarcinoma (Chen et al. 2012, 2013), skin tumours (Mathews-Roth 1982) and prostate cancer (Chen et al. 2012; Soares et al. 2013; Wan et al. 2014). α-Carotene was associated to hepatocarcinoma (Chen et al. 2013). In addition, lutein, zeaxanthin, and cryptoxanthin were also associated with low skin cancer incidence (Heinen et al. 2007; Juin et al. 2018; Logozzi et al. 2019).

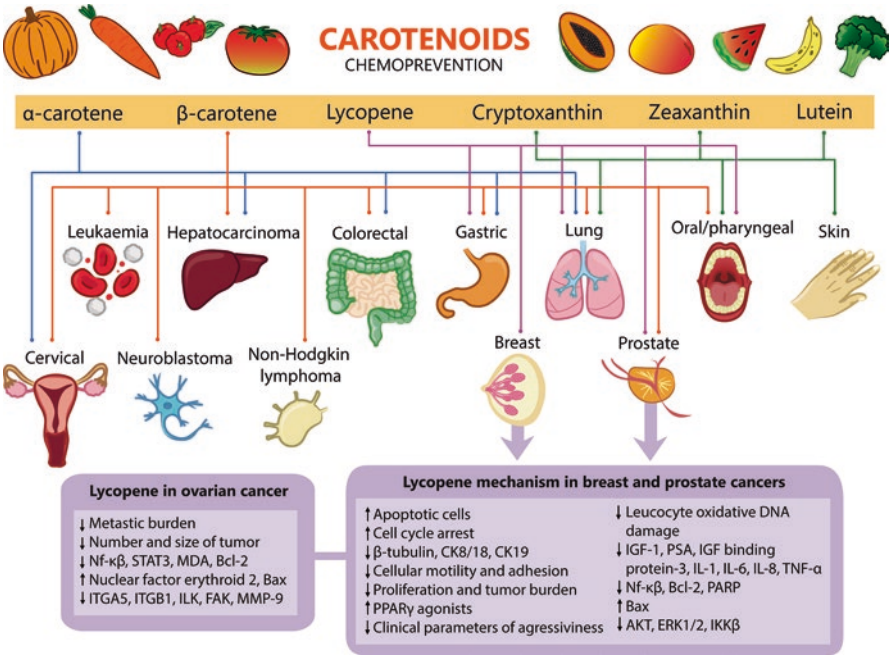

Fig. 10.4 Carotenoids as chemopreventive against cancer and action mechanisms of lycopene in breast, prostate and ovarian cancer. Different carotenoids are involved in the prevention of various types of cancer. Lycopene (Lyc) presents anti-inflammatory effect by reduction of cytokines (IL-1, IL-6, IL-8, TNF-α), by modulating NF-κB signaling pathway, anti-proliferative effect (PPARγ, b-tubulin, CK8/18, CK19), modulates biomarkers of growth and differentiation (IGF-1, IGF-3) and promotes arrest cell cycle. Moreover, reduces oxidative stress (DNA damage) and PSA levels, inhibits motility and cell adhesion leading a reduced metastasis and induction of apoptosis (Bcl-2, PARP, Bax), reducing tumor burden and cancer aggressiveness in breast, ovarian and prostate

Contrarily, there is a great variation in the action of these substances in different types of cancer and many researches present inconclusive or controversial results (Botterweck et al. 2000; Lai et al. 2014; Petimar et al. 2017; Takachi et al. 2010; Umesawa et al. 2014;). This section will focus on studies that show an association between the consumption of carotenoids and their anti-cancer effect, in particular lycopene, which is the most studied carotenoid, but it also includes studies with β-carotene, α-carotene, zeaxanthin, lutein and cryptoxanthin.

4.1 Lycopene and Prostate Cancer

Prostate cancer is one of the most common cancers in men and it is considered a global public health problem. Clinical and epidemiological studies indicate that diet plays a key essential in prevention and development of prostate cancer (Gathirua-Mwangi and Zhang 2014; Mokbel et al. 2019).

A clinical trial that involved prostate carcinoma patients (32 men) which received tomato sauce (30 mg lycopene/day) for 3 weeks before prostatectomy showed that its consumption increased apoptotic cells in benign prostatic hyperplasia (BPH) and in carcinomas (Kim et al. 2003). In first time, in carcinomas the results showed that tomato sauce consumption did not alter Bcl-2 expression and reduced Bax expression. However, in subsequently experiment with prostate carcinoma biopsies was verified the overexpression of genes related to apoptosis (Bcl-2 and Bax), when compared to controls cells (Kim et al. 2003).

These findings were corroborated by *in vitro* studies with lycopene modulating the transcriptional expression levels of Bax and Bcl-2 and this up-regulated process was correlated with the apoptotic effect in cancer cells but no in BPH cells (Soares et al. 2013, 2017). *In vitro*, lycopene induced inhibitory effect on primary prostate epithelial cell (PEC) (Barber et al. 2006). In addition, lycopene reduced the numbers of cells in G0/G1 phase and raised in S and G2/M phases in metastatic prostate cancer cell lineages and promoted cell cycle arrest in G0/G1 phase in a primary cancer cell line (Soares et al. 2013).

In vitro, lycopene presents several properties as reduction of cellular motility and promotes the blocking of cancer cell adhesion (Elgass et al. 2014), reduction of inflammatory cytokines levels, including interleukin-1 (IL-1), IL-6, IL-8, and tumor necrosis factor-alpha (TNF-α) (Jiang et al. 2019). Indeed, lycopene decreased proliferation on prostate cancer (PC-3) cells, alteration of growth and apoptosis associated to biomarkers expression (Rafi et al. 2013). It is worth mentioning that in prostate cancer lycopene reduced the tumor burden (Jiang et al. 2019), and increased the anti-proliferative effect of peroxisome proliferator-activated receptor gamma (PPARγ) agonists, however, neither Doxorubicin or Taxol, were able to do so (Rafi et al. 2013).

Several clinical trials were performed to evaluate the effect of lycopene supplementation on prostate cancer. Lycopene supplementation (30 mg/day), either in the form of tomato sauce or oleoresin tablets, for a short period before radical prostatectomy (36 men) increased lycopene concentrations in prostate tissue and modulated biomarkers of growth and differentiation (insulin-like growth factor-1) (IGF-1), IGF binding protein-3, prostate-specific antigen (PSA)), and decreased clinical parameters of cancer prostate aggressiveness (Kucuk et al. 2001). Analogously, the intervention with tomato sauce based pasta dishes for 3 weeks (30 mg of lycopene per day) on patients with localized prostate adenocarcinoma (32 men) increased serum and prostate lycopene levels and reduced leukocyte oxidative DNA damage, and serum PSA levels (Chen et al. 2001). Confirming previous findings, another pilot phase II clinical study with lycopene dietary supplementation (10 mg/day) reduced cancer prostate progression (Cap) (Barber et al. 2006). In a double-blind crossover study, 30 healthy adults daily consumed 160 g of a high-lycopene tomato sauce which evidenced the putative role of lycopene in the prevention of oxidative stress related diseases (Abete et al. 2013). Thus, there are sufficient evidences that lycopene in diet is inversely associated with risk of prostate cancer and draws attention for potentially prevent cancer.

On the other hand, some studies do not support these evidences. In a multicenter study the association between intake of lycopene and specific tomato products and prostate cancer risk (29,361 men) in a follow-up of 4.2 years with 1338 cases of CaP identified, the reduced risks were not found (Kirsh et al. 2006). In a double-blind, randomized, placebo-controlled trial of 105 African American men veterans, no significant changes in the 8-oxodeoxyguanosine or the lipid peroxidation product malondialdehyde were observed in prostate tissue or plasma, respectively, as a result of 30 mg/day lycopene as a tomato oleoresin supplementation (Van Breemen et al. 2011).

In another phase II randomized, double-blind, placebo-controlled trial carried out in Chicago, the participants consumed lycopene (30 mg/day) for 6 months, but no effect were detected on serum PSA, IGF-1, or IGFBP3 concentrations, neither any effect on proliferation or cell cycle inhibition in benign tissue of men with high grade prostatic intraepithelial neoplasia (HGPIN) was observed (Gann et al. 2015). However, lycopene supplementation increases its levels in serum and prostate tissue (Van Breemen et al. 2011), increases atrophy and decreases in extensive HGPIN (Gann et al. 2015).

Other randomized trials revealed a decreases in prostate cancer risk (Peisch et al. 2017; Perez-Cornago et al. 2017), even if other studies did not demonstrate strong associations between prostate cancer risk and dietary factors (Lane et al. 2017).

4.2 Lycopene and Breast Cancer

Among women, the breast tumour is the most common type. Several factors are related to the risk of developing breast cancer, including diet (Hauner and Hauner 2010; Sauter 2018). In a systematic review, the results suggest that the dietary patterns that include vegetables may reduce breast cancer risk (Dandamudi et al. 2018). Studies indicate that lycopene presents anticancer properties that could be associated to reduced breast cancer risk (Sesso et al. 2005; Yan et al. 2016).

Regarding breast cancer, it has been proposed that carotenoids play a similar role to prostate cancer, such as decrease of the anti-apoptotic protein, Bcl-2, poly ADP-ribose polymerase (PARP), pro-inflammatory and survival protein NF-kB expressions, and activation of the growth signaling proteins, Akt and ERK1/2, where some of them show a clear association with clinical studies (Aune et al. 2012; Rowles and Erdman 2020).

On human breast cancer cells lycopene inhibited cell proliferation through the modulation of cell cycle proteins such as beta tubulin, CK8/18, CK19 (Uppala et al. 2013), arrested cell cycle that can be related to cellular type, time and dose-dependent (Teodoro et al. 2012). Lycopene inhibited the activity of IκB kinase β (IKKβ) (Assar et al. 2016) that consequently modulated the NF-κB signaling pathway and then promoted suppression of TNF-α, a proinflammatory cytokine (Assar et al. 2016), presenting anti-inflammatory effect.

The results of a pilot case-control study performed in Chicago among African, American, and Caucasian women suggest that the plasma lycopene level may be associated with the reduction of reduction of breast cancer risk (Simon et al. 2000). On the other hand, in a prospective cohort study conducted with 39,876 women, the reduced risks to breast cancer development were not found with high dietary lycopene or plasma lycopene levels (Sesso et al. 2005).

4.3 Lycopene and Ovarian Cancer

Ovarian cancer (OvCa) is a leading cause of death among women worldwide. It has been suggested that one of the mechanisms for preventing ovarian cancer is the reduction of oxidative stress. Among carotenoids, lycopene is a well-known natural antioxidant with anti-cancer properties. Considering that fruit and vegetables are a main source of carotenoids, studies were carried out to characterize and analyze dietary patterns in relation to OvCa risk.

The human studies with carotenoids still remain controversy although preclinical studies have been showed promissor findings. A clinical study investigated the plasma carotenoids levels related to ovarian cancer risk in Korean women. The results showed that among antioxidants levels, lycopene was capable to reduce 90% the OvCa risk in the Korean population (Jeong et al. 2009). However, a meta-analysis that included 678,892 subjects and 6127 cases displayed an insignificant inverse association between dietary lycopene consumption and ovarian cancer risk (OR, 0.963; 95% CI, 0.859–1.080) and although the findings are not significantly the authors suggested the importance of lycopene in the diet for OvCa prevention between postmenopausal women (Li and Xu 2014).

In OvCa intraperitoneal animal model the lycopene acted in the mechanisms involved in the development and progression of the tumor. When combined with paclitaxel and carboplatin, it reduced the tumour and metastatic burden of OvCa in vivo. This study shows the relevance of lycopene in the prevention and treatment of OvCa (Holzapfel et al. 2017). The lycopene supplementation reduced ovarian tumour incidence, as well as the number and the size of the tumours in Laying Hens. In this case, lycopene reduced NF-κB expression, increased nuclear factor erythroid 2 expressions, decreased STAT3 expression, which characterize anti-inflammatory mechanisms, and decreased serum malondialdehyde levels, which shows antioxidant effect (Sahin et al. 2018). *In vitro* lycopene inhibits the proliferation of ovarian cancer cells and enhances their apoptosis possibly mediated by up-regulating Bax expression and down-regulating Bcl-2 expression (Xu et al. 2019).

Recently, Zhang et al. (2017b) reported an important study using lycopene nanoparticles associated with low dose of trichostatin A, a chemotherapy agent, and showed positive results against human ovarian cancer cells (SKOV3). The authors showed that their combinatory effects caused excellent cytotoxicity and induced greater apoptosis in SKOV3 cells through regulation of various mechanisms. They

suggested that it could be an alternative in cancers that cannot be submitted to radiation therapy or surgical treatment.

4.4 Other Carotenoids and Cancers

Although *in vivo* and *in vitro* studies suggest differential mechanisms of carotenoid action to protect against prostate cancer (Rafi et al. 2013; Soares et al. 2013; Wan et al. 2014), epidemiological studies are still confusing and controversial. According to Petimar et al. (2017) there is no association between total tomato consumption and reduced risk of prostate cancer. On the other hand, results of a meta-analysis study estimate a 9% reduction in the risk of prostate cancer for every 10 g of cooked tomato/week, due to the combination of lycopene with other carotenoids and their bioavailability through the cooking process (Rowles et al. 2017). In another meta-analysis study, consumption of α-carotene, but not β-carotene, reduced the risk of prostate cancer by 13% and the dose-response association decreased by 2% for each 0.2 mg of α-carotene consumed (Wang et al. 2015). In addition, a correlation was observed between high concentrations of β-carotene serum and low risk of prostate cancer (Karppi et al. 2012).

The relationship between high rates of α-carotene and β-carotene serum demonstrated an inverse association with the risk of cervical cancer in women (Guo et al. 2015). In addition, a review study showed a similar association between 1 mg β-carotene consumption (1000 Kcal dietary) and lower risk (12%) of endotmetrial cancer (Okuyama et al. 2014) and a decrease 16% the ovarian cancer risk with higher consumption of β-carotene (Huncharek et al. 2001).

Diets rich in β-carotene have been associated with a 5% reduction in the rate of breast cancer for every additional 5 mg consumed. Also, blood α-carotene and lutein levels were associated with a decrease in breast cancer (Aune et al. 2012). Another study revealed that plasma α-carotene level seems to be related to a lower rate of invasive breast cancer in the post-menopausal period. Hence, it was associated with a 37% decreased risk of invasive estrogen receptor-positive breast cancer (Wang et al. 2015). Besides, high levels of β-carotene could be involved in reduced risk of breast cancer in women while high levels can also reduce the breast cancer risk (Eliassen et al. 2012). In vitro, the treatment with β-carotene inhibited cell proliferation, arrests cell cycle, and increased apoptosis in human breast cell lines (Gloria et al. 2014).

A higher intake or serum concentrations of total carotenoids (lycopene, β-cryptoxanthin, α and β -carotene, and lutein/zeaxanthin) were strongest associated with a significant decrease in lung cancer risk than individual carotenoids (Gallicchio et al. 2008). It was observed through a meta-analysis study an increase in blood concentrations of lycopene (10 μg/100 mL), β-carotene (20 μg/100 mL), α-carotene (5 μg/100 mL) related to a decreasing in relative risk for lung cancer. Indeed, lower blood concentrations of β-cryptoxanthin (5 μg/100 mL) showed association with lung cancer risk in a non-linear relationship (Abar et al. 2016). In fact,

some animal studies show that β-cryptoxanthin, zeaxanthin and lycopene appear to prevent various types of cancer through NF-κB, RAR/PPARs, RAR/PPARs, SIRT1 signaling pathways and p53 tumor suppressor pathways mediated by their oxidative metabolites (Lim and Wang 2020).

The consumption of β-carotene can decrease the risk of lung cancer by 2% for each extra mg consumed per day and 1% for each 10 μg of β-cryptoxanthin consumed per day as well as 3% for additional consumption of lycopene(mg) per day (Abar et al. 2016; Gallicchio et al. 2008). These data are intriguing because this association only occurred with smokers. On the other hand, supplementation with high doses of β-carotene (about 10–20 times higher than normal dose) increases the risk of lung cancer in smokers and people exposure to asbestos (Goodman et al. 2004; Omenn et al. 1996; Virtamo et al. 2000). In addition, β-carotene in similar doses, can function as a pro-oxidant and/or co-carcinogenic substance (Rowles and Erdman 2020). High levels of β-carotene activate cytochrome P450 enzymes leading to increased activation of tobacco smoke pre-carcinogens and the formation of alternate, harmful, β-carotene and retinol metabolites (Goralczyk 2009). Indeed, it is accepted that while β-carotene and retinol are protective at physiologic doses and derived from fruit and vegetables consumption, high-dose supplementation is harmful particularly in the context of cigarette smoke exposure (Hada et al. 2019).

The carotenoids also showed chemopreventive effects in colorectal cancer. *In vitro* β-carotene had a positive effect against human adenocarcinoma colon cancer cells (Niranjana et al. 2015). In human studies, serum carotenoids including lutein, zeaxanthin, alfa-carotene, β-carotene was inversely associated with a reduced risk of colorectal cancer among Japanese women (Okuyama et al. 2014). Subsequently, in a case-control study performed with Korean population, a high dietary lutein and zeaxanthin intake reduced significantly the colorectal cancer risk (Kim et al. 2019). The possible protective role of xanthophylls especially lutein against carcinogenesis includes the selective modulation of apoptosis, inhibition of cellular differentiation and effects on angiogenesis as well as anti-oxidative activity decreasing reactive oxygen species (ROS) and consequently oxidative stress (Madaan et al. 2017; Ribaya-Mercado and Blumberg 2004).

In general, the consumption of two or more carotenoids associated such as α-carotene, β-carotene, β-cryptoxanthin, zeaxanthin, lutein, lycopene and food rich carotenoids are also involved with a reduced risk of head and neck cancer, oral and pharyngeal cancer, non-Hodgkin lymphoma, skin cancer and gastric cancer (De et al. 2004; Heinen et al. 2007; Kim et al. 2018; Kubo and Corley 2007; Larsson et al. 2007; Leoncini et al. 2015; Lissowska et al. 2004; Pelucchi et al. 2008; Ward et al. 2019). It is known that the consumption of phytochemicals triggers multiple mechanisms, and the phytochemical combinations may have synergistic effects. Additionally, cancer treatments combined with a diet rich in carotenoids may potentiate their anticancer effects and improve prognosis. One study suggests that the combined therapy of 5-FU and β-carotene exerted antitumor effects in vivo and in vitro, in a synergistic way and could mean an innovative therapeutic treatment for ESCC (Zhang et al. 2016). Thus, further research is needed to confirm the applicability of this suggestion.

5 Dietary Fibers

Although the role of dietary fibers (DFs) had been studied a long time ago, regarding their health benefits, only in the last few years, the action mechanisms of DFs have been related to the prevention of different types of cancer (Carlson et al. 2018; Chen et al. 2016b; Conti et al. 2018; de Silva and Alcorn 2019; Encarnação et al. 2018; Grosso et al. 2017; Moen et al. 2016; Navarro et al. 2016; Tajaddini et al. 2015; Trefflich et al. 2020; Xu et al. 2018).

DFs comprises nondigestible carbohydrates, including polysaccharides, oligosaccharides, resistant starch (RS), and noncarbohydrate residues, such as polyphenols, classified according their structure and solubility (Dai and Chau 2017; Slavin 2013). Considering water solubility, dietary fibers can be classified as soluble dietary fiber (SDF) including pectin, inulin, gum, mucilage and insoluble dietary fiber (IDF) that consists mainly of cellulose, hemicellulose, and lignin (Dhingra et al. 2012). DFs from fruits and vegetables have a considerably proportion of SDF which is highly fermented by the human microbiota (Moen et al. 2016).

Lignans comprise a vast group of non-flavonoid polyphenols widespread in plant kingdom (Das and Devi 2019). They share chemical characteristics with some IDFs, such as lignins, but are not classified as dietary fiber, being often found in association with them (Peterson et al. 2010).

Secoisolariciresinol diglycoside (SDG) is one of the most abundant dietary lignans found in flaxseed (Touré and Xueming 2010). When ingested, SDG undergoes hydrolysis to form the aglycone, secoisolariciresinol (SECO) (Adolphe et al. 2010). It has biological activities such as antioxidant and anticancer effect (Alphonse and Aluko 2015). Pinoresinol (PINO) is a high-value plant-derived lignan that can be found in rich concentration in olive oil, with reported antifungal, anti-inflammatory, antioxidant and hypoglycemic activities (López-Biedma et al. 2016). Matairesinol (MAT) is a dibenzyl-butyrolactone lignan found in seeds, vegetables and fruits. It has biological activities such as anti-angiogenic and anti-cancer (Choi et al. 2014; Yamawaki et al. 2011). Lariciresinol (LAR) is a tetrahydrofuran lignan present in Açai Brazilian berry fruits (*Euterpe Oleracea*) and cashew nuts (Chin et al. 2008; Rodríguez-García et al. 2019).

Sesamin is a lignan isolated from sesame seed oil that wields several biological properties, including antitumor activities (Akl et al. 2013; Kong et al. 2014). Another constituent of sesame oil with biological importance is sesamol, a water-soluble lignan with antioxidant, antimutagenic, anti-hepatotoxic, anti-inflammatory, anti-aging and chemopreventive properties (Majdalawieh and Mansour 2019).

Plant lignans and their glycosides are metabolized by gut microbiota to produce different substances that exhibit several biological properties, including anti-inflammatory, antioxidant, estrogenic and anticancer activities (Fig. 10.5), (Kiyama 2016). They are known as enterolignans or mammalian lignans, namely enterolactone (ENL) and enterodiol (END). Several *in vivo* and *in vitro* studies have provided strong evidence that ENL exhibits potent anti-cancer and/or protective properties against different cancers (Mali et al. 2019).

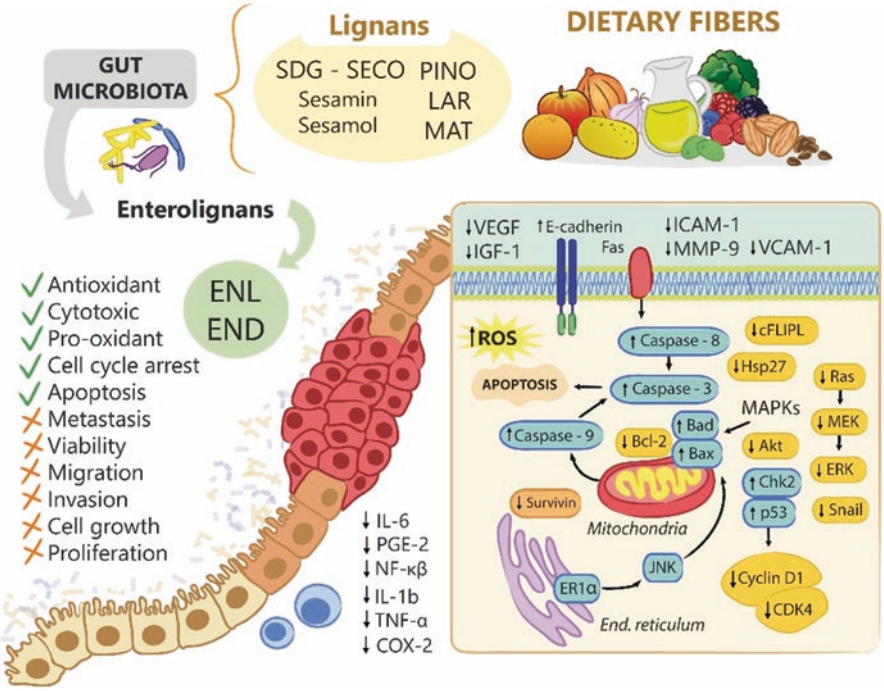

Fig. 10.5 The main anticancer effects and mechanisms of dietary fiber (lignans). Lignans, namely secoisolariciresinol diglycoside (SDG), its aglycone form (SECO), pinoresinol (PINO), Matairesinol (MAT), Lariciresinol (LAR), sesamin and sesamol are associated components of dietary fibers. They are metabolized by gut microbiota to produce actives metabolites, enterolignans known as enterolactone (ENL) and enterodiol (END). These compounds exerts beneficial effects against cancer by several actions mechanisms, such as regulation of enzymes and pro-inflammatory cytokines (COX-2, IL-6, TNF-α), modulation of Bax/Bcl-2 ratio, p53 NF-kb, MMPs and E-cadherin expression, through MAPKs, JNK/ER1a and Ras/ERK pathways. All these anticancer activities (✓) and targets pathways of dietary lignans contribute to inhibitory effect (×) in cancer prevention

Aside from fruits and vegetables, lignans can be found mainly in seeds and its oils (*e.g.,* flax and sesame), also in olive fruit, whole grains and nuts (*e.g.,* almonds and cashew nut). The quantity varies according its food source (Barreca et al. 2020; Chin et al. 2008; de Souza et al. 2017; Durazzo et al. 2018; Kristo et al. 2016; Rodríguez-García et al. 2019).

Rodríguez-García et al. (2019) reported that lignan consumption varies according to the region food-pattern around the world. The major source of lignan ingestion in Latin American diet is flax seed (e.g. secoisolariciresinol); as to Asia, mainly China and India, one has spices, vegetables and Sesame seeds with its oils (e.g. sesamin), whereas in European regions one finds berries (e.g. lariciresinol e matairesinol). Besides, the main lignan sources in the Mediterranean diet are garlic, onions, vegetables, whole grains, virgin olive oil (VOO), and seasonal fruit (Pounis

et al. 2016). Indeed, Offringa et al. (2019) suggested that there is a connection among plant-based diets which contain naturally enriched food of lignans and health benefits, including decrease in cancer risks.

In the last decades, the potential effects of lignans have been the targets of several studies associated with breast, ovarian, prostate, colorectal, cervical, and esophageal cancers as well as hepatocellular carcinoma (Jung et al. 2013). Therefore, this section will focus on lignans effects on different aspects of cancer.

5.1 Lignans and Breast Cancer

In animal studies, it has been observed that SDG supplementation in mice reduces tumor growth in E0771 model of triple-negative breast cancer (TNBC), probably via mechanism involving inhibition of NF-kB. The authors also analyzed an enterolactone (ENL) treatment *in vitro*, which inhibited cell viability, survival, and NF-kB activity (Bowers et al. 2019). Certain tumors have a greater capacity to spread to other organs, which contributes to the difficulty of treatment. In this sense, ENL also has antimetastatic potential activity against TNBC, through inhibition of TGF-β-induced epithelial to mesenchymal transition (EMT) and blocking ERK/NF-κB/Snail signaling pathway *in vitro* (Mali et al. 2018). Another study showed that ENL and SECO enhanced the anticancer activity of therapeutic drugs, such as docetaxel, against breast cancer cell lines, which in combination with ENL could have their doses reduced and still maintain the same effectiveness (Di et al. 2018). Furthermore, ENL could also work on enhancement of radio sensitivity of breast cancer by abrogating X-ray, inducing G2/M arrest, impairing DNA repair processes and increasing apoptosis (Bigdeli et al. 2016). PINO behaved as cytotoxic, anti-proliferative and pro-oxidant in human breast tumor cells (MDA-MB-231; MCF7), without regard to estrogen-receptor expression levels. Indeed, it revealed an antioxidant role preventing DNA damage in MCF10A cells (López-Biedma et al. 2016). In a study with breast cancer cells (SKBr3), LAR and PINO, increased apoptosis induction as well as cell growth, survival and proliferation decrease (Soltani et al. 2020).

In a bioinformatics analysis, MAT had the best binding energy with ER+ receptor for breast cancer cells, compared to PINO, LAR and SECO. It was revealed similar interactions compared to the drug tamoxifen, that could suggest promising biological activity in this type of cancer (Mohamadyar-Toupkanlou et al. 2017). Siao et al. (2015) showed that breast cancer cells (MCF-7), treated with sesamin reduced cell viability by necrosis, apoptosis and cell cycle arrest, promoted by an increase of Bax and caspase-3 expression and sub-G1 arrest related with increased tumor suppressor p53 and Chk2 expression. Indeed, sesamol was able to exert, maintain and augment cytotoxicity on MCF-7 breast cancer cell due association with oleic acid-conjugated gelatin nanoparticles that enhanced sesamol uptake in transdermal delivery through albino mice skin (Elmasry et al. 2018). Both encapsulated and free form of sesamol administration remarkably decreased skin tumor

burden by bcl-2 downregulation and Bax upregulation, inducing apoptosis (Bhardwaj et al. 2016).

In a cross-sectional study, the consumption of phytoestrogens (isoflavones and lignans) by breast cancer patients in different menopausal status was associated with their diet and survival (Boucher et al. 2017). The results showed that the phytoestrogens intake was higher in premenopausal than in postmenopausal consumers, and lignans intake were significantly higher than isoflavones. Among lignans, foods assessed were flaxseed, flaxseed bread, sesame seeds that were associated to provide SECO, PINO, LAR and MAT lignans in diet. Indeed, SECO was the major contributor of diet for all patients. The authors suggested that higher phytoestrogens intake may affect postmenopausal breast cancer survival. In a similar study, no associations were found for SECO intake and breast prognosis (Swann et al. 2013).

In a case-control study, the consumption of flaxseed was associated with a significant reduction in breast cancer risk (OR 0.82, 95% CI 0.69–0.97) among premenopausal women which was attributed to LAR and PINO intake (Lowcock et al. 2013). In addition, evidence for a better prognosis in postmenopausal breast cancer patients who have high estimated enterolignan (ENL and END), dietary intake of lignan-rich foods, and dietary fiber intake exposures was revealed in a large German cohort (Buck et al. 2011). The associations of estimated enterolignans and dietary fiber intake with survival were independent of ER status of the tumour.

Lignans can also act through hormone-independent mechanisms. A population based prospective cohort with 1743 cancer patients revealed that the higher mean ENL concentrations and lower CRP concentrations were inversely associated with mortality and survival (Jaskulski et al. 2018). On the other hand, the association of prognosis with circulating ENL and inflammatory markers changes in post-menopausal breast cancer reveals that enterolignans levels changed over time and were not associated with prognosis (Jaskulski et al. 2019). Thus, these clinical trials show controversy for ENL.

5.2 Lignans and Gastrointestinal Cancers

Lariciresinol lignan has antioxidant and cytoprotective activities and was capable of modulate antioxidant enzymes and up-regulate Nrf-2 via p38 activation pathway in RAW 264.7 (Bajpai et al. 2017). LAR anti-tumor activity was assessed against hepatocellular carcinoma (HepG2 cells) and induced mitochondrial-mediated apoptosis pathway S-phase arrest that contributed to growth inhibition and downregulated Hsp27 expression, a protein that is correlated to chemotherapeutic drugs resistance (Ma et al. 2016, 2018b). LAR also denotes growth inhibition and cell cycle arrest activities against human gastric cancer cells by apoptosis induction through increased Bax/Bcl-2 ratios, ROS generation and decreased MMP (Zhang et al. 2015b). PINO was also associated with mitochondrial apoptosis in hepatocarcinoma cells (HepG2) as well as Fas death receptor pathways, inhibiting migration and invasion through E-cadherin increased expression with VCAM-1, ICAM-1, and

decreasing of MMP-9 expression (Zhang et al. 2018). In hepatocellular carcinoma *in vitro* and *in vivo*, sesamol revealed its anticancer effects by directly altering mitochondrial metabolism, interrupting the S-phase cell cycle and inducing the activation of apoptosis by intrinsic and extrinsic routes (Liu et al. 2017c). In silico analysis with molecular docking suggests that phytoestrogens as SECO, LAR, MAT, and PINO among others, can be considered potential drug candidates for hepatocellular carcinoma while targeting β-catenin in the *Wnt* signaling (Kanahaiya et al. 2017).

Evidence indicates that SDG chemopreventive colon cancer effects may have associated with type 2 diabetes mellitus once it was able to inhibit CDK4 and increase GLUT-1 expression, controlling glycemic parameters. Also, it reduced IL-1b, TNF-a levels and inhibited IGF-1, suggesting action on preventing cell proliferation and cancer progression (Shah and Patel 2016). SDG also presents strong anti-inflammatory properties decreasing IL-6, NF-kB activity, and PGE-2 on human intestinal Caco-2 cells, probably due to its furofuran structure and its intestinal metabolism (During et al. 2012). For instance, Shin et al. (2018) evaluated *in vitro* activity of END on CRC cells. This lignan induced apoptosis mechanism through MAPK signaling pathway and reduced metastatic capacity, exhibiting cytotoxic effect to cancer cells. Sesamol induced apoptosis in human colon HCT116 cells, exerted both antioxidant and pro-oxidant activities, and suppressed cell viability through S-phase arrest (Khamphio et al. 2016).

A Spanish case-control study showed that the lignans and total flavonoids intake were inversely related to CRC risk (Zamora-Ros et al. 2013). In the other hand, the results of EPIC Cohort showed no significant association between total polyphenol intake and CRC risk (Zamora-Ros et al. 2018). It could be said that polyphenols have multiple targets and different actions such as immunomodulation, anti-angiogenesis, anti-proliferative, apoptosis induction and metastasis suppression that depend on gut-microbiota-modulation for therapeutic potential promoting in CRC (Cueva et al. 2020).

Lignans are safe (Bedell et al. 2014), however, clinical studies should be developed in order to identify therapeutically relevant doses to understand their role in cancer prevention (De Silva and Alcorn 2019).

5.3 Lignans and Lung Cancer

Radiotherapy is a treatment widely used in patients with the most diverse types of cancer. However, it causes several adverse effects on the patient. SDG demonstrated a potential protective activity against radiation-induced oxidative damage to non-malignant lung cells, mediated by free-radical scavenging or increasing of endogenous antioxidant defenses, like trans-resveratrol (Velalopoulou et al. 2016). Similar result was obtained where SDG successfully scavenged active chlorine species (ACS) preventing radiation-induced DNA damage (Mishra et al. 2016). These findings may present SDG as a novel radioprotective agent in cancer therapy, which has already been patented (Christofidou-Solomidou 2018).

In lung cancer cells, ENL was able to suppress metastases through disruption of F-actin cytoskeleton dynamics, inhibition of the focal adhesion kinase and steroid receptor coactivator/paxillin signaling cascade and expression of motility regulators. It was also observed cycle arrest activity in the G1 and downregulation of cyclin D1 and CDK4 mRNA (Chikara et al. 2017a, b). In a brand new study by Chen et al. (2020), sesamin suppressed cell proliferation in non-small cell lung cancer (NSCLC) by induction of cell cycle arrest via inhibiting cyclin D1 expression, up-regulated p53 expression and Akt activity inhibition, both *in vitro* and *in vivo*, without severe side effects. As a matter of fact, sesamin also ameliorates the survival of cardiac muscle cells impaired by doxorubicin, an important chemotherapy drug and sesamin combined with cisplatin synergistically suppressed lung cancer cells (H460) proliferation (Liu et al. 2019a; Su et al. 2014).

5.4 Lignans and Other Cancers

In both *in vitro* and *in vivo* assays PINO inhibited ovarian cancer cell growth by autophagy via MMP levels reduction, cell invasion inhibition and Ras/MEK/ERK signaling pathway (Ning et al. 2019).

Not only that, high concentrations of both END and ENL could inhibit ovarian cancer cell proliferation, as well as viability, migration and invasiveness i*n vitro* and *in vivo*, but ENL was considered more effective particularly in *the in vivo* analysis (Liu et al. 2017a). On cervical cancer cells (HeLa cells) sesamin inhibits proliferation and migration dose-dependently, inducing cell autophagy, and modulated apoptosis with increased expressions of Bax/Bcl-2 ratio and ER-stress related proteins through IRE1α/JNK pathway (Dou et al. 2018). In prostate cancer (PC3 cells) under LPS stimulation, sesamin significantly decreased TNF-α, IL-6, cyclin D1, COX-2, Bcl-2 and Survivin expressions. Other effects of sesamin included decreasing in MMP-9, ICAM-1 and VEGF proteins through p38-MAPK signaling pathway, NF-κB activation and inhibition of tumor growth *in vivo* (Xu et al. 2015).

Indeed, PINO sensitized glioblastoma cancer cells against TNF-related apoptosis-inducing ligand (TRAIL) therapy, increasing apoptosis, caspase-8 and downregulation of FLICE-inhibitory protein ($cFLIP_L$) by a mechanism involving *de novo* protein synthesis (Lee et al. 2019b). In the light of these results, PINO has potential to enhance the effectiveness of usual anticancer drugs. MAT had promising effects on C6 glioma cells on rats, reducing proliferation and inducing apoptosis without affect astrocytes, as does arctigenin (ARC), another lignan (Baetas et al. 2018).

Furthermore, PINO promoted a doxorubicin resistance-reversing effect on human myelogenous leukemia cells with low cytotoxicity (González et al. 2017). In T-cell lymphoma, MAT and ARC caused antiproliferative effects with selective cytotoxicity. Importantly, they cause cell cycle arrest in the S phase and apoptosis activation, mainly by upregulation of Bax (MAT), Bad (ARC), and caspase-9 (Both) expressions, besides increasing intracellular ROS levels. (Su et al. 2015).

6 Evidences in Focus

The consistent protective effects of fruits and vegetables should reflect the fact that they are the largest source of fiber and contain several phytochemicals with anticancer properties. There are many studies that show the anticancer effects of phytochemicals (quercetin, resveratrol, carotenoids and dietary fiber) and some of these studies are summarized in Tables 10.1, 10.2, 10.3 and 10.4.

The data listed in Tables 10.1, 10.2, 10.3, and 10.4 show that phytochemicals from fruits and vegetables exert their anticancer protective effects through different mechanisms and may act in a synergic way been strongly related to prevent several cancers. There are many evidences in cancer research that suggest quercetin, resveratrol, carotenoids and dietary fibers (lignans) as anticancer agents. This represents the advance on acknowledgment on chemopreventive role fruits and vegetables besides their functional properties, already well established.

The search for scientific work carried out *in vitro*, *in vivo* and in human studies brings together a wide range of evidence to ratify the role of these natural compounds envisaging cancer combat. Furthermore, published pre-clinical trials represent an endless search to information. However, despite positive associations in epidemiological studies, there are still limitations of clinical studies.

In fact, the evidence from clinical trials is advantageous because in intervention studies, the results represent controlled approaches; on the other hand, epidemiological studies have strengths and limitations (WCRF/AICR 2018).

All these researches results show the diet importance in order to maintain a healthy life associated to fruits and vegetables intake. Indeed, particularly this chapter emphasizes bioactive phytochemicals role in cancer prevention. Furthermore, these phytochemicals could be used as a tool to be associated to traditional cancer treatments searching alleviate the side effects (Clinton et al. 2019).

7 Final Remarks

Despite the technological and pharmaceutical advances, cancer remains to be a global concern. Herbal medicine or phytotherapics has been used for a long time as complementary therapy to cancer treatment. In addition, the side effects caused by the chemotherapy are well known and the search for new natural agents that decrease these effects is fundamental. In this sense, natural compounds, such as quercetin, resveratrol, carotenoids and dietary fibers, have been gaining attention of scientists and community.

Quercetin is considered the most abundant antioxidant in the human diet and its easy accessibility, great efficacy and low toxicity make it a promising biological compound that can be used alone or in combination with other chemotherapeutic drugs in the treatment of cancer, including colorectal, breast, prostate, gastric, oral

Table 10.1 *In vitro* evidences of phytochemicals from fruit and vegetables in cancer prevention

Phytochemical	Treatment	Anticancer effect	Reference
Quercetin	Mouse colon cancer cells (CT26; MC38) and human colon adenocarcinoma cell (HT29) [10–100 μM] 24–72 h	Inhibition of cell viability by induction of intrinsic apoptosis through the ERK, JNK and p38 MAPKs pathway in CT26 cells. Inhibition of metastasis by decreasing MMP-2 and MMP-9 activity and regulating expression of EMT markers, such as E-cadherin (increased) and N-cadherin, β-catenin and snail (decreased).	Kee et al. (2016)
Quercetin	Human oral cancer SAS cells [40 μM] 12–48 h	Reduction of cell viability due ROS and Ca^{2+} production increase and decrease of mitochondrial membrane potential (ΔΨm) levels. The treatment also increased Fas, FasLigand, caspase 8, ATF6β and gastrin releasing peptide78.	Ma et al. (2018a)
Quercetin	Human prostate cancer cell line (PC3) and doxorubicin-resistant PC3 cells (PC3/R) [10 μM] for 48 h in combination with doxorubicin	Induction of apoptosis in PC3/R cells through mitochondrial/ROS pathway by inhibition of c-met expression and PI3K/AKT pathway downstream.	Shu et al. (2018)
Resveratrol	Human Intestinal cell Caco-2 [10–50 μM] 1 h	Reduction, in a concentration-dependent manner, the induction of COX-2. Cells treated with 50 μM, exhibited a less evident reduction of COX-2 expression.	Cianciulli et al. (2012)
Resveratrol	Oral squamous cancer cells (OSCC: SCC-VII, SCC-25, and YD-38) [0.1–1.5 μg/mL] 24–48 h	Induction of cell cycle arrest in the G2/M phase and enhanced expression of phospho-cdc2 (Tyr 15), cyclin A2, and cyclin B1 in the (OSCC) cells.	Yu et al. (2016)
Resveratrol	Breast cancer cell with different ER status (MCF-7 and MDA-MB-231) [10 μM] 96 h	DHT interfered with RESV-induced anti-proliferation in both ER-α-positive MCF-7 and ER-α-negative MDA-MB-231 cells.	Chin et al. (2015)
Lycopene	Prostate and Breast cancer cells (PC3 and MDA-MB-231) [0.5–5 μM] 20–48 h	Suppression PC3 and MDA-MB-231 with proliferation inhibition. Inhibits IκB phosphorylation and inhibits TNFα-induced NF-κB p65 nuclear translocation in PC3 and MDA-MB-231 cells	Assar et al. (2016)
Lycopene	Human prostate cancer cells and benign prostate hyperplastic cells Bone metastasis-derived PCA human cells (PC-3), and brain metastasis-derived PCA human cells (DU-145) [0.5–20 μM] 48–96 h	Decreased the percentage of cells in G0/G1 phase and increased in S and G2/M phases in metastatic prostate cancer cell lineages; cell cycle arrest in G0/G1 phase in a primary cancer cell line; apoptosis in prostate cancer cells with altered gene expression of Bax and Bcl-2.	Soares et al. (2013)

β-carotene	Rat melanoma cells and rat aortic ring assay (in vitro angiogenesis study model) (1–10 μg/mL) for 6 days	A significant and dose-dependent inhibition of microvessel outgrowth from the rat aorta ring induced by the conditioned medium from B16F-10 cells with the microvessel outgrowth totally stopped at 10 μg/mL	Guruvayoorappan and Kuttan (2007)
Lycopene + Beta-carotene	Human breast cell lines (0.5–10 μM) for 48 and 96 h.	Inhibition of cell proliferation; cell cycle arrest in different phases, induction of apoptosis. Decrease the expression of antiapoptotic protein Bcl-2 and PARP130 decrease the activation of the growth signaling protein Akt and ERK1/2.	Gloria et al. (2014)
END	Mouse colon adenocarcinoma cells (CT26) [0.1–100 μM] 24–72 h	Increase 40% of apoptosis rate in CT26 cells and reduced metastatic capacity of the cells in a concentration-dependent manner. Down-regulation of phosphorylation of ERK, JNK, and p38.	Shin et al. (2018)
SECO ENL	Breast cancer cell with different ER status (SKBR3 and MDA-MB-231) [50 μM] 72 h in combination with chemotherapeutic agents	SECO with docetaxel, presented moderate antiproliferative properties against metastatic cancer cells lines. ENL with docetaxel, increased 50% cytotoxicity to MDA-MB-231 more efficiently than SECO. SECO with ENL and Metformin, increased fourfold the cytotoxicity in a concentration dependent-response.	Di et al. (2018)
Pectin-rich in phenolics extracts	Colon carcinoma (Caco-2) and the leukemia monocytic (THP-1) cell lines Caco-2 (7 days treatment) THP-1 (4 days treatment) Pectolivs and MCP [0.37–10 mg/mL]	Pectolivs rich in phenolics, inhibit cells proliferation in both models of cancerous cells and the inhibitory effect was higher than control MCP. Caco-2 cells were more susceptible to inhibition of proliferation than THP-1 cells. Pectolivs induced apoptosis as determined by activation of caspase-3, and inhibited agglutination of red blood cells by galectin-3.	Bermúdez-Oria et al. (2019)

COX-2 cyclooxygenase-2, *END* enterodiol, *ENL* enterolactone, *ERK* extracellular signal-regulated kinase, *EMT* epithelial-mesenchymal transition, *JNK* jun N-terminal kinase, *IκB* inhibitor of kappa B, *MAPKs* mitogen-activated protein kinases, *MMP-2* matrix metalloproteinases 2, *MCP* modified citrus pectin, *Pectolivs* pectin-rich olive extracts, *SECO* secoisolariciresinol, *ATF6β* transcription factors-6β, *TNFα* transcription necrosis factor-α

Table 10.2 *In vivo* evidences of phytochemicals from fruits and vegetables in cancer prevention

Phytochemical	Type of cancer	Model	Rout/dose/duration	Effects observed	Mechanism	Reference
Quercetin	Pancreatic cancer	nu/nu mice	40 mg/kg (v.o.) daily Mon–Fri for 3 weeks	Suppress tumor growth	Increase pro-apoptotic effects of BET inhibitors by decreasing hnRNPA1 and downregulated protein levels of cIAP-2, Hsp70 and Survivin.	Pham et al. (2019)
Quercetin	Lung cancer	C57 mice	50, 100 and 200 μg/mL (v.o.) daily for 15 days	Decrease of tumor volume	Upregulation of apoptosis related genes including p53, Bax and Fas and the ratio of Bax/Bcl-2 increase.	Li et al. (2019)
Quercetin	Gastric carcinoma	NOD/SCID mice	30 mg/kg (v.o.) daily for 2 weeks	Decrease of tumor volume	Induction of p53-dependent apoptosis by increasing expression of the cleaved forms of caspase-3, -9, and Parp.	Lee et al. (2016)
Resveratrol	Breast cancer	BALB/c nude mice	RESV-loaded NPS (30 mg/kg) (i.v.) with DOX (5 and 10 mg/kg), every 5th day, for 25 days.	Suppress tumor growth	Effects in dose-dependent manner, pro-apoptotic effects via caspase-3 activity and cytotoxicity.	Zhao et al. (2016)
Resveratrol	Ovarian cancer	nu/nu mice	Cisplatin (6 mg/kg) RESV (160 mg/kg) (i.p.) for 25 days.	Antineoplastic effect	Inhibition of glucose uptake.	Tan et al. (2016)
Resveratrol	Pancreatic cancer	nu/nu mice	RESV (40 mg/kg), (p.o.) once daily or/with gemcitabine (25 mg/kg) (i.p.) twice weekly	Potentiate the cytotoxic effects of gemcitabine	Downregulation of Ki-67 and the micro vessel density CD31 markers of proliferation index. Suppressed NF-κB activation and expression of cyclin D1, COX-2, ICAM-1, MMP-9 and surviving.	Harikumar et al. (2010)
Lycopene	Breast cancer	Rats	LYC (50 mg/Kg) alone or combined with melatonin (SC) (2.5 mg/kg) for 120 days	Decreases the tumor number and suppress tumor growth	Antioxidant mechanism with inhibition of lipid peroxidation and decreased SOD, CAT and GPx activities.	Moselhy and Al Mslmani (2008)

(continued)

Table 10.2 (continued)

Phytochemical	Type of cancer	Model	Rout/dose/duration	Effects observed	Mechanism	Reference
Lycopene	Ovarian cancer	NOD/SCID mice	15 mg/kg (v.o) Preventative effects: 2 weeks PI of OV-MZ-6 cells in mice Therapeutic effects:4 weeks AS	Anti-metastatic Antiproliferative	Downregulated of ITGA5, ITGB1, ILK, FAK and MMP9 expression in metastatic tissue. Decreased levels of CA125 in serum and ascites.	Holzapfel et al. (2017)
Lycopene	Prostate cancer	Rat	4 g lycopene/kg diet for 4-week	Antiproliferative Suppress tumor growth	Downregulation of 5-a-reductase, reduced steroid target genes expression and prostatic insulin-like growth factor-1 (IGF-1) and interleukin-6.	Siler et al. (2004)
END ENL	Ovarian cancer	BALB/c nude mice Xenograft model	END/ENL (0.1 mg/kg or 1 mg/kg) Once per 2 days for 32 days	Antiproliferative Suppress tumor growth	END performed estrogenic activity at lower concentration, while in higher concentration cause side effects on weight loss. ENL at higher dose was more effective as anti-cancer agent than END	Liu et al. (2017a)
SDG	Breast cancer	C57BL/6 mice	100 mg SDG/kg diet for 7 weeks	Suppress tumor growth	Significant decrease in phosphorylated (Ser276) p65 as well as NF-κB target gene expression in the tumors.	Bowers et al. (2019)
Sesamol	Hepato-carcinoma	BALB/c nude mice Xenograft model	100 mg/kg or 200 mg/kg via i.p. every day from day 10 thru day 44	Suppress tumor growth	The Bcl-2/Bax ratio in tumor tissues decreased and levels of the cell proliferation marker Ki76 were down-regulated, while levels of the cell apoptosis marker cleaved-caspase 3 were increased. The expression of LC3 protein was remarkably decreased by sesamol in a dose-dependent manner.	Liu et al. (2017c)

AS after surgery, *CAT* catalase, *GPx* glutathione peroxidase, *HCC* human hepatocellular carcinoma cells, *i.g* intragastrically, *i.p* intraperitoneally, *LYC* lycopene, *v.o.* via oral, *PI* prior implantation, *SC* subcutaneously, *TNBC* triple-negative breast cancer, *RESV* resveratrol, *SOD* superoxide dismutase

Table 10.3 Clinical evidences of phytochemicals from fruits and vegetables in cancer prevention

Phytochemical	Cancer type	Study design	Subjects	Intervention treatment/ duration	Outcomes	Reference
Quercetin	Colorectal cancer	Observational prospective	5 patients with FAP (men and women) aging 21–54 years	Quercetin 20 mg and curcumin 480 mg orally 3 times a day for 6 months	Decrease polyp number and size of ileal and rectal adenomas with minimal adverse side effects	Cruz-Correa et al. (2006)
Quercetin	Blood malignancies	Double-blind, randomized placebo controlled	20 adult patients who underwent high dose chemotherapy for blood malignancies	250 mg quercetin capsules twice daily for 4 weeks	Lower oral mucositis incidence in treated group	Kooshyar et al. (2017)
Trans-resveratrol	Breast	Placebo-controlled	39 adult women at increased breast cancer risk	5 or 50 mg, twice daily, 12 weeks	Decrease in the fraction of methylated RASSF-1α DNA Decrease in the expression of PGE2 in the breast	Zhu et al. (2012)
Resveratrol	Colorectal	Intervention	20 patients with confirmed colorectal cancer	0.5 or 1.0 g, eight daily doses, before surgical resection	Reduced tumor cell proliferation	Patel et al. (2010)
Plant-derived resveratrol	Colon	Clinical Trial phase I	8 patients diagnosed with colon cancer	80 g/day containing 0.07 mg of resveratrol, 2 weeks	Inhibition of Wnt target gene expression in normal colonic mucosa, with no effect on cancerous mucosa	Nguyen et al. (2009)
Lycopene	Prostate adenocarcinoma	Clinical Trial phase I	32 patients with localized prostate adenocarcinoma	Tomato sauce-based pasta dishes for the 3 weeks (30 mg of lycopene per day) preceding radical prostatectomy	Serum PSA levels decreased, from 10.9 ng/mL (95% CI = 8.7–13.2 ng/mL) to 8.7 ng/mL (95% CI = 6.8–10.6 ng/mL) (P < .001).	Chen et al. (2001)
Lycopene	Prostate	Randomized clinical trial	Benign prostate Hyperplasia patients	Administration of 15 mg lycopene every day for 6 months	Reduced disease progression with decreased serum PSA concentration	Schwarz et al. (2008)

(continued)

Table 10.3 (continued)

Phytochemical	Cancer type	Study design	Subjects	Intervention treatment/ duration	Outcomes	Reference
Flaxseed	Breast cancer	Randomized Intervention Placebo controlled	24 postmenopausal women (ER^+) BC	25 g/day ground (FS) with 1 mg/day anastrozole (AI) 13–16 days	FS + AI treatments decrease 40% the ERβ expression and reduce serum steroid hormone DHEA production, as well as urinary lignan excretion	McCann et al. (2014)

AI aromatase inhibitor, *BC* breast cancer, *ER* estrogen receptor, *FS* flaxseed, *FAP* familial adenomatous polyposis, *PGE2* prostaglandina-2

Table 10.4 Observational epidemiology evidences of phytochemicals from fruit and vegetables in cancer prevention

Phytochemical	Population	Cancer type	Aim	Methods	Outcomes	Reference
Quercetin	505 cases and 1116 controls (40–79 years)	Gastric cancer	To study the impact of the dietary antioxidant quercetin on risk of gastric adenocarcinoma	FFQ	High dietary quercetin intake protects to this adenocarcinoma, and the effect is stronger for women exposed to oxidative stress, such as tobacco smoking	Ekström et al. (2011)
Quercetin	1163 cases and 1501 controls participants, between 45 and 80 years	Colon canscer	Associate the dietary quercetin with proximal colon cancer risk	FFQ	Protective effect of quercetin on risk of proximal colon cancer were significant only to high fruit intake, or low tea intake.	Djuric et al. (2012)
Resveratrol	Patients over 18 years diagnosed with cancer in the first 6 months of 2018 vs. healthy individuals	Head and neck cancer	Evaluate the relationship between traditional Mediterranean diet and the risk of developing head and neck cancer	Mediterranean Diet Adherence Screener questionnaire	The consistent medium-high adherence to Mediterranean diet is associated with a decrease in the risk of developing head and neck cancer	Salvatore Benito et al. (2019)
Resveratrol	181 cases of oesophageal adenocarcinoma, 158 cases of oesophageal squamous-cell carcinoma, 255 cases of gastro-oesophageal junctional adenocarcinoma and 806 controls from a Swedish nationwide population-based case–control study	Oesophageal adenocarcinoma, oesophageal squamous-cell carcinoma and gastro-oesophageal junctional adenocarcinoma	To verify the association of a dietary pattern rich in lignans, quercetin, and resveratrol with the risk of oesophageal cancer development.	FFQ	Lignans, quercetin, and resveratrol synergistically act to prevent the development of all types of esophageal cancer investigated	Lin et al. (2014)

(continued)

Table 10.4 (continued)

Phytochemical	Population	Cancer type	Aim	Methods	Outcomes	Reference
Lycopene	39 women with breast cancer and 31 control women. Evaluation of the relationship between plasma micronutrient levels and breast cancer risk.	Breast cancer	To evaluate the potential for accrual to a study of the association of plasma levels of b-carotene, retinol, lycopene, a-tocopherol, and g-tocopherol with breast cancer risk among African American and Caucasian women	FFQ and micronutrients plasma levels	Among the lowest lycopene tertile, the risk of breast cancer among Caucasian women was 0.76 and the risk of breast cancer among African American women was 2.29, although these odds ratios were not statistically significant.	Simon et al. (2000)
Lycopene	43,851 PCa cases reported from 692,012 participants (32 studies in North America), 6 from Europe, 2 from Australia, 2 from Asia (China and Singapore) and 1 from South America (Uruguay).	Prostate cancer	To determine the impact of dietary and circulating concentrations of lycopene on PCa risk and to investigate potential dose response associations.	Meta-analysis using randomized control trials, cohort, cross-sectional, retrospective, prospective and case–control studies	Higher dietary and circulating lycopene concentrations are inversely associated with PCa risk	Rowles et al. (2017)
β-carotene	9 studies including 5 in USA and 3 in Europe	Pancreatic cancer	To evaluate the association between antioxidants intake and pancreatic cancer risk	Meta-analysis with relevant articles retrieved from PUBMED and EMBASE databases and standard methods	It was significantly associated with reduced pancreatic cancer risk	Chen et al. (2016b)
Flaxseed and flax bread	2999 cases and 3370 controls (25–74 years)	Breast cancer	To investigate associations between phytoestrogens-containing foods and BC	FFQ	Dietary lignans intake was associated with 20–30% reductions in BC risk in postmenopausal	Lowcock et al. (2013)

BC breast cancer, *FFQ* food frequency questionnaire

and others. Thus, the variety of published evidence suggests that quercetin is a flavonoid with great anticancer potential.

In vitro, *in vivo*, clinical and epidemiological studies reveal that resveratrol is a promising molecule with anticancer activity, in prevention and treatment, especially in synergisms with other chemotherapeutic agents, in addition to reducing the associated side effect during treatment. It is well tolerated at low doses, but its beneficial effects remain controversial in humans or have only been shown to a limited extent so far, due to several factors such as achieving consensus on dose-response profiles, biomarker or conclusive result, since a small number of patients included in clinical studies and the diversity of resveratrol doses used. Further clinical trials should be carried out to clarify the effects of resveratrol in cancer patients and to determine its therapeutic and chemopreventive potential.

Researches indicate that different carotenoids such as lycopene and beta-carotene are involved in the anticancer action. Despite this, there are still controversial studies for some types of cancer. Thus, this analysis requires attention and important aspects need to be considered such as the availability of these carotenoids, their processing before ingestion and the interaction with other phytochemicals present in the tested foods. Those factors can influence the presence or absence of anticancer properties, as well as side effects of this combination, and influence the results of research since little is known about phytochemical/phytochemical interactions. Another subject that deserves attention is the role of bacteria microflora involved in the biotransformation of these phytochemicals, changing the nutrition and the functions of these molecules in human organisms. In general, a diet rich in fruits and vegetables, important sources of carotenoids, is recommended to maintain health and prevent diseases like cancer.

Dietary fibers provide a beneficial physiological effect through their functional mechanisms besides to playing a protective role in various types of cancer. Among them, lignans stand out, whose ingestion in the diet produces bioactive metabolites related to the prevention of carcinogenesis through the efficiency of the intestinal microbiota in the metabolism of these components. Thus, lignans can interfere directly or indirectly in various metabolic pathways, acting as antioxidants/pro-oxidants, phytoestrogenic agents in various types of cancer, mainly gastrointestinal, breast and lung cancer.

The main phytochemicals agents presented in this chapter reveal their anti-cancer mechanisms through multiple intracellular targets, triggering metabolic pathways and molecular signals that modulate genes and proteins associated to inflammatory, antioxidant/pro-oxidant, and carcinogenic processes.

These phytochemicals are also capable of increasing the effectiveness of drugs already established in treatments, without toxicity, reinforcing the importance of indicating the consumption of fruits by the population. However, further clinical studies must be developed to identify therapeutically relevant doses to prevent cancer.

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Chapter 11
Garlic: Allyl Sulfur Compounds and Cancer Prevention

Sumeyra Cetinkaya and Ipek Süntar

Abstract Garlic, *Allium sativum* L., is a plant within the family Alliaceae that has been widely used for its culinary and medicinal properties. This plant contains organosulfur compounds with allyl groups such as allyl mercaptan (AM), S-allyl cysteine (SAC), diallyl trisulfide (DATS) and has been responsible for different health benefits such as antihypertensive, anticoagulant, anti-inflammatory, antimicrobial and anticancer. Especially some lipid-soluble allyl sulfur compounds can inactivate carcinogens and reduce cancer risk and regulate the cellular processes. Epidemiological studies have shown that garlic and its components can decrease the incidence of human stomach, colon, prostate, brain, skin, breast, lung, uterine, and esophagus cancers. These anticarcinogenic effects appear to be achieved by modifying common signaling pathways. But allyl sulfur compounds have different effect in supressing tumor proliferation. Therefore, the compounds that are responsible for the cellular and molecular effects, the stages which they suppress neoplasia and interactions with other drugs should be very well known. Tumor supression ability of allyl sulfur compounds of garlic is attributed the stimulation of detoxification enzymes, protection from oxidative stress, induction of cell apoptosis and cell cycle arrest, prevention of chromosomal damage, induction of immune system and supression of nitrosamine bioactivation. On the other hand, not only the genetic mechanisms, but also the epigenetic mechanisms can be associated with the cancer prevention. Garlic and its several allyl sulfur compounds can be modified by both DNA methylation and histon acetylation. In this chapter, preclinical and clinical studies on the effects of garlic consumption in reducing cancer prevalence will be presented in detail. Furthermore, studies involving the use of allyl sulfur compounds individually or in combination will be discussed and their mechanisms of action will be interpreted at cellular and molecular level.

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Abbreviations

AGE	Aged garlic extract
AM	Allyl mercaptan
AMD	Allyl methyl disulfide
AMPK/TSC2	AMP-activated protein kinase/tuberous sclerosis complex
AMS	Allyl methyl sulfide
Bak	Bcl-2 homologous antagonist killer
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra large
Bip	Binding immunoglobulin protein
BP	Benzo[a]pyrene
Cdc2	Cell division cycle-2
Cdc25	Cell division cycle-25
Cdk	Cyclin dependent kinases
CHOP	CCAAT-enhancer-binding protein homologous protein
DADS	Diallyl disulfide
DAS	Diallyl sulfide
DATS	Diallyl trisulfide
DATTS	Dialyl tetrasulfide
DMBA	7,12-Dimethylbenz[a]anthracene
DMH	1,2-Dimethylhydrazine
DNA	Deoxyribonucleic acid
DNMTi	DNA methyltransferase inhibitors
DNMTs	DNA methyltransferases
eIF2α	Eukaryotic translation initiation factor 2α
EMT	Epithelial-mesenchymal transition
ER	Endoplasmic retikulum
ERK1/2	Extracellular signal–regulated kinases1/2
FAK	Focal adhesion kinase
FOXM1	Forkhead box protein M1
GADD153	G1 arrest and DNA damage 153
GPx	Glutathione peroxidase
GRP78	Glucose-regulated protein78
GSH	Glutathione
GSK-3β	Glycogen synthase kinase 3β

GST	Glutathione-S-transferase
H_2O_2	Hydrogen peroxide
H_2S	Hydrogen sulfide
HCC	Hepatocellular carcinoma
HDAC	Histone deacetylase
HER2	Human epidermal growth factor receptor2
H-RAS	Harvey rat sarcoma viral oncogene homolog
IFNγ	Interferon-gamma
IL10	Interleukin 10
IL12	Interleukin 12
IL1α	Interleukin 1α
IL1β	Interleukin 1β
IL2	Interleukin 2
IL6	Interleukin 6
IL8	Interleukin 8
JNK	c-Jun terminal kinase
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MEK	MAPK/ERK kinase
MMP	Matrix metallopeptidases
NF-κB	Nuclear factor kappa light chain enhancer of activated B cells
NO	Nitric oxide
NQO	NAD(P)H:quinone acceptor oxidoreductase
Nrf2	Nuclear factor erythroid 2-related factor 2
OSCs	Organosulfur compounds
P13k/Akt/mTOR	Phosphoinositide-3-kinase/protein kinase B
p38 MAPK	p38 mitogen-activated protein kinases
PCNA	Proliferation cell nuclear antigen
PUMA	p53 upregulated modulator of apoptosis
ROS	Reactive oxygen species
SAC	S-allyl cysteine
SAMC	S-allylmercaptocysteine
Slug (SNAI2)	Snail family transcriptional repressor 2
SOD	Superoxide dismutase
TGF-β	Transforming growth factor beta 1
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
TPA	12-O-tetradecanoylphorbol-13-acetate
UGT	UDP-glucuronosyl transferase
UPR	Unfolded protein response
VEGF	Vascular endothelial growth factor

1 Introduction

Garlic *(Allium sativum* L.*)* is among the most widely used plants in the Alliaceae family, which has more than 850 different species (Sharifi-Rad et al. 2016). Bulbs of garlic are used as food and spice. Apart from its use as food, it has been used for centuries to treat various diseases such as gastrointestinal (Nicastro et al. 2015) and cardiovascular (Bradley et al. 2016) system disorders, diabetes (Bayan et al. 2014), Alzheimer's disease (Borek 2006) and for wound healing (Srimuzipo et al. 2009). In addition, previous bioactivity studies have reported that garlic displays hepatoprotective (Ajayi et al. 2009), antihypertensive (Ried et al. 2008), antihelmentic (Worku et al. 2009), antimicrobial (Yin et al. 2003), antifungal (Kutawa et al. 2018), immune modulation (Kyo et al. 2001) and anticancer (Ejaz et al. 2003) effects. Epidemiological studies have shown that garlic consumption reduce the risk of disease development. This also supports the ethnobotanical use of the plant.

Phytochemical studies on garlic have shown that various types of chemical compounds are present especially in the bulbs of this plant, including a high water content (approximately 65%). Carbohydrates (28%) (mainly fructans), proteins (2%) (mainly alliin), amino acids (1.2%) (mainly arginine), fibers (1.5%), sulfur compounds (2.3%), trace elements and phenols (Butt et al. 2009) were reported (Fig. 11.1). According to USDA database, 63.535 search results are available on garlic containing food. The main ingredients mentioned above are in different

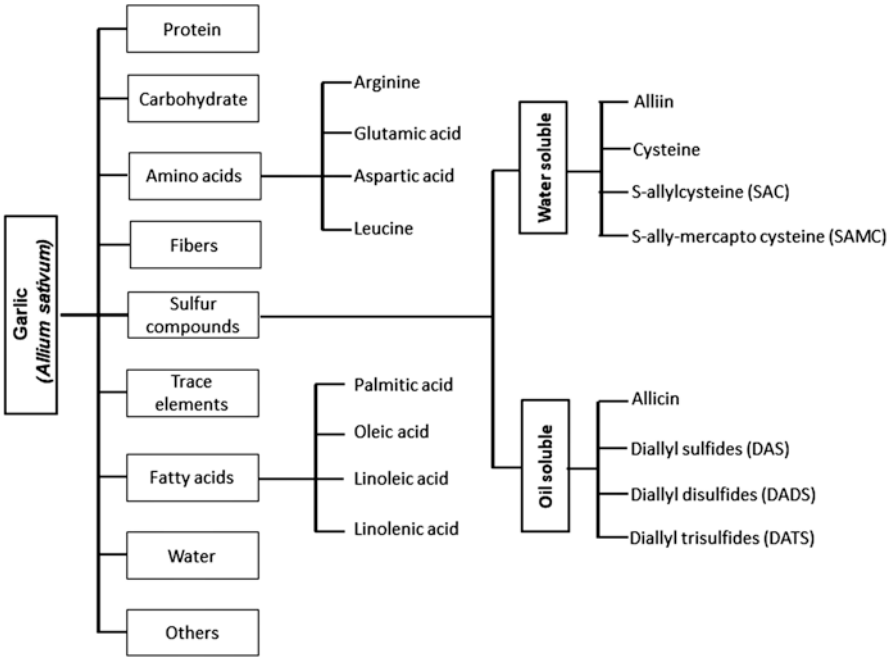

Fig. 11.1 Schematic representation of classification of the main bioactive constituents in garlic

amount for each type of food (USDA, https://fdc.nal.usda.gov). Several biological effects of garlic from wound-healing properties to anticancer effect are mainly attributed to allyl sulfur compounds and flavonoids (Putnik et al. 2018).

In vivo and *in vitro* preclinical studies on tumorigenesis have showed that garlic and its components are effective against human colorectal (Zhang et al. 2018), skin (Wang et al. 2010), prostate (Chu et al. 2006a), brain (Das et al. 2007), gastric (Ling et al. 2006), nasopharyngeal (Zhang et al. 2006), stomach (Fleischauer et al. 2000), lung (Li et al. 2012), breast (Kaschula et al. 2016), liver (Chu et al. 2013) and thyroid (Shin et al. 2010) cancers. Various mechanisms such as stimulation of detoxification enzymes, cells protection from oxidative stress, induction of cell apoptosis and cell cycle arrest, enhancement of immune system and epigenetic mechanisms are attributed to the anticancer activities of allyl sulfur compounds (Lea et al. 1999; Bruck et al. 2005; Melino et al. 2011; Upadhyay 2017). Understanding the mechanisms through which these compounds exert their biological activities is particularly important for the development of anticancer agents. It should be well known which compound or compounds are responsible for the cellular and molecular effects.

2 Organosulphur Compounds (OSCs)

The characteristic aroma of garlic is due to sulfur-containing volatile compounds that compose 1% of its dry weight (Fenwick and Hanley 1985). These volatile compounds are produced from their non-volatile precursors namely γ-glutamyl-S-alk(en)yl-L-cysteines and S-alk(en)yl-L-cysteine sulfoxides (Butt et al. 2009). OSCs are generally divided into two groups as oil-soluble and water-soluble OSCs. Unharmed cells of garlic bulbs contain alliin (S-allylcysteine sulfoxide). When the garlic is crushed, chopped or chewed, thiosulfinates whose general formula is R_1-S(O)-S-R_2 (where R_1 and R_2 are methyl, allyl, 1-propenyl) are formed (Zalepugin et al. 2015). The half-life of thiosulfinates is about 5 min in the blood (Okada et al. 2005). It triggers biochemical transformations by reacting with thiols in the cells which are in the blood or plasma. It is thought that these transformations and additions of thiol functional groups to the proteins may be related to anticancer activity (Bhuiyan et al. 2015). With crushing, chopping or chewing of the fresh garlic an enzyme known as alliinase is released and converts alliin to allicin (diallyl thiosulfinate) which is a well-known thiosulfinate. Allicin is the main chemical compound of these family, however it is stability depends on its concentration, the temperature, and the solvent in the surrounding environment (Okada et al. 2005). It is rapidly metabolized in aqueous solutions into mono-, di- and trisulfides or other organosulfide compounds such as ajoene and vinilditins (Lanzotti 2006).

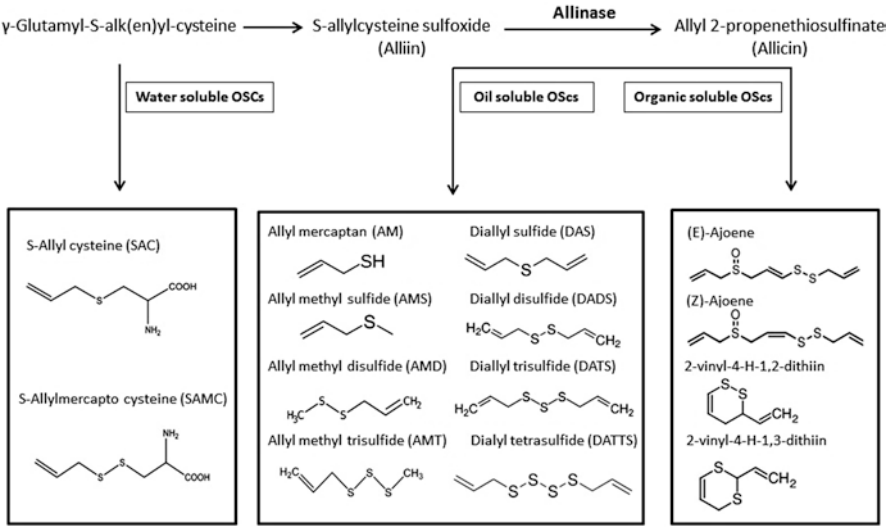

Fig. 11.2 General classification and chemical structures of commonly studied organosulfur compounds of garlic

2.1 Water-Soluble Organosulphur Compounds: S-Allyl Cysteine (SAC), S-Allylmercaptocysteine (SAMC)

γ-Glutamyl-S-alk(en)yl-cysteine is converted to SAC through γ-glutamyl transferase during the long-term incubation of crushed garlic in aqueous solutions named as aged garlic extract (AGE) (Block 1985; Jiang et al. 2019) (Fig. 11.2). One of the major bioactive components in AGE is SAC. SAC is a stable compound that prevents cardiovascular (Chuah et al. 2007), neurodegenerative diseases (Ray et al. 2011), diabetes mellitus (Sathibabu Uddandrao et al. 2017) and cancer (Ho et al. 2018). In previous studies, it was reported that SAC acts as an antiproliferative agent against some cancer types in both *in vitro* and *in vivo* models (Tang et al. 2010). It has been revealed that another water-soluble organosulfur compound, SAMC, stimulates apoptosis in breast and gastric cancer cells (Sigounas et al. 1997; Yan et al. 2013), inhibits ROS formation and DNA damage in lung cancer cells (Wang et al. 2016), changes the expression of prostate biomarkers in prostate cancer cells (Pinto et al. 2000), activates JNK1 pathway and microtubule depolymerization in colon cancer cells (Xiao et al. 2003). Moreover, both SAC and SAMC were demonstrated to inhibit vascular endothelial cell growth and suppress the effect of colony-forming, development, and invasion rate of cancer cells (Chu et al. 2006b). In addition, SAC has a 30-fold lower toxicity than allicin and DADS (Amagase et al. 2001).

2.2 Oil-Soluble Organosulphur Compounds

Steam distillation of garlic produces an oil with different allyl sulfur components such as dialyl tetrasulfide (DATTS), dialyl trisulfide (DATS), dialyl disulfide (DADS), dialyl sulfur (DAS), allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD), allyl methyl sulfide (AMS) and allyl mercaptan (AM). The oil obtained by maceration contains vinilditins such as 2-vinyl-4-H-1,2-dithiin and 2-vinyl-4-H-1,3-dithiin and ajoens such as E-Ajoene and Z-Ajoene (Yoo et al. 2014) (Fig. 11.2).

The allyl sulfur compounds, which are more commonly studied in anticancer studies, are DAS, DADS, DATS and DATTS. Therefore, in this chapter we will focus specifically on anticancer activities of these compounds.

3 Cancer Chemopreventive Effects of Organosulfur Compounds of Garlic

3.1 In Vitro *Studies*

Carcinogenesis, also called oncogenesis or tumorigenesis, consists of three different stages: initiation, promotion, and progression involving invasion and metastasis. In this process, cancer hallmarks including cell proliferation, inhibition of apoptosis, invasion and metastasis, angiogenesis, immortalization, inflammation, immunity, genome instability and mutation, cell energetics and metabolism are involved (Hanahan and Weinberg 2011). Therefore, agents with therapeutic effect focus on targeting these mechanisms.

The first study to suggest that garlic can prevent the growth of malignant cells belongs to Weisberger and Pensky (1958). Table 1 presents the results of biological activity of various allyl sulfur compounds on human cell lines. Allicin, one of the most studied compounds in cancer research, induce apoptosis and interfere with cell growth signaling pathways (Lawson et al. 1992; Rose et al. 2019). However, some studies have showed that the treatment of alliin alone does not exert an antiproliferative effect on the growth of tumor cells. Therefore, it has been considered that the alliin should be broken down for maximum tumor inhibition (Scharfenberg et al. 1990).

It has been reported that SAC and SAMC regulate the expression of E-cadherin and decrease the expression of Snail, E-cadherin suppressor, in prostate, ovarian, nasopharyngeal and esophageal cancer cells (Chu et al. 2006b). E-cadherin is a transmembrane protein that plays a role in cell adhesion and is an important factor for epithelial-mesenchymal transition (EMT). Decreased level of its expression is associated with an invasive phenotype. SAC and SAMC are shown as potential agents in suppressing invasive growth (Chu et al. 2006b). Ng et al. (2012) reported that SAC significantly suppresses the expression of proliferation markers Ki-67 and

proliferation cell nuclear antigen (PCNA) and apoptosis-related B-cell lymphoma-extra large (Bcl-xL) and B-cell lymphoma 2 (Bcl-2), as well as stimulates the cell cycle arrest at S phase by decreasing cell division cycle-25 (Cdc25), cell division cycle-2 (Cdc2) and cyclin B1 expressions in the hepatocellular carcinoma cell line. They confirmed that SAC increases the level of E-cadherin and decreases the level of VEGF, similar to the studies of Chu et al. (2006b). In addition, SAC mediates the suppression of motility and invasion by stimulating E-cadherin and downregulation of MMP-2 in the breast cancer cell line, MDAMB231 (Gapter et al. 2008). According to a detailed study, DATS inhibits metastasis by inhibition of focal adhesion kinase (FAK), extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38 in colon cancer cell line (Lai et al. 2015).

It was previously mentioned that all allyl sulfur compounds obtained from garlic are not equally effective in reducing tumor proliferation (Dion et al. 1997; Sakamoto et al. 1997). Similarly, fat-soluble DATS has been shown to be more effective in suppressing cell growth than DAS and DADS in HCT-5 and DLD-1 (colorectal cancer cell lines) and A549 (lung cancer cell lines) (Seki et al. 2008; Wang et al. 2014). Furthermore, DATS treatment has been reported to reduce the activity of Wnt/β-catenin by stimulating apoptosis in colorectal cancer stem cells (Zhang et al. 2018). A study on proliferation and cell cycle progression by Shirin et al. (2001) showed that SAMC, but not SAC, stops the cell cycle in the G2/M phase and activates caspase-3, triggering apoptosis in colon cancer cell lines. In addition, coadministration SAC with sulindac sulfide (SS), a chemotherapy agent in colon cancer, apoptotic and growth-inhibiting effect increased. DADS has been reported to inhibit metastasis through the SRC/RAS/ERK signaling pathway by increasing the expression of miR-34a in the breast cancer cell line, MDM-MB-23 (Xiao et al. 2014). DATS inhibits gastric cancer cell growth by regulating the expression of MMP-9 and E-cadherin proteins in BALB/c(nu/nu) mice (Jiang et al. 2017). A recent study has shown that SAC reduces viability of MCF-7 cells by decreasing the 3-mercaptopyruvate sulfur transferase (MPST) expression, H_2S/sulfane sulfur endogenous formation from L-cysteine, and sulfate sulfur level (Bronowicka-Adamska et al. 2020).

Garlic allylsulfide compounds also play a role in the cytotoxicity of cancer cells through ER stress. Ajoene, increases the level of GRP78 (Glucose-Regulated Protein 78 kDa) protein by activating the unfolded protein response (UPR) in the MDA-MB-231 breast cancer cell line and WHCO1 esophageal-cancer cells (Kaschula et al. 2016). In this way, it triggers ER stress by causing unfolded protein aggregates. A study on the colon cancer cell line showed that DATTS activates eIF2α and Nrf2/HO-1, one of the signal molecules associated with ER stress (Saidu et al. 2013). In the human malignant neuroblastoma cell line, SH-SY5Y, DAS and DADS have been demonstrated to stimulate $Ca^{(2+)}$-dependent protease calpain (Karmakar et al. 2007). Wang et al. (2012b) showed that DATS increased intracellular Ca^{2+} mobilization and expression of ER stress sensors GRP78/Bip and CHOP (CCAAT-enhancer-binding protein homologous protein)/GADD153 (G1 arrest and DNA damage 153). These studies have revealed that allyl sulfur compounds have an anticancer effect through ER stress.

In addition, oil-soluble allyl sulfur compounds in garlic are possibly more toxic than water-soluble compounds. Indeed, studies have shown that oil-soluble allyl sulfur compounds including DADS reduce the growth of neoplasms, while a water-soluble compound SAC has no effect on established tumors (Sundaram and Milner 1993; Hong 2004). Detailed information about the possible activities of allyl sulfur compounds in various human cancer cell lines is presented in Table 11.1.

Table 11.1 Selected studies that show the anticancer effects of various organosulfur compounds of garlic on human cancer cell lines

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Colon cancer	Allicin (3–6μg/mL)	LoVo	Supression of adhesion, migration and invasion	Gao et al. (2009)
	SAMC (~160–175μM)	SW-480, HT-29	Inhibition of cell growth, G2/M cell cycle arrest	Shirin et al. (2001)
	SAMC (300μM)	SW480	Apoptosis via JNK1 and caspase-3 signaling pathways	Xiao et al. (2003)
	SAMC (150μM) DADS (56μM)	SW480	Microtubule depolymerization by arresting cells in mitosis	Xiao et al. (2005)
	DAS (50μM)	Colo201, Colo320, Colo320	Inhibition of N-acetyltransferase activity	Chung et al. (2004)
	DATS (40μM)	SW480, DLD-1	Supression of cell proliferation, Wnt/β-catenin pathway inhibition	Zhang et al. (2018)
	DADS (200μM)	Caco-2, HT-29	Inhibition of cell proliferation trough epigenetic mechanism; inhibition of HDAC activity, histone hyperacetylation and upregulation of p21	Druesne et al. (2004b)
	DATS (11μM)	HT-29	Inhibition of migration and invasion through MMP-2,-7,-9 and VEGF downregulation	Hosono et al. (2008)
	DATS (25μM)	HT-29, HUVEC	Inhibition of migration and angiogenesis via FAK, Src and Ras	Lai et al. (2015)

(continued)

Table 11.1 (continued)

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Skin cancer	Ajoene (50μM)	B16F10 (murine)	Inhibition of tumor formation and cell proliferation	Ledezma et al. (2004)
	SAC (2.7–4.6 mM)	UCLASO-M7, M10, M12, M14, M16, M24, M25, M210, M223 (human), F10, BL6 (mouse)	Supression of cell proliferation and clonogenicity	Takeyama et al. (1993)
	DATS (50 mM)	A375	Increases intracellular ROS generation, activation of p53 pathway	Wang et al. (2010)
Prostate cancer	SAC (10–15 mM)	PC-3	Inhibition of cell proliferation, cell cycle arrest at the G0/G1 phase and induction of apoptosis through downregulation of Bcl-2 and upregulation of Bax, caspase 8	Liu et al. (2012)
	DADS (40μM)	PC-3	Induction of apoptosis and histone hyperacetylation	Arunkumar et al. (2007)
	DATS (10–40μM)	LNCaP, LNCaP-C81, LNCaP-C4-2	ROS generation, mitochondria-mediated apoptosis; upregulation of Bak and, downregulation of Bcl-2 and Bcl-xL protein levels	Kim et al. (2007)
	SAC (2.16 ± 0.32 mM), SAMC (86.34 ± 6.25μM)	PC-3	Supression of invasive growth of cancer cells through regulation of E-cadherin	Chu et al. (2006a, b)
	SAC (4.59 ± 0.93 mM), SAMC (145.79 ± 16.18μM)	DU145		

(continued)

Table 11.1 (continued)

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Brain tumor	SAC (600μg/mL)	LA-N-5	Inhibition of cell growth	Welch et al. (1992)
	DAS, DADS (100μM)	T98G, U87MG	ROS production, apoptosis correlated with intracellular Ca^{+2} promotion, activation of JNK1 pathway	Das et al. (2007)
Nasopharyngeal carcinoma	SAC (10–40 mM)	HNE1, HONE1	Inhibition of invasion and migration; downregulation of FAK, Slug (SNAI2) and MMP2/9	Cho et al. (2015)
	DADS (50–150μM)	CNE-2	Cell cycle arrest at the S phase, increase of MAPK phosphorylation	Zhang et al. (2006)
Gastric cancer	DADS (30 mg/L)	MGC803	Cell cycle arrest at the G2/M phase, an alteration of the ERK1/2 signaling pathway	Ling et al. (2006)
Lung cancer	Allicin (1–20μM)	A549, H1299	Supression of adhesion, invasion and migration through decreasing the activity of the PI3K/AKT signaling pathway	Huang et al. 2017
	DADS (25–200μM)	A549	Oxidative stress mediated cell cycle arrest at G2/M and apoptosis	Wu et al. (2005)
	DATS (25–100μM)	A549	Stimulation of apoptosis through upregulation of Bax/Bcl-2 ratio and caspase-3, -8, and -9	Li et al. (2012)
	DATS (20–40μM)	LNCaP, HCT-116	Cell cycle arrest via induction of cyclin B1 and down-regulation of CDK	Xiao et al. (2009)

(continued)

Table 11.1 (continued)

Cancer type	Effective compounds	Cancer cell type	Mechanisms of action	References
Breast cancer	Ajoene (20–60μM)	MDA-MB-231	ER stress-mediated cell death via activation of UPR and upregulation of GRP78 protein	Kaschula et al. (2016)
	DATS (2.5–160μM)	MDA-MB-231, HS 578t	Inhibition of metastasis through downregulation of MMP2/9 activity by increasing the NF-κB pathway	Liu et al. (2015a), Anwar et al. (2018)
	DADS (200μM)	MCF-7	Inhibition of ERK and activation of the SAPK/JNK and p38 pathways	Lei et al. (2008)
	DADS (100–400μM)	MCF-7	Inhibition of invasion and metastasis; downregulation of vimentin, MMP9 and upregulation of E-cadherin	Chen et al. (2016)
Liver cancer	Allicin (15–50μM)	HepG2	p53 protein expression mediated apoptosis	Chu et al. (2013)
	SAC (5–50 mM)	MHCC97L	Inhibition of cell proliferation by downregulation of Ki-67 and PCNA. Inhibition of cell invasion and migration with upregulation of E-cadherin and downregulation of VEGF	Ng et al. (2012)
	SAC (0.1–100 mM)	HepG2	Stimulation of apoptosis related with caspase-8, upregulation of p38 MAPK signalling	Sengupta et al. (2017)
	DATS (10–100μM)	J5	Cell cycle arrest through accumulation of cyclin B1 and down-regulation of CDK	Wu et al. (2004)
Thyroid cancer	DAS (50–400μM)	ARO	Inhibition of cell growth and apoptosis with increase in the level of Bax, activation of caspase-9 and -3	Shin et al. (2010)
	SAMC (0.02–0.1 mg/mL)	HPACC-8305C	Apoptotic cell death and inhibit telomerase activity	Liu et al. (2015b)

3.2 In Vivo *Studies*

Effective evidence has been obtained in animal models that allyl sulfur compounds can inhibit the tumor formation as well as cancer cell growth. The intraperitoneal (i.p.) application of raw garlic extract (RGE) completely improved the mice implanted with the murine sarcoma cancer cell S180 (100 mg of the RGE for 21 days), but the same findings could not be obtained in oral application (Li et al. 2018a). Although a meta-analysis of 18 studies found a negative association between garlic consumption and reduced risk of gastric cancer (OR = 0.51, 95% CI = 0.44–0.57), prospective study results were not significant (OR = 0.95, 95% CI = 0.66–1.24) (Li et al. 2018b). These results have shown that garlic extract should not pass through the gastrointestinal tract. Garlic treatment in mice with bladder cancer has been shown to inhibit tumor growth and reduce mortality (Rigs et al. 1997). It has been reported that allicin improved liver damage and increased chemotherapy response in tamoxifen-induced mice by i.p. injection at a dose of 45 mg/kg for 7 days (Suddek 2014). On the other hand, SAMC treatment inhibited hepatocarcinogenesis by targeting the LRP6/Wnt pathway in hepatocellular carcinoma (HCC) nude mice model by daily oral gastric lavage feeding at a dose of 300 mg/kg SAMC (Xiao et al. 2018).

Administration of DAS by orally at a dose of 200 mg/kg for 7 days with Se-methylselenocysteine or quercetin to animals with 7,12-dimethylbenz[a]anthracene (DMBA)-induced breast tumor has been shown to have a greater antitumor effect than alone treatment (Ip and Ganther 1991). DAS also inhibits tumor formation in mice and rats with benzo[a]pyrene (BP) and 1,2-dimethylhydrazine (DMH)-induced colon tumors as well as inhibits lung tumor formation in mice by reducing the metabolic activation of nitrosamine. According to these results it can be said that DAS has shown antitumor effect by affecting defective signaling pathways of various types of cancer. Similarly, DADS has been reported to decrease the NF-κB phosphorylation in azoxymethane and dextran sulphate sodium (DSS)-induced mice (60 mg/kg for 5 weeks) and prevent colitis-induced colon cancer by inhibiting GSK-3β (Saud et al. 2016). However, when DADS was given orally to H-*ras* oncogene transformed tumors in mice at a dose of 33μmol for three times per week, H-RAS mutant cancer cells growth decreased (Singh et al. 1996). In addition, when U2OS cells were subcutaneously injected to BALB/c nude mice and then treated 100 mg/kg DADS with miR-134 inhibitor for 35 days, DADS was shown to suppress forkhead box protein M1 (FOXM1)-mediated proliferation and invasion by upregulating miR-134 in osteosarcoma (Li et al. 2018c).

According to the researchers, allyl sulfur compounds reduce or suppress the growth, proliferation, invasion and metastasis of cancer when administered alone. But little is known about its clinical implications, and it should be supported by epidemiological studies. First of all, the molecular mechanism studies, including the application of both alone and combination with chemotherapy drugs, should be investigated in detail in the prevention of metastasis, which is known as the primary cause of cancer deaths.

Lai et al. (2015) treated BALB/c (nu/nu) mice with 50 mg/kg DATS for 32 days after subcutaneously injected HT-29 cells. As a result of this study, DATS was found to reduced tumor growth, tumor weight, and angiogenesis. Ajoene significantly reduced the incidence of tumors in 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted mouse skin, treated with 250μg ajoene for 18 weeks (Nishikawa et al. 2002). In a similar study ajoene has been shown to reduce LPS-induced TNF-α and IL6 stimulation and inhibit lung metastasis with B16/BL6 melanoma tumor model in C57BL/6 mice by i.p. injection at a dose of 25μg/g for 28 days (Taylor et al. 2006). Another study indicated that it exerts antimetastatic effect by the suppression of viable circulating tumor cells (Howard et al. 2007). In addition, DATS (6μmol orally, thrice weekly) significantly reduced tumor growth without any side effects in PC-3 xenografts in athymic mice, as well as these results correlated with the increased expressions of Bax and Bak (Xiao et al. 2006a).

In DMBA-stimulated mouse skin tumor DAS (applied topically 10 mg/kg body-weight for 24 h) suppressed the growth of tumor cells by decreasing the expression of p21/Ras oncoprotein and H-RAS mRNA level (Arora et al. 2005). Similarly, it was reported that tumor size and number decreased and p53wt and p21/Waf1 were upregulated in mice where liposomized DAS formulation (250μg, three times a week for 12 weeks) was applied against DMBA-induced skin papilloma (Khan et al. 2007). In addition, DAS (applied topically 10 mg/kg body-weight for 48 h) provided effective protection against DNA strain fractures in the DMBA-stimulated skin tumor model (Nigam and Shukla 2007). In an *in vivo* study in androgen-independent prostate cancer xenografts, oral SMAC administration (300 mg/kg for 28 days) reduced the growth of primary tumors and the number of metastases to the lung and adrenal gland (Howard et al. 2007). In the study conducted by Ng et al. (2012), it was determined that treating SAC alone or in combination with cisplatin (1 mg SAC/kg/day + 1 mg cisplatin/kg/day for 6 weeks) in the *in vivo* xenograft liver tumor model suppressed the progression and metastasis of hepatocellular carcinoma.

4 Epidemiological Studies

Researchers or scientific authorities adopt the view that nutrition can reduce the risk of cancer. 80% of cancers are associated with environmental factors, only 1% are caused by cancer syndromes and up to 5% are caused by single gene mutations. Therefore, it is predicted that 35–40% of cancers can be prevented by nutrition and physical activity (Wilson et al. 2002; Tandon et al. 2008). Although several epidemiological studies provide evidence that garlic consumption changes the course of the disease by affecting the molecular pathogenesis of cancer, long-term intervention studies are lacking. For example, stomach cancer mortality was 13 times lower in those who consumed 20 g of garlic per day than those who consumed only 1 g/day in Shandong province of China (Han 1993). According to the Chinese Academy of Medical Sciences, there was a negative relationship between the consumption of

garlic with the incidence of gastric cancer (Setiawan et al. 2005; Li et al. 2018a). Kodali and Eslick (2015) also reported a significant association with an elevated allium consumption and reduced gastric cancer risk in a meta-analysis study consisting of 8621 cancer cases and 14,889 controls. In a case-control and meta-analysis study of 230 cancer cases and 547 controls, a negative relationship was reported between increased garlic consumption and reduced risk of gastric cancer (Turati et al. 2015). However, in a study conducted in the Korean population, there was no significant relationship between garlic intake and decreased stomach cancer incidence (Kim et al. 2002). Dorant et al. (1996) and You et al. (2006) also adopted the same view. In another study in China, the intake of more than 10 g daily of allium vegetables in men reduced risk of prostate cancer compared to those who used less than 2.2 g daily (Hsing et al. 2002). Kirsh et al. (2007) reported that more than once intake per week was not associated with prostate cancer risk. In the case-control study conducted in China, it was observed that consumption of raw garlic more than twice [OR of 0.78 (95% CI: 0.62–1.01)] per week was negatively related to risk of liver cancer (Liu et al. 2019). These outcomes explain that daily dose should be determined according to cancer types in reducing the incidence.

Several studies have demonstrated that high garlic consumption is negatively related to the risk of prostate (Salem et al. 2011; Zhou et al. 2013), esophagus (Chen et al. 2004), larings, ovarian, renal and oral (Galeone et al. 2006), breast (Desai et al. 2019), multiple myeloma (Wang et al. 2012a), endometrium (Galeone et al. 2009), liver (Zhang et al. 2013), primary invasive epithelial ovarian and colon cancer (Steinmetz et al. 1994; Levi et al. 1999; Galeone et al. 2006). One of the most impressive studies have revealed that when garlic is consumed over 10 years, the incidence of hematological malignancy can lead to a 45% reduction (Nicastro et al. 2015). Nevertheless, there are studies claiming that there is no significant relationship between garlic consumption and cancer incidence. For instance, it was stated that the use of garlic is not related to the risk of colon (Tanaka et al. 2004; Giovannucci et al. 1994), lung (Dorant et al. 1995) and breast cancer (Galeone et al. 2006). In another study, after topical ajoene application in 21 patients with nodular or superficial basal cell carcinoma, it was reported that tumor size was reduced in 17 patients, Bcl-2 expression was significantly reduced, thus mitochondria-mediated apoptosis was stimulated (Tilli et al. 2003).

In some studies showing that garlic consumption has decreased cancer incidence, it was understood that the number of subjects was low, a low dose-control group was used instead of the placebo group, or the garlic intake was determined with qualitative questions without quantitatively measuring. On the other hand, the results may be directly related to the countries' diet (cooked or raw) and consumption amounts, and the reason for the differences between studies. Therefore, there is a need for advanced epidemiological studies based on larger populations and quantitative data.

5 Mechanisms of Action

5.1 Stimulation of Detoxification Enzymes

One of the mechanisms mediating the anticarcinogenic effect of garlic and some allyl sulfur compounds is the induction of detoxification enzymes. In mammalian systems, these enzymes are generally divided into two classes, phase I and phase II enzymes. Phase I metabolism largely occurs through cytochrome P450 (CYP450s) enzymes. Xenobiotics including drugs, toxins, carcinogens, mutagenes and toxic chemicals are metabolized by the CYP450s. In the phase II, the metabolized products are conjugated with molecules such as glucoronic acid, sulfate and glutathione, so that they can be excreted from the body through gall and urine.

Various allyl sulfur compounds have been reported to suppress or activate the expression of CYP450 genes (Srivastava et al. 1997). This suppression or activation can provide some benefits, such as preventing DNA damage, removing various carcinogens from the body. In a study by Davenport and Wargovich (2005), it was determined that DAS and DADS decreased rat liver protein level, while propyl-derived compounds and water-soluble SAC were not effective. However, DAS has been shown to increase liver CYP1A1 and CYP1A2 protein levels in time and dose depending manner. In addition, Wargovich (2006) demonstrated that DAS and DADS are the compounds that block CYP2E1 protein synthesis but the compounds of propyl origin cannot show the same effect. DADS increased the expression levels of liver and intestine CYP2B1 and CYP2B2 in rat. Although DAS had similar effect with DADS in the liver, only CYP2B1/2 protein levels were increased in the intestine.

Nitrosamines (NA) are potential carcinogens that affect the risk of cancer in humans and play a role in increasing this risk. Suppression of nitrosamine formation has been proposed as one of the possible anticancer action mechanisms of garlic, and allyl sulfur compounds. (Atanasova-Goranova et al. 1997; Dion et al. 1997). They also regulate phase I and II enzymes and DNA repair (Wattenberg 1990). Several studies have shown that DAS is a competitive inhibitor of N-nitrosodimethylamine (NDMA), a highly carcinogenic NA (Yang et al. 2001; Fasolino et al. 2015). Studies have shown that SAC is more effective than DAS and DADS in suppressing nitrosamine formation (Dion et al. 1997; Milner 2001). These effects of allyl sulfur compounds can be associated with inhibition of carcinogen activation by the P450s.

Activation of detoxification pathways through the induction of phase II enzymes (glutathione S-transferase (GST), UDP-glucuronosyl transferase (UGT), quinone reductase) is suggested as one of the main anti-tumor mechanisms of allyl sulfide compounds (Hu et al. 1997; Andorfer et al. 2004). Although DADS significantly increases GST and glutathione (GSH) levels in rats, SAC does not show the same effect. It has been suggested that an allysulfur-rich diet can alter chemotherapy treatment by increasing the expression of genes associated with multiple drug resistance (Demeule et al. 2004). GSH activity has also been shown to increase in the

DAS-treated mice stomach (Maurya and Singh 1991). There is a positive relationship between chemopreventive effects of the allyl sulfur compounds such as DAS, DADS and DATS, and increased NAD(P)H:quinone oxidoreductase (NQO) expression in benzo(a)pyrene (BP)-induced forestomach and lung cancer (Singh et al. 1998).

Garlic OSCs are H_2S donors, gaseous signaling molecules, and release H_2S through mainly GSH-dependent mechanism. Also, some OSCs such as DATS perform this secretion much faster than others (Liang et al. 2015). Under physiological conditions, while endogenous H_2S or relatively low exogenous H_2S takes part of maintaining homeostasis or limiting tissue damage, prolonged or high amount of H_2S exposure is thought to cause cancer cell death due to cellular toxicity (Han et al. 2019). Therefore, exogenous H_2S sources could be used as powerful therapeutic agents against a variety of diseases, including cancer.

As a result, some CYP450 enzymes and GSH mediate the anticancer effect of allyl sulfur compounds. However, compounds carrying allyl and oil-soluble groups are more effective in stimulation of detoxifying enzymes than those carrying propyl and water-soluble groups (Chen et al. 2004). Moreover, some components are not effective at the mRNA level, but at the protein level. This is an example of translational-level mechanisms of action, including epigenetic mechanisms.

5.2 Cell Protection from Oxidative Stress

Increased intracellular level of reactive oxygen species (ROS) causing oxidative stress is closely related to the pathogenesis of many diseases, including cancer (Liou and Storz 2010). Antioxidant system (involved in enzymes such as glutathione peroxidase (GPx), GST, catalase, superoxide dismutase (SOD)) neutralizes the oxidative damage. Garlic and its allyl sulfur compounds display free radical scarvening activity and protect the cell from lipid, protein and DNA damage (Sowjanya et al. 2009; Upadhyay 2017). Oral ingestion of garlic in animal models has been shown to reduce lipid peroxidation, increase circulating antioxidants, reduce glutathione and glutathione peroxidase (Balasenthil et al. 2000), and exhibit antimutation effect against gamma radiation (Chang et al. 2012). SAC has been found to have antioxidant properties in *in vitro* and *in vivo* models, improve $K_2Cr_2O_7$-induced toxicity (Medina-Campos et al. 2007) and reduce DNA damage (You et al. 2006). Similarly, SAMC plays a role in reducing ROS formation, preventing DNA damage, increasing SOD activity, and preventing NF-κB activity (Wang et al. 2016). DADS and DATS have also been shown to fight cellular stress by activating antioxidant enzymes (Awan et al. 2019). However, DAS, DADS and DATS stimulate the production of ROS (Antosiewicz et al. 2006; Das et al. 2007) by triggering cellular apoptosis and arresting the cell cycle (Yang et al. 2009).

5.3 *Induction of Cell Death and Cell Cycle Arrest*

Possible anticancer activity mechanisms of garlic's OSCs are summarized in Fig. 11.3. Cellular death and regulation of the cell cycle are among the most studied anticancer mechanisms of garlic and its OSCs. Although the only cell death mechanism is considered to be apoptosis (type I cell death), autophagy (type II cell death) and necrosis (type III cell death) are also included in this classification nowadays. In multicellular organisms, the main cellular death mechanism is apoptosis which is required for homeostasis in the development process from embryonic period to aging. However, apoptosis is triggered by intracellular (caspases) and extracellular pathways (death receptors) in immune response or cellular damage (Norbury and Hickson 2001). On the other hand, autophagy is a process for cellular homeostasis and cell survival that involved the remove of misfolded or aggregated proteins and damaged organelles as well as eliminated intracellular pathogens.

In some studies, involving the effects of garlic allyl sulfur compounds on cell death, SAMC has been shown to inhibit cell growth in gastric cancer through apoptotic proteins (Katsuki et al. 2006) and trigger MAPK-induced apoptosis by TGF-β

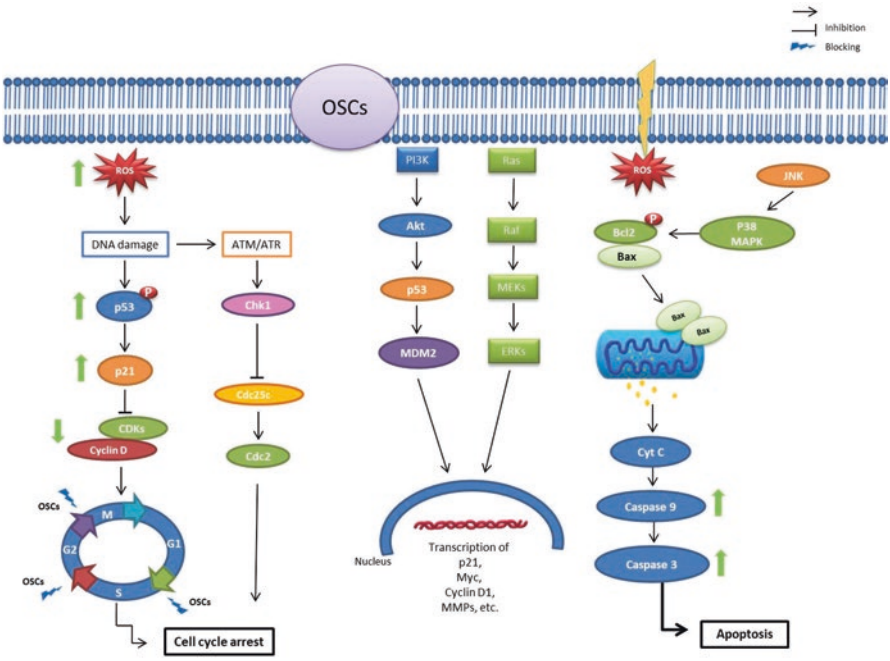

Fig. 11.3 Molecular mechanisms of action of OSCs-induced cell cycle arrest and apoptosis in cancer cells. OSCs induce ROS generation and DNA damage. It results the activation of phospho-53 and p21, and p21 inhibits the regulatory proteins, and then blocks the cell cycle at G1/S, S/G2 and G2/M. Also, OSCs activates ERK and PI3K for transcription of some survival genes such as p21, Cyclin D1. And they activate the JNK and p38 MAPK, and then upregulate of Bax and down-regulate of anti-apoptotic protein Bcl-2 gene. Decreasing of mitochondrial membrane potential trigger the release of Cyt C from mitochondria, and it results activation of caspase 9, caspase 3 and PARP that induce the caspase dependent apoptosis

activation in colon and hepatocellular carcinoma cells (Tong et al. 2014). Although SAMC is more effective than SAC, it is reported that it suppresses the proliferation and invasion of prostate, ovarian, nasopharyngeal and esophagal cancer cells and rearranges the cell cycle (Chu et al. 2006a). However, SAC has been shown to upregulate caspase-3 in ovarian cancer lines and inhibit cell proliferation by stimulating DNA methylation via DNA methyltransferases (DNMTs) (Xu et al. 2014, 2018). After SAC treatment, it was demonstrated that the antiapoptotic proteins Bcl-2 and Bcl-xL expression decreased and apoptotic proteins Bak and PUMA expression increased (Velmurugan et al. 2005; Ng et al. 2012). In HepG2 cells, it stimulates the apoptosis and cell cycle arrest through p53/p21 and JNK/c-Jun pathways (Knowles and Milner 2003). Based on these data, water-soluble allyl sulfur compounds promote cell death through both intracellular and extracellular apoptotic proteins.

Hong et al. (2000) found that DAS, DADS, and ajoen direct cancer cells to apoptosis by increasing the expression of apoptotic proteins (such as p53 and Bax) and decreasing the expression of antiapoptotic Bcl-2 through DNA fragmentation and intracellular free calcium. In addition, DADS inhibits cell proliferation by inducing cell cycle arrest in G2/M phase by decreasing cyclin B, Cdc2 and Cdc25C in ECA109 esophageal squamous cell line. Then caspase-mediated apoptosis accompanied by Bcl-2 and Bax proteins and inhibition of MAPK/ERK pathway takes place (Yin et al. 2014). Kelkel et al. (2012) suggested that DATS exhibits anticancer properties by inhibiting tubulin polymerization, in particular, this effect is related to the number of sulfur atoms.

Xiao et al. (2009) showed that DATS stimulates apoptosis by arresting cell cycle in G2/M phase through checkpoint kinase 1 (CDK1) by phosphorylation of its Tyr 15 residue in LNCaP and HCT-116 human cancer cells. On the other hand, DATS has been shown to be effective in preventing the angiogenic properties of human umbilical vein endothelial cells (Xiao et al. 2006b), stimulating human epidermal growth factor receptor2 (HER2) or p53-induced apoptosis, cell growth, migration and cell viability in MCF-7 and MDA-MB-231 breast cancer cell line (Antony and Singh 2011; Chandra-Kuntal et al. 2013).

The integrity of the cell cycle is essential to maintain healthy cell proliferation. It is mainly regulated by cyclin dependent kinases (CDKs) and inhibitors. Any problem in cell division can initiate the tumor process by causing uncontrolled cell divisions. Therefore, agents that inhibit tumor growth at phases of the cell division are among the therapeutic targets. It has been reported that SAMC, DAS, and DADS cause an increase in the percentage of cells blocked in the G2/M phase (Knowles and Milner 2001). In another study, DADS has been shown to block the cell cycle in the G2/M phase on osteosarcoma cells, thereby stimulating apoptosis and autophagy (Knowles and Milner 2001). This anticancer effect of DADS is due to the blocking of the phosphoinositide 3-kinase/mammalian target of rapamycin (PI3K/Akt/mTOR) signal pathway, one of the major pathways involved in the growth and proliferation of many cancer cell types (Yue et al. 2019). Similarly, Chu et al. (2013) suggested that allicin inhibits cell viability by decreasing the level of Bcl-2, cytoplasmic p53 and PI3K/Akt/mTOR signaling pathway and stimulates autophagy by

increasing tumor-suppressor AMP-activated protein kinase/tuberous sclerosis complex (AMPK/TSC2) expression and Beclin-1 signaling pathways.

5.4 Immune System Enhancement

Immune system is an incredibly complex host defence mechanism involved many biological structures and processes within an organism (Bourgeon et al. 2007). The host defense cells in the inflammatory system secrete many cytokines, chemokines and similar molecules to suppress malignant cells (Korniluk et al. 2017). When the defense system encounters a stimulus, it activates intracellular signaling pathways especially NF-kB, MAPK and JAK/STAT pathways, releasing inflammatory mediators (Chen et al. 2017). Expression of proinflammatory cytokines (such as IL1β, IL6, TNF-α) with the activation of these pathways supports tumor development. In addition, anti-inflammatory cytokines such as IL10 reverse this condition. Therapeutics that affect inflammatory pathways are being investigated in detail as they can potentially change the cancer process.

Evidence for both preventive and therapeutic effects of garlic on anticancer activity has been presented so far. Garlic and its OSCs have been shown to inhibit cancer progression by influencing inflammatory responses or by regulating cytokine production (Guan et al. 2018). For instance, garlic extract decreases the release of IL12, TNF-α, IL1α, IL6, IL8, IFNγ, IL2 cytokines, while increases the level of IL10 (Hodge et al. 2002).

It has been determined that SAC inhibits NF-κB activation in human T lymphocytes stimulated by TNF-α and H_2O_2 (Geng et al. 1997). In another study, it was reported that garlic extract inhibits NF-κB and the molecules in TLRs and LPS receptor signaling pathway cascades (Youn et al. 2008). DADS supresses NF-κB thought blocking the glycogen synthase kinase 3β (GSK-3β) activity, and inhibits tumor growth (Saud et al. 2016) in colitis-induced colorectal cancer, stimulates the release of IL1β, TNF-α and IL6, inhibits the release of IL10 in LPS-stimulated human whole blood (Keiss et al. 2003; Chang et al. 2005). As a result, garlic and its allyl sulfur compounds can regulate inflammation by modulating cytokines and leading to inhibition of NF-κB activity.

5.5 Epigenetic Mechanisms

Not only genetic mechanisms, but epigenetic mechanisms also get involved in the cancer process. Epigenetic mechanisms are all changes in gene expression without modifying the DNA base sequence. There are three defined mechanisms involved epigenetic: histone modification (acetylation, methylation, phosphorylation, ubiquitination and sumolation), DNA methylation at the transcriptional level and noncoding ribonucleic acid regulation at the post-transcriptional level. These

mechanisms affect the binding of transcription factors to DNA. For example; low level of methylation (hypomethylation) activates gene expression by DNA methyltransferase inhibitors (DNMTi), while its high level suppresses gene expression by preventing transcription factors from binding to the promoter. Hypomethylation is a common condition in the early stages of cancer. Therefore, agents targeting epigenetic mechanisms in developing cancer therapeutics remain the focus of attention. In addition, histone deacetylases (HDAC) are considered as potential drug targets because they affect cellular processes such as differentiation, apoptosis, angiogenesis, invasion, and metastasis. Studies show that garlic and various allyl sulfur compounds act as HDAC inhibitors and activate epigenetically silenced genes, leading to apoptosis and cell cycle arrest. Indeed, the allyl mercaptan and DADS are examples of potent HDAC inhibitors (Nian et al. 2009; Druesne-Pecollo and Latino-Martes 2011). DADS has been shown to inhibit cell proliferation related with increased $p21^{WAF1}$ expression, HDAC inhibition, and histone acetylation in colon cancer cell lines (Druesne et al. 2004a, b). DADS also stimulates cellular apoptosis as a result of increased histone acetylation in prostate cancer cells and inhibits the growth of H-RAS oncogene-transformed tumors (Singh et al. 1996). Apart from the fat-soluble allyl sulfur compounds, water soluble garlic extract has also been reported to inhibit tumor proliferation related with histone hyperacetylation in the T-cell lymphoma cell line (Bhuiyan et al. 2015).

6 Future Directions for Research on the Anticancer Effects of Garlic

Even though garlic and its organosulfur compounds have been used in food or pharmaceutical industry throughout history, detailed research has been undertaken for the past few decades involving mechanisms of action. It is very difficult to treat cancer after it has spread throughout the body which is called as metastasis. Various allyl sulfur type compounds were shown to have a decreasing effect on the frequency of cancer occurrence and progression. Therefore, they are potential agents in anticancer therapy, alone or in combination with antitumor drugs. Antioxidant, apoptotic, proliferative, cell cycle regulating, anti-inflammatory and detoxifying mechanism are among their described mechanisms of action. However, more studies are needed to understand the mechanism of action at both molecular and biochemical levels.

According to the literature, all isolated allyl sulfur compounds do not show the same anticancer effect when evaluated separately. In addition, garlic components, especially the lipophilic ones having allyl groups, exhibited higher anticarcinogenic activities via those mentioned mechanisms. However, there are many *in vitro* studies showing the anticancer properties of water soluble components, especially SAMC or allicin. Accordingly, when developing therapeutically effective compound/compounds from garlic or its preparations, this should be taken into

consideration and its clinical implications should be evaluated. Due to the fact that many of the recent studies do not contain quantitative results the data on garlic's effects on metabolism is limited. Although the studies carried out so far have provided sufficient data on a cellular basis, in clinical trials more attention should be drawn into the factors such as the applied dose, the route of administration and the type of the cancer as well as the diet style. It is important to know that individuals will have different respond to garlic intake, as the causes of cancers depend on variable factors including genetic and environmental. Detailed *in vitro* and *in vivo* studies on allyl sulfur compounds are still in need for further clarification between epigenetic mechanisms of action, especially in tumor inhibition, proliferation, invasion and metastasis.

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Chapter 12
Ginger (Gingerols and 6-Shogaol) Against Cancer

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Abstract Ginger, the rhizomes of *Zingiber officinale* Roscoe (Zingiberaceae), is one of the most widely consumed spices worldwide due to its pungent smell and taste. It has also traditionally been used since antiquity for the treatment of ailments like common colds, fever, arthritis, rheumatism, gastrointestinal complications, motion sickness, diabetes, infectious diseases, asthma, stroke, cancer, etc. Ginger is composed polyphenolics, such as gingerols, paradols and shogaols, which are associated with its remarkable bioactivity, including cancer. Various laboratory investigations have shown that ginger and its bioactive compounds especially 6-gingerol and 6-shogaol exhibit anticancer activities. In experimental studies, several mechanisms related to the chemopreventive effects of ginger and its active ingredients have been stated. Although ginger is a promising resource for the prevention and treatment of cancer, larger-scale clinical trials are required to support experimental studies.

Keywords Cancer · Ginger · Gingerols · Shogaols · Mechanisms

1 Introduction

Ginger is the rhizome of *Zingiber officinale* Roscoe, a monocotyledonous perennial plant, and it is one of the most well-known and commonly used spices, especially in Asia, due to its pungent smell and taste (Beristain-Bauza et al. 2019; Mascolo et al. 1989).

In addition to its use as a condiment in foods, it also has been a traditional medicine since ancient times for many ailments such as colds, fevers, sore throats, infectious diseases, catarrh, nausea, vomiting, colic, diarrhea, stomachache, gastritis, dyspepsia, asthma, respiratory disorders, toothache, gingivitis, cramps, muscular aches, pains, sprain, bleeding, rheumatism, arthritis, cardiovascular diseases, hypertension, stroke, diabetes, dementia, nervous diseases and metabolic diseases (White

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2007; Grzanna et al. 2005; Haniadka et al. 2013; de Lima et al. 2018; Tuntiwechapikul et al. 2010; Nedungadi et al. 2019; Khan and Abourashed 2011).

It has been reported with some population-based investigations that people in South East Asian countries have a lower risk of being diagnosed with various types of cancer than their Western counterparts, and this may be related to their dietary habits that contain chemopreventive dietary agents. Ginger is one of these important constituents of their diet (Dorai and Aggarwal 2004).

Various studies have shown that ginger has anticancer properties thanks to its many important bioactive ingredients. Many volatile and non-volatile compounds have been identified in ginger. The essential oil mainly consists of high proportion of sesquiterpene derivative compounds such as zingiberene, α-curcumene, α-farnesene, β-bisabolene, β-sesquiphellandrene, and relatively small percentage of monoterpene derivative compounds such as 1,8-cineole, linalool, borneol, neral, geraniol (Govindarajan and Connell 1983). Many of these ingredients have been associated with ginger flavour and taste (Shukla and Singh 2007). The main non-volatile compounds that contribute to its pungent taste are gingerols, shogaols, paradols and zingerone. Additionally, these phenolic ingredients, especially gingerols and shogaols (Fig. 12.1), are responsible for various important biological activities of ginger (Haniadka et al. 2012).

Gingerols and shogaols possess an unbranched alkyl chain length of six carbon atoms (Lu et al. 2014). Gingerol analogues bear a β-hydroxy keto moiety inside chain structure that's why they are thermally labile and undergo dehydration readily with thermal processing or long-time storage (Bhattarai and Duke 2001; Koo et al.

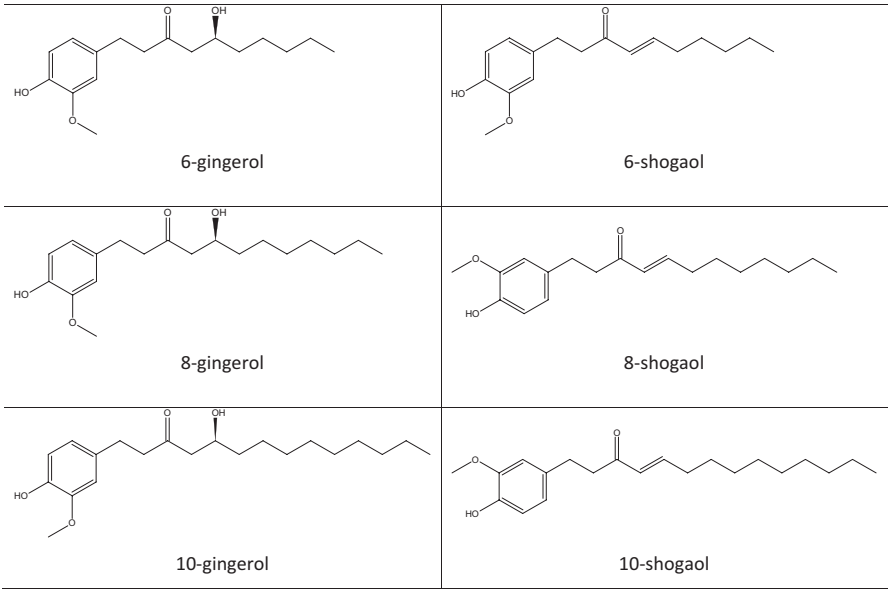

Fig. 12.1 Chemical structures of the some gingerol and shogaol analogues

2001). At the end of this reaction corresponding shogaols occur as dehydration products of gingerol, by the hydrogenation of shogaols, paradols may be formed too (Grzanna et al. 2005; Bhattarai and Duke 2001). Therefore, the amount of gingerols, which are the main components of raw ginger, is relatively less in dry ginger whereas the shogaols percentage increase during drying process (Jolad et al. 2005).

Numerous pharmacological studies have stated that ginger, gingerols and shogaols derivatives have many biological activities such as anti-ulcerogenic (Anosike et al. 2009), antiemetic (Sharma et al. 1997), antimicrobial (Akintobi et al. 2013; Park et al. 2008; Mohamedin et al. 2018), anti-allergic (Sohn et al. 2013), antidiabetic (Adefegha et al. 2010), anti-oxidant (Kikuzaki and Nakatani 1993), anti-inflammatory (Anosike et al. 2009; Levy et al. 2006), antidepressant (Farzin et al. 2013) and anticancer (Akimoto et al. 2015).

In an *in vitro* study that gingerol and shogaol were tested for their antioxidant and anti-inflammatory activities, 6-shogaol was reported to exhibit the strongest antioxidant and anti-inflammatory features, which was associated with the existence of α, β-unsaturated ketone group. Since gingerols are compared among themselves, it is stated that 10-gingerol shows the strongest activity thanks to its long carbon chain (Dugasani et al. 2010).

Similarly, in another study comparing anticarcinogenic and anti-inflammatory activities of three major gingerols (6, 8, and 10-gingerol) and corresponding shogaols (6, 8, and 10-shogaol), 6-shogaol has been identified to possess stronger anti-inflammatory activity. It also has been showed that shogaols have much stronger growth inhibitor effect on human lung cancer cells (H-1299) and human colon cancer cells (HCT-116) than gingerols (Sang et al. 2009).

The chemopreventive and chemotherapeutic potentials and mechanisms of action of ginger extract and its bioactive compounds have been evaluated by giving details of current *in vitro*, *in vivo,* and clinical studies in the following sections.

2 Ginger in Cancer Treatment and Prevention

2.1 In Vitro *Cytotoxicity Studies*

Ginger has attracted the attention of researchers due to its biological activities and important components, and its various extracts, essential oil and active ingredients have been the subject of many cancer research studies. The favourable effects of ginger on cancer have been known for many years. There are many cytotoxicity studies with various cell lines such as leukemia (HL 60, K562, CCRF-CEM), cervix (HeLa, SiHa), ovary (SKOV-3, A2780, HEY, OVCAR-3), breast (MDA-MB-23, MCF-7), prostate (C4-2, PC-3, DU145, LNCaP), lung (A549, H1299), liver (HepG-2 BEL-7404), kidney (786-O, 769-P, ACHN), pancreas (BxPC-3, HPAC), colon (HCT116, SW480, LoVo, HT29, HCT-15), among others. These studies are summarized in Table 12.1.

Table 12.1 *In vitro* cytotoxicity studies of ginger extracts and its active ingredients

Extract/compound	Cell line	Exposure time	Method	Results	References
Aqueous extract	HL60/ADR (acute human myeloid leukemia cell line)	12 and 24 h	MTT (3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay	Significantly decreased cell viability at the high concentrations (100 and 1000μg/mL)	Al-Abbas (2019)
Aqueous extract	HL60 (acute human myeloid leukemia cell line)	12 and 24 h	MTT assay	Significantly decreased cell viability at the high concentrations (100 and 1000μg/mL)	Al-Abbas (2019)
Chloroform extract	L929 (mouse fibroblastic cell line)	48 h	MTT assay	IC_{50}: 87.28μg/mL	Karaboz (2010)
Chloroform extract	HeLa (human cervical carcinoma cell line)	48 h	MTT assay	IC_{50}: 74.32μg/mL	Karaboz (2010)
Ethanolic extract	HeLa	48 h	MTT assay	IC_{50}: 33.78μg/mL	Karaboz (2010)
Ethanolic extract	L929	48 h	MTT assay	IC_{50}: 101.0μg/mL	Karaboz (2010)
Ethanolic extract (70%)	K562 (chronic myelogenous leukemia cell line)	24 h	MTT assay	IC_{50}: 67μM	Tiber et al. (2019)
Ethanolic extract (70%)	HT1080 (human fibrosarcoma cell line)	4 h	MTT assay	Significantly decreased cell viability at 1000μg/mL	Romero-Arias et al. (2019)
Ethanolic extract (70%)	HT1080	4 h	Propidium iodide assay	Significantly decreased cell viability at 400μg/mL	Romero-Arias et al. (2019)
Ethanolic extract (95%)	MDA-MB-231 (human breast cancer cell line, triple negative)	48 h	MTT assay	IC_{50}: 70.55 ± 5.5μg/mL	Nedungadi et al. (2019)
Ethanolic extract (95%)	A549 (nonsmall cell lung cancer cell line)	48 h	MTT assay	IC_{50}: 77.5 ± 11.5μg/mL	Nedungadi et al. (2019)

Extract/compound	Cell line	Exposure time	Method	Results	References
Ethyl acetate fraction of ginger leaf	HCT116 (human colorectal cancer cell line)	24 and 48 h	MTT assay	Reduced the cell viability after treatment for 24 h: At 50μg/mL by 24% At 100μg/mL by 53% At 200μg/mL by 79% Reduced the cell viability after treatment for 48 h: At 50μg/mL by 59% At 100μg/mL by 79% At 200μg/mL by 88%	Park et al. (2014)
Ethyl acetate fraction of ginger leaf	SW480 (human colorectal cancer cell line)	24 and 48 h	MTT assay	Reduced the cell viability after treatment for 24 h: At 50μg/mL by 23% At 100μg/mL by 42% At 200μg/mL by 55% Reduced the cell viability after treatment for 48 h: At 50μg/mL by 40% At 100μg/mL by 57% At 200μg/mL by 76%	Park et al. (2014)
Ethyl acetate fraction of ginger leaf	LoVo (human colorectal cancer cell line)	24 and 48 h	MTT assay	Reduced the cell viability after treatment for 24 h: At 50μg/mL by 20% At 100μg/mL by 34% At 200μg/mL by 95% Reduced the cell viability after treatment for 48 h: At 50μg/mL by 33% At 100μg/mL by 55% At 200μg/mL by 80%	Park et al. (2014)

(continued)

Table 12.1 (continued)

Extract/compound	Cell line	Exposure time	Method	Results	References
Ethyl acetate fraction of ginger leaf	MCF-7 (human breast cancer cell line)	24 h	MTT assay	Reduced the cell viability at 100μg/mL by 36%	Park et al. (2014)
Ethyl acetate fraction of ginger leaf	MDA-MD-231	24 h	MTT assay	Reduced the cell viability at 100μg/mL by 44%	(Park et al. 2014)
Ethyl acetate fraction of ginger leaf	HepG-2 (human hepatocellular carcinoma cell line)	24 h	MTT assay	At 100μg/mL reduced the cell viability by 30%	Park et al. (2014)
Ginger extract (Sigma-Aldrich., W252108)	SKOV-3 (human epithelial ovarian cancer cell line)	24, 48 and 72 h	MTT assay	At 24 h: IC_{50} = 97μg/mL At 48 h: IC_{50} = 60μg/mL At 72 h: IC_{50} = 40μg/mL	Pashaei-Asl et al. (2017)
Methanolic extract	C4-2 (prostate cancer cell line)	72 h	Alamar blue cell proliferation assay	IC_{50}: 512μg/mL	Karna et al. (2012)
Methanolic extract	PC-3 (Caucasian prostate adenocarcinoma cell line)	72 h	Alamar blue cell proliferation assay	IC_{50}: 250μg/mL	Karna et al. (2012)
Methanolic extract	C42-B (human prostate cancer cell line, bone metastatic)	72 h	Alamar blue cell proliferation assay	IC_{50}: 240μg/mL	Karna et al. (2012)
Methanolic extract	DU145 (human prostate cancer cell line)	72 h	Alamar blue cell proliferation assay	IC_{50}: 95μg/mL	Karna et al. (2012)
Methanolic extract	LNCaP (human prostate cancer cell line)	72 h	Alamar blue cell proliferation assay	IC_{50}: 75μg/mL	Karna et al. (2012)
Methanolic extract	HeLa	24 and 48 h	MTT assay	IC_{50} = 46.5μg/mL (24 h) IC_{50} = 37.5μg/mL (48 h)	Ansari et al. (2016)
Methanolic extract	MDA-MB-231	24 and 48 h	MTT assay	IC_{50} = 86.7μg/mL (24 h) IC_{50} = 57.5μg/mL (48 h)	Ansari et al. (2016)

Extract/compound	Cell line	Exposure time	Method	Results	References
Methanolic extract	• A549 • H1299 (non-small lung carcinoma cell line) • C6 (glioma cancer cell line)	24 h	MTT assay	IC_{50}: 2.16 ± 0.08 to 8.53 ± 0.62μg/mL	Gezici (2019)
Ginger oil/water soluble	CCRF-CEM (human Caucasian acute lymphoblastic leukaemia)	72 h	MTT assay	IC_{50}: 167μg/mL	Babasheikhali et al. (2019)
Essential oil	L929	48 h	MTT assay	IC_{50}: 41μg/mL	Jeena et al. (2015)
Essential oil	HeLa	24 h	MTT assay	IC_{50}: 46.0 ± 0.9μg/mL	Lee (2016)
Essential oil	SiHa (cervix cancer cell line)	24 h	MTT assay	IC_{50}: 38.6 ± 0.87μg/mL	Lee (2016)
Essential oil	MCF-7	24 h	MTT assay	IC_{50}: 82.6 ± 3.2μg/mL	Lee (2016)
Essential oil	HL60	24 h	MTT assay	IC_{50}: 39.1 ± 4.2μg/mL	Lee (2016)
Essential oil	HT29-19 (A) (human colon adenocarcinoma cell line, non-muco secreting)	24 h	MTT assay	IC_{50}: 60μL/mL	Al-Tamimi et al. (2016)
Essential oil	HT-29 (human colon adenocarcinoma cell line muco secreting)	24 h	MTT assay	IC_{50}: 40μL/mL	Al-Tamimi et al. (2016)
Essential oil	HepG-2	24 h	MTT and NRU (Neutral Red Uptake) assays	By MTT assay; IC_{50}: 635.1μg/mL By NRU assay; IC_{50}: 635.1μg/mL (NRU assay)	Santos et al. (2016)

(continued)

Table 12.1 (continued)

Extract/compound	Cell line	Exposure time	Method	Results	References
Essential oil	HeLa	24 h	MTT and NRU assays	IC_{50}: 141.4μg/mL (MTT assay) IC_{50}: 129.9μg/mL (NRU assay)	Santos et al. (2016)
Essential oil	MCF-7	72 h	MTT assay	IC_{50}: 10.12μg/mL	El-Rahman et al. (2017)
6-Shogaol	A549	48 h	XTT assay	IC_{50}: 55.4μM	Hung et al. (2009)
6-Shogaol	HeLa	24 h	MTT	IC_{50}: 14.75 ± 0.94μM	Liu et al. (2012)
6-Shogaol	A549	72 h	MTT assay	IC_{50}: 22.9 ± 2.1μM	Peng et al. (2012)
6-Shogaol	BEL-7404 (human hepatocellular carcinoma)	72 h	MTT assay	IC_{50}: 11.8 ± 2.6μM	Peng et al. (2012)
6-Shogaol	CNE (human nasopharyngeal carcinoma)	72 h	MTT assay	IC_{50}: 43.8 ± 5.0μM	Peng et al. (2012)
6-Shogaol	HeLa	72 h	MTT assay	IC_{50}: 62.5 ± 4.7μM	Peng et al. (2012)
6-Shogaol	HL-60 (human promyelocytic leukemia)	72 h	MTT assay	IC_{50}: 7.9 ± 2.0μM	Peng et al. (2012)
6-Shogaol	K562	72 h	MTT assay	IC_{50}: 24.2 ± 2.8μM	Peng et al. (2012)
6-Shogaol	KB (human oral epidermal carcinoma)	72 h	MTT assay	IC_{50}: 7.4 ± 2.2μM	Peng et al. (2012)
6-Shogaol	PC-3	72 h	MTT assay	IC_{50}:100.0 ± 13.1μM	(Peng et al. 2012)
6-Shogaol	P388D1 (murine macrophage-like lymphoid)	72 h	MTT assay	IC_{50}: 95.9 ± 9.6μM	Peng et al. (2012)

Extract/compound	Cell line	Exposure time	Method	Results	References
6-Shogaol	LNCaP	24, 48, and 72 h	MTT assay	At a dose of 40 mmol/L reduced the cell viability: At 24 h by 67%, At 48 h by 85%, At 72 h by 96%	Saha et al. (2014)
6-Shogaol	DU145	24, 48, and 72 h	MTT assay	At a dose of 40 mmol/L reduced the cell viability: At 24 h by 64%, At 48 h by 80%, At 72 h by 90%	Saha et al. (2014)
6-Shogaol	PC-3	24, 48, and 72 h	MTT assay	At a dose of 40 mmol/L reduced the cell viability: At 24 h by 66%, At 48 h by 78%, At 72 h by 76%	Saha et al. (2014)
6-Shogaol	A2780 (human ovarian cancer cell line)	24 and 48 h	MTT assay	IC_{50} = 30μg/mL (24 h) IC_{50} = 25μg/mL (48 h)	Liang et al. (2019)
6-Shogaol	HT1080	24 h	MTT assay	IC_{50}: 52.8μM	Romero-Arias et al. (2019)
6-Gingerol	BxPC-3 (human pancreatic cancer cell line)	72 h	MTT assay	IC_{50}: 387.4μM	Park et al. (2006)
6-Gingerol	HPAC (human glucocorticoid-sensitive pancreatic ductal adenocarcinoma cell line)	72 h	MTT assay	IC_{50}: 405.3μM	Park et al. (2006)
6-Gingerol	H1299	24 h	MTT assay	IC_{50}: 136.73μM	Lv et al. (2012)
6-Gingerol	HCT-116	24 h	MTT assay	IC_{50}: 160.42μM	Lv et al. (2012)

(continued)

Table 12.1 (continued)

Extract/compound	Cell line	Exposure time	Method	Results	References
6-Gingerol	SW-480	72 h	MTT assay	IC_{50}: 205 ± 5μM	Radhakrishnan et al. (2014)
6-Gingerol	HCT-116	72 h	MTT assay	IC_{50}: 283 ± 7μM	Radhakrishnan et al. (2014)
6-Gingerol	HCT-15 (human colonadenocarcinoma)	24 h	MTT	IC_{50}: 100μM	Kumara et al. (2017)
6-Gingerol	L929	24 h	MTT assay	IC_{50}: 102μM	Kumara et al. (2017)
6-Gingerol	RAW 264.7 (mouse monocyte macrophage cell line)	24 h	MTT assay	IC_{50}: 102μM	Kumara et al. (2017)
6-Gingerol	HeLa	48 h	CCK-8 (Cholecystokinin Octapeptide) assay	IC_{50}: 96.32μM	Zhang et al. (2017a)
6-Gingerol	LNCaP	24 h	MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium]-assay	At 150 and 300μg/mL concentration the cell viabilities were 79.90 ± 3.56% and 53.06 ± 7.82% respectively	Silva et al. (2020)
6-Gingerol	A549	24 h	MTT assay	IC_{50}: 63.6 ± 3.7μg/mL	Wang et al. (2020)
6-Gingerol	HepG2	24 h	MTT assay	IC_{50}: 82.7 ± 4.1μg/mL	Wang et al. (2020)
6-Gingerol	MDA-MB-231	24 h	MTT assay	IC_{50}: 74.9 ± 2.3μg/mL	Wang et al. (2020)
6-Gingerol	786-O (hypertriploid renal carcinoma cell line)	72 h	MTT assay	IC_{50}: 31.05μM	Xu et al. (2020)
6-Gingerol	769-P (human kidney carcinoma cell line)	72 h	MTT assay	IC_{50}: 30.48μM	Xu et al. (2020)
6-Gingerol	ACHN (human renal carcinoma cell line	72 h	MTT assay	IC_{50}: 27.41μM	Xu et al. (2020)

Extract/compound	Cell line	Exposure time	Method	Results	References
10-Gingerol	SW480	24 h	TBE (Trypan blue exclusion) method	The cell viability decreased in a concentration-dependent manner at 10–100μM	Chen et al. (2009)
10-Gingerol	A549	72 h	MTT assay	IC_{50}: 85.4 ± 10.2μM	Peng et al. (2012)
10-Gingerol	BEL-7404	72 h	MTT assay	IC_{50}: 95.2 ± 12.2μM	Peng et al. (2012)
10-Gingerol	CNE	72 h	MTT assay	IC_{50}: 88.1 ± 7.3μM	Peng et al. (2012)
10-Gingerol	HeLa	72 h	MTT assay	IC_{50}: 52.4 ± 7.1μM	Peng et al. (2012)
10-Gingerol	HL-60	72 h	MTT assay	IC_{50}: 75.4 ± 10.3μM	Peng et al. (2012)
10-Gingerol	K562	72 h	MTT assay	IC_{50}: 112.5 ± 8.7μM	Peng et al. (2012)
10-Gingerol	KB	72 h	MTT assay	IC_{50}: 89.5 ± 8.7μM	Peng et al. (2012)
10-Gingerol	PC-3	72 h	MTT assay	IC_{50}: 59.7 ± 8.2μM	Peng et al. (2012)
10-Gingerol	P388D1 (mouse lymphoid tumour cell line)	72 h	MTT assay	IC_{50}: 99.7 ± 11.7μM	Peng et al. (2012)
10-Gingerol	HEY (ovarian cancer cell line)	24 and 72 h	MTT assay	At 100μM inhibited the cell growth by 34 ± 6% at 24 h of treatment. At 200μM inhibited the cell growth by 31 ± 14% at 72 h of treatment.	Rasmussen et al. (2019)
10-Gingerol	OVCAR-3 (ovarian cancer cell line)	72 h	MTT assay	At 200μM inhibited the cell growth by 33 ± 5%.	Rasmussen et al. (2019)
10-Gingerol	SKOV-3	72 h	MTT assay	At 200μM inhibited the cell growth by 38 ± 7%.	Rasmussen et al. (2019)

(continued)

In some *in vitro* experimental studies, it was found that various ethanolic extracts of ginger rhizome have cytotoxic activity against MDA-MB-231 (IC_{50}: 70.55 ± 5.5μg/mL), A549 cells (IC_{50}: 77.5 ± 11.5μg/mL) and K562 (IC_{50}: 67μM), and 70% ethanol extract reduced cell viability at high doses in HT1080 cell line (Nedungadi et al. 2019; Tiber et al. 2019; Romero-Arias et al. 2019).

In another research, it was reported that ethanol extract (IC_{50}: 33.78μg/mL) of ginger exhibited more cytotoxic effects compared to chloroform extract (IC_{50}: 74.32μg/mL) in cervical cancer cells (HeLa), however it was less cytotoxic (IC_{50}: 101.0 and 87.28μg/mL respectively) against non-cancer mouse fibroblast cells (L929) (Karaboz 2010).

Additionally, the cytotoxic effects of ginger's methanol extract (IC_{50}: 46.5μg/mL) and essential oil (IC_{50}: 46.0 ± 0.9μg/mL), 6-shogaol (IC_{50}: 14.75 ± 0.94μM) and 10-gingerol (IC_{50}: 52.4 ± 7.1μM) were also shown against the HeLa cell line with different studies (Ansari et al. 2016; Lee 2016; Liu et al. 2012; Peng et al. 2012).

An ethyl acetate fraction prepared from ginger leaf was reported that it causes 30–53% decrease in cell viability in six different cell lines (HCT116, SW480, LoVo, MCF-7, MDA-MD-231 and HepG-2) at a dose of 100μg/mL and with a 24-h exposure (Park et al. 2014).

In another study to investigate the effects of ginger on prostate cancer, the antiproliferative activity of methanol extract against five different prostate cancer cell lines (C4-2, PC-3, C42-B, DU145, LNCaP) was investigated by the alamar blue cell proliferation test and it was shown LNCaP is the most sensitive cell line among them with the IC_{50} value of 75μg/mL (Karna et al. 2012).

Similarly, in a study conducted with three different prostate cancer cell lines, including LNCaP, DU145 and PC-3, it was shown by the MTT assay that 6-shogaol reduced cell viability by 96%, 80% and 76%, respectively, at a dose of 40 mmol/L and with a 72-h exposure (Saha et al. 2014).

Rasmussen et al. (2019) was determined that 10-gingerol, at 200 mm dose, inhibited cell growth in various ovarian cancer cells such as HEY, OVCAR-3 and SKOV-3 cell lines by 31 ± 14%, 33 ± 5% and 38 ± 7% respectively (Rasmussen et al. 2019).

In fine, the promising results have been obtained from in vitro studies on many different cancer cell lines with both ginger extracts and its phenolic compounds. These data have formed the basis for further studies to clear up their mechanism of action.

2.2 In Vivo *Anticancer Studies and Mechanism of Action*

According to several studies, ginger's and its purified components anticancer activity have been attributed to their ability to modulate various mechanisms including MAPK, PI3K/Akt/mTOR, JAK/STAT, AP1, TRAIL and NF-κB (Prasad and Tyagi 2015) (Table 12.2).

Table 12.2 *In vivo* anticancer studies of ginger extracts and its active ingredients

Extract/ compound	Study design (experimental design)	Results	Reference
Ethanolic extract	**Model:** 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and 7,12-dimethylbenz(a)anthracene (DMBA) induced mouse skin tumorigenesis model. **Animal:** SENCAR mice **Dose:** 1, 2 or 4 mg/animal ginger extract (topical application, twice a week). **Period:** 20 weeks	Significantly inhibits of TPA-cause induction of epidermal ornithine decarboxylase (ODC), activities of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes and expression of ODC mRNA in a dose-dependent manner.	Katiyar et al. (1996)
Ethanolic extract	**Model:** Ethionine-induced hepatoma rats **Animal:** Male Wistar albino rats **Dose:** 100 mg/kg body weight (by the gavage method) **Period:** 8 weeks	Decreased the high expression of NFκB and TNF-α.	Habib et al. (2008)
Ethanolic crude extract	**Model:** Ethionine induced hepatocarcinogenesis. **Animal:** Wistar albino rats **Dose:** 100 mg/kg ginger extract **Period:** 8 weeks	Reduced the SOD activity and MDA level, increased catalase activity but cause no change for GPx activity. So, suppressed hepatocarcinogenesis by scavenging the free radicals and by reducing lipid peroxidation.	Yusof et al. (2009)
Methanolic extract	**Model:** Prostate tumor model (induced by injecting PC-3 cells subcutaneously) **Animal:** Male Balb/c nude mice **Dose:** 100 mg/kg ginger extract (oral feeding) **Period:** 8 weeks.	Significantly inhibited the proliferation and cell-cycle and showed pro-apoptotic activity and strongly suppressed *in vitro* and *in vivo* expression of cyclins/ cdks.	Karna et al. (2012)
Hydroalcoholic extract	**Model:** 1,2-dimethylhydrazine (DMH) induced colon carcinogenesis. **Animal:** Wistar rats **Dose:** Diet containing ginger extract at 0.5 or 1.0%. **Period:** 10 weeks.	Although 1% dietary consumption of ginger extract reduced the serum cholesterol levels significantly, cell proliferation and apoptosis rates did not change significantly by ginger treatment.	Dias et al. (2006)

(continued)

Table 12.2 (continued)

Extract/ compound	Study design (experimental design)	Results	Reference
Ginger extract (solvent: ethanol, hexane and ethyl acetate mixture)	**Model:** Solid tumors induced by injecting the Ehrlich Ascites Carcinoma (EAC) subcutaneously. **Animal:** Swiss albino mice **Dose:** 120 mg/kg bw ginger extract (orally, three times a week) **Period:** 1 month	Ameliorated ALT, AST, urea, creatinine, MDA, SOD and CAT values and significantly reduced the tumour volumes and the destructed genomic DNA retained the normal pattern.	Badr et al. (2016)
Aqueous extract of ginger	**Model:** *N*-nitroso *N*-methylurea (MNU) induced gastric cancer. **Animal:** Albino Wistar rats **Dose:** 100 mg/kg aqueous extract of ginger (by intragastric route) **Period:** 16 weeks.	Reduced the oxidative stress and pro-inflammatory markers levels (NF-kB, TNF-α, IL-6, PGE_2) related to gastric cancer.	Mansingh et al. (2020)
Powdered ginger	**Model:** 1,2 dimethylhydrazine (DMH)-induced colon cancer **Animal:** Wistar rats **Dose:** 50 mg/kg body weight ginger (daily, orally). **Period:** 32 weeks.	Significantly decreased the number of tumors and the incidence of cancer. Also significantly reduced circulating lipid peroxidation, increased the enzymic (GPx, GST, GR, SOD and CAT) and non-enzymic (GSH, vitamins C, E, and A) antioxidants.	Manju and Nalini (2005)
6-Shogaol	**Model:** Tumor induced by injecting SMMC-7721 subcutaneously. **Animal:** SCID mice (male, 2 group × 8 animal) **Dose:** 10 mg/kg or 50 mg/kg (i.p.) **Period:** 28 days	Tumor growth was inhibited by induction of apoptosis, activation of caspase-3, and inactivation of eIF2α.	Hu et al. (2012)
[6]-Shogaol	**Model:** Allograft model using HMVP2 cells **Animal:** Syngeneic FVB/N male mice. **Dose:** 50 and 100 mg/kg body weight (intraperitoneally) **Period:** 32 days.	Significantly inhibited tumor growth by inhibition of STAT3 and NF-kB signaling.	Saha et al. (2014)

(continued)

Table 12.2 (continued)

Extract/ compound	Study design (experimental design)	Results	Reference
6-Shogaol	**Model:** Xenograft Breast Cancer Mouse Model (MDA-MB-231 cells were implanted subcutaneously in the right flank of nude mice) **Animal:** athymic nu/nu female mice **Dose:** 10 or 50 mg/kg (intraperitoneally) **Period:** 4 weeks	Significantly reduced the tumor volume and showed anti-proliferative and pro-apoptotic effects by the regulation of STAT3 and MAPKs signaling pathways.	Kim et al. (2015)
[6]-Shogaol	**Model:** 7,12-dimethylbenz[a] anthracene (DMBA) induced hamster buccal pouch carcinogenesis **Animal:** Syrian hamsters **Dose:** 20 mg/kg body weight **Period:** 16 weeks	Reduced the inflammation and cell proliferation by inhibition of NF-κB and AP-1 activation.	Annamalai and Suresh (2018)
[10]-Gingerol	**Model:** Metastatic triple negative breast cancer induced by injecting 4T1Br4 cells. **Animal:** Mice **Dose:** 5 mg/kg, 10 mg/kg (i.p.) **Period:** 14 days	Induced a prominent increase in caspase-3 activation and inhibited orthotopic tumour growth.	Martin et al. (2017)
[6]-Gingerol	**Model:** Azoxymethane-induced intestinal carcinogenesis **Animal:** F 344 rat **Dose:** Diet containing 6-gingerol at 0.02%. **Period:** 3 weeks	Showed inhibitory effect on intestinal carcinogenesis.	Yoshimi et al. (1992)
[6]-Gingerol	**Model:** Two-stage mouse skin carcinogenesis model induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) and 7,12-dimethylbenz(a)anthracene (DMBA). **Animal:** ICR mice **Dose:** 2.5μmol (topically, 30 min before each TPA treatment) **Period:** 22 weeks	Showed inhibitory effect on skin papillomagenesis, inflammation and epidermal ornithine decarboxylase activity.	Park et al. (1998)

(continued)

Table 12.2 (continued)

Extract/ compound	Study design (experimental design)	Results	Reference
[6]-Gingerol	**Model:** VEGF-induced angiogenesis and lung metastasis induced by intravenous injection of B16F10 melanoma cells. **Animal:** Male C57BL/6 mice (6 and 8 weeks old) and Sprague–Dawley rats (6 weeks old) **Dose:** Hydron pellets (P) containing 200 ng [6]-gingerol (angiogenesis assay). Every 2 days with 3 or 5 mg/kg [6]-gingerol in a volume of 200μL for 2 weeks (lung metastasis assay)	Inhibited the formation of lung metastases of B16F10 melanoma and showed anti-angiogenic activity via inhibition of proliferation and differentiation of endothelial cells in response to VEGF.	Kim et al. (2005a)
[6]-Gingerol	**Model:** Tumor induced by injecting HCT116 colon cells into the right flank of each mouse. **Animal:** Athymic mice (NIH Swiss nude) **Dose:** 500μg (three times a week) **Period:** 75 days.	Suppressed tumor growth by inhibiting of LTA4H activity in colorectal cancer.	Jeong et al. (2009)
[6]-Gingerol	**Model:** Tumor induced by injecting K562 cells subcutaneously. **Animal:** Male (nu/nu) nude mice **Dose:** 5 mg/kg body wt, (intraperitoneal) **Period:** 45 days	Inhibited tumor cell proliferation and induced apoptosis.	Rastogi et al. (2014)
[6]-Gingerol	**Model:** HeLa xenograft model. **Animal:** Nude mice **Dose:** 2.5 mg/kg and 5.0 mg/kg body weight. **Period:** 45 days.	Caused significant reduction of tumor volume, tumor weight, inhibition of proteasome and accumulation of p5.	Rastogi et al. (2015)

The anticarcinogenic effects of ginger, gingerol and shogaol analogues against various cancer types such as skin, liver, colon, stomach, intestinal, breast, cervix, prostate, etc. were investigated using different *in vivo* experimental models.

Data obtained from two different studies using the same hepatocarcinoma experimental model (ethionine-induced hepatocarcinoma) in rats have revealed that the ethanolic extract reduced the high expression of NFκB and TNF-α and suppressed liver cancer by scavenging free radicals and reducing lipid peroxidation (Habib et al. 2008; Yusof et al. 2009).

Consistent with *in vitro* studies, methanol extract has been reported to exhibit significant antiproliferative, cell cycle inhibitory and pro-apoptotic activity in the prostate tumour model induced by PC-3 cell injection (Karna et al. 2012).

Ginger, traditionally used in gastrointestinal system diseases, has shown to have promising curative effects on stomach, intestine and colon cancers by various *in vivo* experiments. In a DMH-induced colon cancer experimental model, it was determined that the consumption of powdered ginger orally significantly decreased the number of tumour and cancer incidence in rats (Manju and Nalini 2005).

In addition, studies on 6-gingerol have shown that this compound also has inhibitory effects on both intestinal and colorectal carcinogenesis (Yoshimi et al. 1992; Jeong et al. 2009).

3 Anticancer Mechanisms of Ginger

Anticancer action mechanisms of ginger and its bioactive compounds are discussed below in detail.

3.1 MAPK (Mitogen-Activated Protein Kinase) Signaling

The MAPK signalling pathway consists of RAF, MEK and ERK (extracellular signal regulated protein kinase) proteins, which are essential for proliferation in many cancer cells also for normal cells. MAPK transmits signals generated by receptor tyrosine kinases activated by growth factors to the nucleus in normal cells and leads to gene expression activation. When activated ERK transported to the nucleus, it phosphorylates certain nuclear transcription factors which administer cellular responses. Phosphorylated transcription factors induce the transcription of genes responsible for encoding different proteins, comprising those necessary for cell cycle progression, such as cyclin D. In oncogenic cells, MAPK signalling is frequently increased as a result of oncogenic activation of RTKs or RAS (Pazarbaşı et al. 2011; Arkun and Yasemi 2018).

In a study by Kim et al., 6-gingerol was studied at the molecular level in mouse skin (*in vivo*) to clarify the mechanisms underlying its antitumor effect. The effects of 6-gingerol on mouse skin have been described as inhibiting the activity of MAPK, which regulates COX-2 expression and phosphorylation induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) (Kim et al. 2005b).

Joo et al. (2016) investigated the mechanism of action of 10-gingerol in regulating the occupation of breast cancer cells (MDA-MB-231) at the molecular level. 10-Gingerol was found to inhibit cell propagation by down-regulation of cyclin-dependent kinases and cyclines followed by initiation of G1 phase interruption. Treatment with 10-gingerol in response to mitogenic stimulation stopped cell

occupation, and this effect was mediated by inactivation of Akt and p38MAPK activity and interception of epidermal growth factor receptor expression (Joo et al. 2016).

Pathways that regulate MMP expression are MAPK and phosphoinositide-3 kinase/protein kinase B (PI3K/Akt). Weng et al. (2012) demonstrated the inhibition of MMPs in Hep3B (human hepatoma cell line) cells by 6-gingerol and 6-shogaol and at molecular level the activity of these natural chemicals on MAPK and PI3K/Akt pathways were studied. Analysis showed that 6-gingerol and 6-shogaol (≥10, ≥2.5μM, respectively) significantly inhibit phosphorylation of MAPK and PI3K/Akt signal against controls and it was 6-shogaol, which more strongly inhibits the invasion, migration and suppression of MMPs and uPA of HCC cells (Weng et al. 2012).

The chemopreventive and hepatoprotective activities of 6-shogaol and 6-gingerol were investigated *in vitro* and *in vivo* by nuclear erythroid-associated factor 2 (Nrf2)/antioxidant response element (ARE). The results showed that ARE-luciferase activity as well as the related molecular events such as Nrf2 and heme oxygenase-1 (HO-1) inductions were more strongly induced by 6-shogaol than 6-gingerol. 6-shogaol stimulated the phosphorylations of MAPKs such as ERK, JNK and p38. The induction of Nrf2/ARE-mediated phase II detoxifying enzyme via p38 pathway resulted in that 6-shogaol could be used as a promising natural chemopreventive agent (Ok 2012).

Inhibition of MAPK, pJNK and pERK protein expression after 6-gingerol treatment in HPAC (wild type) and BxPC-3 (p53 deficient) pancreatic tumor cell lines has been reported by Park et al. It is also one of the other results of the study that phosphorylation of AKT elevated in HPAC but the phosphorylation in BxPC-3 cells does not increase (Park et al. 2006).

In a research investigating the efficacy of 6-gingerol on colon cancer (SW-480 cell line) it was found that 6-gingerol inhibits cell proliferation and initiated apoptosis in SW-480 cell line, while healthy colon cells are not affected. Induction of caspases 8, 9, 3 and 7 and PARP cleavage proving the stimulation of apoptotic cell death have been correlated with the sensitivity of SW-480 cells to gingerol. 6-Gingerol had little act on phosphorylation and activation of NF-kappa B and p38 MAP kinase but down-regulated the phosphorylation of ERK1/2 and JNK MAP kinases and AP-1 transcription activation stimulated with Phorbol Myristate Acetate (PMA) (Radhakrishnan et al. 2014).

An *in vitro* experimental study about colon carcinogenesis has been aimed to explain the possible mechanism of zingerone on inflammatory and Nrf-2 signalling cascade. Rats were divided into four groups. Saline was given orally to the control group (Group I) and 1,2-dimethylhydrazine (DMH) was given to the Group II. Group III and IV received both DMH at 20 mg/kg bw dose and zingerone oral therapy at 50 and 100 mg/kg bw dose. Applying zingerone in DMH treated rats caused changes in the activity of cytochrome P450-2E1 and CEA. While zingerone contributed to the protection of the mucous layer, it also lowered the amounts of IL-6 and TNF-a. Additionally, NF-kB-p65, COX-2, iNOS, PCNA and Ki-67 have been reported to be suppressed by zingerone. In the light of the results of the study,

zingerone promises as an acceptable chemopreventive agent in the experimental colon carcinogenesis model (Ganaie et al. 2019).

Li et al. (2019) reported that paradol was discovered as a potential agent against hepatocellular carcinoma, inhibiting proliferation and migration by inducing apoptosis, partially by HepG-2 (hepatocellular carcinoma) cells, by MAPK signal, by stopping the G0/G1 phase. High p38 MAPK and JNK activation after paradol treatment as a result of flow cytometry and western blot analysis for Bcl-2 and Bax are important outcomes of the study (Li et al. 2019).

3.2 PI3K/Akt/mTOR Signaling

The phosphatidylinositol-3-kinase (PI3K) pathway is especially responsible for controlling cellular metabolism in glucose transport and use, modulation of cell growth, protein synthesis, and prevention of apoptosis. The PI3K/Akt and the mammalian target of rapamycin (mTOR) signaling pathways are two important pathways for many attitudes of cell growth and survival both in physiological and pathological conditions. The act of this pathway in cancer has gained importance since tumors appear spontaneously in a stressful environment with restricted nutrient and oxygen supplement, and low pH. mTOR is a serine/threonine kinase and receives and combines signals triggered by nutrient input, growth factors, and other cellular stimuli to arrange downstream signaling and protein synthesis (Porta et al. 2014).

Zhang et al., evaluated the anti-cancer effect of 10-gingerol in HeLa cells and the outcomes clearly showed the apoptotic activity on the cells could not reveal any difference after 12 h of application with 10-gingerol. It has been reported that the count of apoptotic cells in the 10-gingerol group is greater than 5-Fluorouracil as the exposure increases, and that this is related to the control of PI3K/Akt pathway signal transduction, and may be associated with anti-cancer effects of 10-gingerol. 10-Gingerol initiated mTOR-mediated cell apoptosis by blocking PI3K/Akt and activating AMPK in HeLa cells (Zhang et al. 2017b).

Hung et al., assessed the effect of 6-shogaol in A549 cells (human non-small cell lung cancer) and reported that 6-shogaol blocks cell propagation by stimulating autophagic cell death. Pre-administration of 3-methyladenine (autophagy inhibitor) to the cells suggested that induction of 6-shogaol's autophagy is favorable to cell death by suppressing 6-shogaol-mediated antiproliferation activity. They also reported that 6-shogaol inhibits survival signal via the AKT/mTOR signaling pathway by restricting the activation of AKT and downstream targets, mTOR, forkhead transcription factors (FKHR) and glycogen synthase kinase-3β (GSK-3β) (Hung et al. 2009).

Potential synergistic chemopreventive efficacy of raw ginger extract and Gelam honey combination against colorectal cancer cells (HT29) with molecular mechanisms comprising of KRAS/ERK and PI3K/AKT pathways was studied by Tahir et al. (2015). KRAS protein acts a crucial role in keeping down the activity of

downstream signaling pathways such as P13K/AKT/mTOR and Ras/Raf/ERK. The results showed that both single and co-treatment with ginger and Gelam honey initially resulted in downstream regulation of high KRAS gene expression in the untreated group, but a major effect was observed in co-treatment group at a concentration of ginger (3 mg/mL) with Gelam honey (30 or 50 mg/mL) (Tahir et al. 2015).

The activity of 6-shogaol on reactive oxygen species (ROS) generation, glucose uptake, and protein expression of the signaling pathway PTEN/Akt/mTOR was measured by Romero-Arias et al. (2019). With the administration of 6-shogaol, expression of MTOR-p and Akt-p proteins increased, and PTEN was effective in HT1080 fibrosarcoma cells (Romero-Arias et al. 2019).

Evaluating the potential for zerumbone to prevent hepatocellular carcinoma, Wani et al. explained that zerumbone inhibits the survival of liver cancer cells and inhibits PI3K/AKT/mTOR and STAT3 signaling pathways by arresting in the G2/M phase and inducing apoptosis (Wani et al. 2018).

3.3 JAK (Janus Kinase)/STAT (Signal Transducer and Activator of Transcription)

It has been shown that JAK/STAT pathway plays a crucial role in cancer development. Ligand-receptor interaction ultimately triggered the rapid activation of receptor-associated JAKs by phosphorylated STAT proteins. Functionally active phosphorylated STAT proteins are transferred into the nucleus and transcriptionally regulated expression of numerous genes. There has been increased interest in identifying molecules that have potent activity against different STAT proteins to target the JAK-STAT signaling pathway therapeutically (Farooqi et al. 2018).

Since abnormal activation of STAT3 and MAPKs is relevant to controlling of proliferation, invasion and metastasis of tumors, 6-shogaol has been assigned to modulate activation of STAT3 and MAPK in tumor cells. Both on MDA-MB231 and DU145 cells 6-shogaol strongly inhibited the constructional phosphorylation of STAT3 by inhibiting the activation of upstream JAK2 and c-Src kinases and nuclear translocation of STAT3. In addition, 6-shogaol has been reported to cause activation of JNK, p38 MAPK and ERK. Compared to other analogs of 6-shogaol such as 6-gingerol, 8-gingerol and 10-gingerol, 6-shogaol has been found to be the strongest inhibitor of STAT3 activation. It was sighted that only 6-shogaol treatment significantly reduced tumor growth (Kim et al. 2015).

6-Shogaol has been shown to inhibit cell proliferation by modulation of JAK/STAT-3 signaling in A2780 (ovarian cancer cell line). 6-Shogaol inhibited STAT-3 translocation by inhibiting over expression of PCNA, cyclin-D1, Bcl-2, and decreased Bax, caspase-9 and 3 expression in ovarian cancer cell line (Liang et al. 2019).

Gingerenone A (Gin A) has been reported to exhibit minimal toxicity to normal cells but selectively kill cancer cells. In the study, GinA significantly inhibit the growth of EJ, HCT116, OVCAR-8, MDA-MD-468 and A549 cancer cells, showing relatively weak toxicity to SW480 and HuCCT1 cancer cells. The effect of GinA on JAK2 and S6K1 signaling pathways was investigated to further examining the mechanism. GinA treatment in EJ and HCT116 cells represented a significant targeting of the JAK2-STAT3 pathway, with less or no effect against STAT1 and STAT6 phosphorylation levels, resulting in a significant decrease in STAT3 phosphorylation. GinA significantly inhibited S6 phosphorylation in E6 and HCT116 cells without reducing MAPK signal. Application lead to an increment in Akt phosphorylation in HCT116 cells as a result of negative feedback between S6K1 and Akt pathways. Sensitive cancer cells to GinA harboured comparatively elevated levels of phosphorylated JAK2 and S6K1, indicating a relationship between the activation levels of target kinases and cytotoxic effect of GinA (Byun et al. 2015).

3.4 Activator Protein-1 (AP-1) Transcription Factor

The AP-1 transcription factor family be composed of several components such as C-JUN, c-FOS and ATF, and plays an important role in mediating several biological processes like proliferation, differentiation, and cell death. Promoting cell propagation by stimulating the cyclin D1 gene and repressing tumor suppressor genes, such as p53, p21cip1/WAF1 and p16 by AP-1 transcription factors have been emphasized in multiple studies. Increasing information about the role of AP-1 in diseases and especially in cancer shows that these transcription factors are considered as promising therapeutic targets for various malignancies (Alonso et al. 2018).

Phosphorylation of a number of substrates, including ERKs and p38 kinases involving to AP-1 activation, results from activation of the EGF receptor. The activity of 6-gingerol on the phosphorylation of EGF-induced ERK and p38 kinases was investigated by Bode et al. and they reported that 6-gingerol had no effect on EGF-induced ERKs or p38 kinase phosphorylation. AP-1 DNA binding activity was assessed by gel shifting assay to further investigate the molecular basis of inhibition on AP-1 transactivation of 6-gingerol. According to the results of the study, 6-gingerol blocked AP-1 DNA binding activity caused by EGF in a dose dependent manner (25–300μM) (Bode et al. 2001).

In *in vitro* and *in vivo* experimental models, 6-Shogaol was proven to activate proapoptotic factors and prevent oral squamous cell carcinoma. Annamalai and Suresh examined the activity of 6-shogaol on inflammation and cell proliferation in 7,12-dimethylbenz[a]anthracene (DMBA) induced hamster buccal pouch carcinogenesis. They determined upregulation of COX-2, iNOS, TNF-α, IL-1 and IL-6 inflammatory markers, Cyclin D1, PCNA and Ki-67cell proliferative markers and abnormal activation of NF-κB, AP-1, IKKβ, c-jun, c-fos and lowered IκB-α in

DMBA induced hamsters. Strongly inhibition of phosphorylation and degradation of IκB-α and phosphorylation of c-jun, c-fos resulted in inhibition of nuclear translocation of NF-κBp65 and AP-1 after oral administration of 6-shogaol. 6-shogaol has been identified to reduce inhibition of NF-κB and AP-1 activation, inflammation and cell proliferative response in DMBA-induced hamsters (Annamalai and Suresh 2018).

3.5 TNF-Related Apoptosis-Inducing Ligand (TRAIL) Signaling

It is becoming increasingly clear that cancer cells develop resistance to apoptosis. Inhibition of pro-apoptotic proteins, over-expression of anti-apoptotic proteins, inactivation of intrinsic and extrinsic pathways, and down regulation of death receptors on the surface of cancer cells are multiple examples of scientifically proven metabolism underlying apoptosis loss. TRAIL's discovery in the mid-90s revolutionized molecular oncology and became a focus of attention due to its capacity to kill cancer cells, so researchers identified the molecule that could be effective in cancer treatment. Down regulation of death receptors is a frequently reported mechanism in TRAIL-resistant cancer cell lines and recent studies identified several proteins which severely disrupt TRAIL-induced apoptosis in different cancer cell lines (Farooqi et al. 2018).

TRAIL-mediated glioblastoma cell apoptosis induced by gingerol has been proven in the study of Lee et al. (2014). It has been disclosed that gingerol elevated the death receptor levels in a p53-dependent manner and reduced the expression level of anti-apoptotic proteins (survivin, c-FLIP, Bcl-2 and XIAP) by creating reactive oxygen species. Also, the sensitizing effects of gingerol in TRAIL-induced cell death are inhibited by ROS excretion or overexpression of anti-apoptotic protein (Bcl-2). This study reported the possibility of administering gingerol as an antitumor compound that can be utilised for combination therapy with TRAIL in the treatment of TRAIL-resistant glioblastoma tumor (Lee et al. 2014).

It has been reported by Nazım and Park that 6-shogaol shows anti-inflammatory and anticancer features in liver cancer cells, reduces tumor cell spread and induces TRAIL-mediated cell death. Co-treatment of TRAIL and 6-shogaol at different doses significantly increased cell death compared to only 6-shogaol or TRAIL. Results showed that 6-shogaol pretreatment makes liver cancer cells susceptible to TRAIL-stimulated apoptosis. LC3-II and p62 expressions enhanced in a dose-dependent fashion following 6-shogaol treatments. Co-treatment with TRAIL and 6-shogaol raised split cas8 and split cas3 levels compared to untreated or single treatments. Cell morphology showed enhanced cell death by treatment with TRAIL and 6-shogaol (20μM) or chloroquine (20μM). Combination treatment method employing chloroquine and TRAIL, significantly augmented cell death and markedly reduced cell survival compared to untreated and single treated groups. Findings

suggest that 6-shogaol makes susceptible to TRAIL-induced cell death by alleviating the autophagy flux and 6-shogaol may be a beneficial therapeutic method for the treatment of TRAIL-resistant Huh7 liver cells (Nazim and Park 2019).

A study showed that treatment of A549 cells with TRAIL showed slightly stimulated cell death, but gingerol therapy increased TRAIL-induced cell death in A549 cells. The co-treatment of gingerol and TRAIL has been reported to increase microtubule-associated protein light chain 3-II and p62 accumulation, as well as to confirm inhibited autophagy flux (Nazim et al. 2015).

3.6 NF-kB Signaling Pathway

Analogous to the STAT pathway, activation of the NFKB pathway indisputably does not induce cancer, and has different functions in hematopoietic cells such as the STAT pathway compared to other cell types. The primary function of the NFKB pathway is the regulation of lymphoid cells, inflammation and apoptosis. In some cell types, however, it is specifically involved in the arrangement of cell proliferation in response to cytokines (Sel and Erbaş 2018). The nuclear factor-kappa B protein complex comprise of five different sub proteins (NF-kB1 (p50/p105), NF-kB2 (p52/p100), RelA (p65), RelB and c-Rel) (Savinova et al. 2009). Through these proteins, it can bind to DNA and interact with its intracellular inhibitor, IkB. Nuclear factor-kB activation enables activation of interleukin (IL) -2, stimulating Janus kinase 3 (JAK3) protein by auto phosphorylation. Janus kinase 3 (JAK3) provides the stimulation of protein by auto phosphorylation. Activated JAK3 activates the protein known as STAT3. Activated JAK3 activates the protein known as STAT3 (signal transducer and activator of transcription 3). It has been demonstrated in colon cancers that JAK3/STAT3 is significantly expressed *in vivo* and *in vitro* experiments (Sel and Erbaş 2018; Lin et al. 2005).

Expression of COX-2 was restricted by topical application of 6-gingerol in the mouse skin stimulated with tumor inducer TPA. 6-gingerol caused a tail off in both the DNA binding caused by TPA and the transcription activities of NF-κB by suppressing IκBα degradation and p65 nuclear translocation.

In addition, one of the experimental outputs is to prevent TPA-stimulated phosphorylation and catalytic activity of the MAPK, which regulates COX-2 expression of 6-gingerol in mouse skin (Kim et al. 2015).

In a study investigating the effect of 6-shogaol on inflammation and cell proliferation by inhibiting NF-κB and AP-1 translocation in DMBA induced hamster buccal pouch carcinogenesis, 6-shogaol has been reported to be strongly inhibiting the constitutive phosphorylation and degradation of IκB after oral treatment and inhibiting the nuclear translocation of NF-κB p65 and AP-1 by inhibiting the phosphorylation of c-jun, c-fos (Annamalai and Suresh 2018).

In human LNCaP, DU145, and PC3 and mouse HMVP2 prostate cancer cell lines 6-shogaol, 6-gingerol, and 6-paradol were investigated. Studies have demonstrated that 6-shogaol decreases interleukin-6 (IL-6) stimulated STAT3 activation

and inhibits both constitutive and TNF-a-induced NF-kB activity in the studied cells. 6-Shogaol lowered the protein level of target genes such as Cyclin D1, survivin and cMyc regulated by STAT3 and NF-kB. 6-Shogaol also modulated the mRNA levels of IL-7, CCL5, BAX, BCL2, p21, and p27 apoptosis regulatory genes. 6-Shogaol has been found to be more effective than 6-gingerol and 6-paradise in alleviating the STAT3 and NF-kB signal and lowering the survival levels of prostate cancer cells. 6-Shogaol also demonstrate distinct tumor growth inhibitory activity in an allograft model using HMVP2 cells, and in the light of all these results, 6-shogaol has the potential to be used for chemopreventive and/or therapeutic purposes in prostate cancer (Saha et al. 2014).

Mohd Habib et al. (2008) evaluated the effect of ginger extract on liver cancer rats and clarified that extract of ginger significantly lessened the expression of high NFκB and TNF-a in rats (Habib et al. 2008).

4 Clinical Trials

Unlike many *in vivo* studies on ginger and its purified components, the number of clinical trials is very limited. In a study with people at normal risk for colon cancer, levels of eicosanoid in colon biopsies of participants who received 2.0 g of ginger extract daily (30 days) were evaluated. Zick et al. (2011) reported that the extract was well tolerated but there was no marked alteration in the concentrations of pro-inflammatory compounds (Zick et al. 2011). In a randomized study with patients at high risk of colon cancer, 28 people received 2.0 g of ginger or placebo daily for 28 days. Expression was measured per cryptal dissociation of Bax, Bcl-2, p21, hTERT (human telomerase reverse transcriptase) and MIB-1 in colorectal biopsies. They found that ginger decrease the propagation of normal-appearing colorectal epithelium and differentiation of augmented apoptosis and crypts. While this advantageous efficacy of ginger was correlated with the down regulation of Bax, hTERT and MIB-1, p21 and Bcl-2 expression remained relatively unchanged (Citronberg et al. 2013).

A clinical study has also been conducted showing that ginger and coconut oil used during massage improves cellular immunity in 66 colorectal cancer patients undergoing chemotherapy (Khiewkhern et al. 2013).

In a clinical trial with effects of ginger, 30 normal volunteers and 20 patients at increased risk for colorectal cancers were evaluated and the anti-inflammatory activity of ginger was reported.Interestingly, ginger has significantly reduced COX-1 protein expression in individuals with a high risk of colorectal cancer, but not significantly in individuals at normal risk. However, ginger was found not to alter protein expression of 15-hydroxyprostaglandin dehydrogenase (PGDH) in both groups (Jiang et al. 2013).

5 Conclusion

Ginger is very popular traditional medicine as well as being a widely used spice thanks to its pungent smell and taste. Ginger, which is used in the treatment of many ailments such as colds, fevers, infectious diseases, respiratory disorders, rheumatism, arthritis, cardiovascular diseases, nervous diseases and metabolic diseases, also draws attention due to its rich phytochemical content.

Although hundreds of secondary metabolites have been identified in the plant, phenolic compounds such as gingerols and shogaols are important secondary metabolites associated with its many bioactivities, including its anticarcinogenic effect.

It has been declared in various population-based studies that dietary habits are regarding with the risk of developing cancer, and vegetables, fruits and spices may be natural chemopreventive agents that reduce the risk of cancer development. Ginger is one of these important agents. Its extracts, essential oil and even its powder have been researched for their cytotoxic effects in different types of cancers such as leukemia, cervix, ovary, breast, prostate, lung, liver, kidney, pancreas, colon etc. and promising results have been obtained.

In vitro and *in vivo* experiments have shown that ginger and its active ingredients affect inflammation, cancer cell apoptosis and proliferation, tumor metastasis and invasion by modulating enzymes and intracellular signaling molecules linked with cancer progression. It has been demonstrated that especially gingerol and shogaol analogues are potential chemoprotective and chemotherapeutic agents which show promising anticarcinogenic activity by affecting mechanisms such as PI3K/Akt/mTOR, JAK/STAT, AP1, TRAIL and NF-κB.

Although there are many preclinical studies on the anticarcinogenic effects of ginger, clinical trials are very limited and insufficient. Most of the clinical studies with ginger have focused on its antiemetic efficacy, and although it has been reported that it may also be beneficial against chemotherapy-induced nausea and vomiting, the results of clinical trials are not yet convincing (Ansari Damavandi et al. 2019; Zick et al. 2009; Panahi et al. 2012; Lua et al. 2015).

A restricted number of clinical studies aiming to investigate the effects of ginger on cancer have focused on colorectal cancer.

In these studies, it has been shown that ginger has anti-inflammatory activity and reduces COX-1 protein expression in participants with a high risk of colorectal cancer, decreases the propagation of normal-appearing colorectal epithelium and differentiation of augmented apoptosis and crypts by down-regulating of Bax, hTERT and MIB-1.

Although ginger appears to be an effective and safe natural anticarcinogenic agent when ethnobotanical and preclinical studies are evaluated, more evidence based on clinical studies is needed for it and its active ingredients to be used as a chemotherapeutic and chemopreventive agents.

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Chapter 13
Saffron (Crocins) Against Cancer

Mohammed Bhia, Huda Fatima Rajani, Niloufar Mohammadkhani, and Seid Mahdi Jafari

Abstract Saffron is a spice which is isolated from the dried stigmas of *Crocus sativus* L. with plentiful of beneficial pharmacological effects including chemopreventive and anticancer properties that are mainly linked to the rich presence of carotenoids in particular crocin, crocetin, picrocrocin and safranal. The mechanism of carotenoids chemopreventive features includes apoptosis, antioxidant activity, improving cell differentiation, modulating carcinogen metabolism, immune modulation, regulating the progression of cell cycle and growth, stimulating the communications of cell-to-cell gap junctions, and inhibiting cell proliferation. Numerous preclinical studies were performed on cancer animal models and cancerous cell lines which confirmed the favorable anticancer properties of saffron on a variety of cancers. Additionally, saffron has been shown to provide protective effects against toxicities associated with several cancer chemotherapeutic agents. Moreover, the key saffron compounds crocin and crocetin can have a synergetic effect when used with other conventional anticancer drugs, thus, improving the efficacy of these drugs in cancer therapy. Furthermore, several studies encapsulated crocin, crocetin, or safranal within nanocarriers to enhance their effectiveness in cancer therapy by overcoming physicochemical limitations. In the present chapter, we aim to give an overview of the mechanism involved in the promising anticancer effects of saffron

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and its main carotenoids and their applications in preventing and treating several types of cancers.

Keywords Saffron · Crocin · Crocetin · Carotenoids · Cancer · Natural products · *Crocus sativus* · Antioxidant · Safranal

1 Introduction

Cancer contributes to the largest cause of mortality globally, affecting about eight million individuals each year (Gutheil et al. 2015), whereas till 2020, 15 million people are estimated to be affected by cancer (Safarzadeh et al. 2014). In 2019, more than 1.5 million cases of cancer were reported in the United States, and the number of mortality is expected to be ~606,000 patients per annum in this region (Siegel et al. 2019). More than 100 types of cancers have been identified. It is a multistage disease that includes irreversible cell changes followed by clonal proliferation and aggressive metastasis. The malignancy of cancer is determined by its spread to other organs and tissues. The initial stage of cancer is usually managed surgically; nonetheless, depending on the stage and the spread of cancer, chemotherapy with anticancer drugs, and radiotherapy or an augmentation of both is performed. These treatments come with a variety of side effects such as damage to organs like heart, kidney, and lungs, bone marrow suppression and alopecia areata (Liu et al. 2021). Cancer cells can also get resistant to these treatments. Based on data provided by WHO, over 80% of the global population relies on herbal medicine for primary care. Herbal medicine has been extensively studied and utilized for cancer therapy. From 121 anticarcinogenic drugs, 90 are herbal derivatives (Safarzadeh et al. 2014). From 1981 to2002, 48 cancer drugs were derived from natural extracts such as vinca alkaloids, taxanes, podophyllotoxin, and anthracyclines. In addition to primary cancer treatment, 60% of cancer patients are estimated to take herbal medicine (Mohammadi et al. 2017).

2 Composition of Saffron

Saffron spice comes from dried stigmas of *Crocus sativus* L. flower, from Iridaceae family that is mainly found in Iran, China, and India, in the Mediterranean Sea (Zhang et al. 2013). Stigma saffron is composed of more than 150 ingredients (Bhandari 2015). Mainly, its constituents are 15% moisture, volatile matter including ashes 8%, raw fiber 6%, silica 2%, and 3.5–14.5% fats. As shown in Fig. 13.1, the chemical composition of saffron is actively based on crocetin esters, picrocrocin, and safranal (Sarfarazi et al. 2019; Shahi et al. 2016). Moreover, nutritional elements such as carotenoids, carbohydrates, anthocyanins, fats, flavonoids,

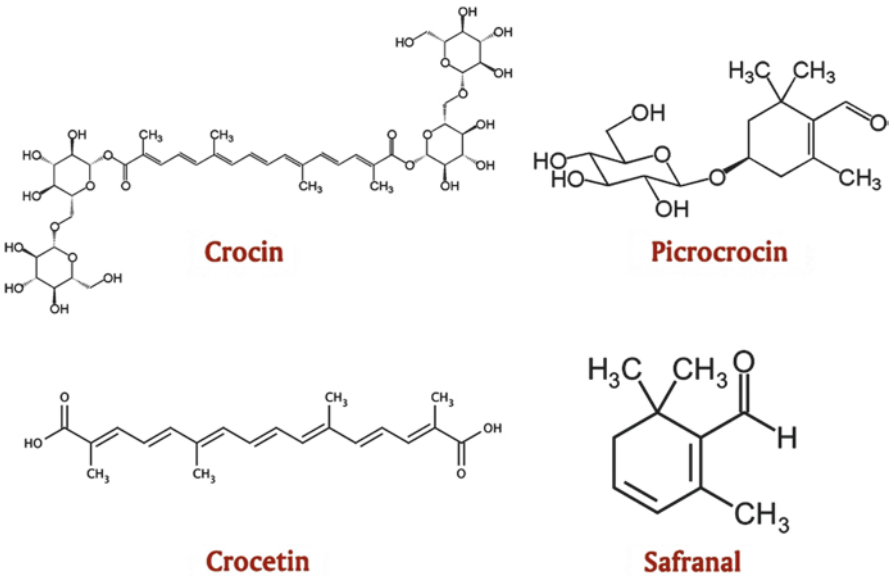

Fig. 13.1 The chemical structure of the key active compounds of saffron

proteins, and vitamins (riboflavin and thiamine) also form its composition. It is extensively used as a flavoring and food coloring agent, where its organoleptic properties make it one of the expensive spices (José Bagur et al. 2017).

Aqueous methanol, ethanol and water extracts of saffron majorly composed of bioactive components such as crocin, a water-soluble carotenoid, can be performed using solvent-assisted extraction and phenolic and flavonoid contents are commonly extracted using methanolic extraction method (Rahaiee et al. 2015). Crocin is transformed into crocetin before entering the bloodstream when taken orally (Broadhead et al. 2016).

3 Pharmacology of Saffron

Many animal-based, preclinical and clinical studies have revealed the therapeutic benefits of saffron and its main phytochemicals in various diseases, predominantly owing to its anti-apoptotic, antioxidant, antifibrotic, and anti-inflammatory effects (Pashirzad et al. 2019). *Trans* sodium crocetinate is seen as effective against hypoxia, and crocin induces spermatogenesis and is effective against skin wrinkles. A combination of saffron with several other herbs is known for its protective effects against cardiovascular disease, cancer, urinary and inflammatory dysfunction, and dermatological problems (Rameshrad et al. 2018). A recent double-blind randomized study has shown its efficacy against depression in combination with saffron, which aligns with several other studies confirming these effects (Lopresti and Drummond 2017).

Clinical studies have also demonstrated the effectiveness of saffron in treating erectile dysfunction (Maleki-Saghooni et al. 2018), Alzheimer's disease (Akhondzadeh et al. 2010), asthma (Zilaee et al. 2019), and age-related macular degeneration (Broadhead et al. 2019). Other benefits of saffron include antinociceptive, antidiabetic, antihyperlipidemic, anxiolytic, anticonvulsant, and antiallergic (Hosseini et al. 2018).

4 Anticancer Molecular Mechanisms of Saffron and Its Active Ingredients

Chemopreventive effects of crocin and crocetin extracts are marked by the downregulation of human telomerase catalytic subunit expression, modulation of topoisomerase II activity due to carotenoids causing cytotoxic effects, detoxification (removal of free radicals), reversing epithelial to mesenchymal transition (EMT), suppression of DNA (DNA fragmentation, C to B conversion) and RNA synthesis but no effect on protein synthesis, epigenetic modifications, metabolic transformation of carotenoids to retinoids, interaction with lectin (type II programmed cell death/autophagy), reducing NO (iNOS levels, nitric oxide production), peroxynitrite ion generation and inhibiting the release of cytochrome c, increasing glutathione-s-transferase activity and scavenging free radicals, Fig. 13.2 gives an overview in the mechanisms involved in anticancer activity of saffron (Boskabady and Farkhondeh 2016; Colapietro et al. 2019; Hoshyar et al. 2008).

4.1 Antioxidant Effect

Crocetin application on skin tumor inhibits the 12-O-tetradecanoyl phorbol 13-acetate-mediated expression of hydrogen peroxide and myeloperoxidase while up surging the activity of several antioxidant enzymes like glutathione peroxidase (GPx), glutathione-S transferase (GST), catalase (CAT) and superoxide dismutase (SOD) and suppresses the activity of myeloperoxidase and malondialdehyde and the production of protein carbonyl in the liver (Colapietro et al. 2019; Das et al. 2010). A combination of hyperthermia and crocin in breast cancer cells reduced the expression of heat shock proteins (Hsp70 and Hsp900) (Mostafavinia et al. 2016). Essential oils of saffron, including safranal as a key constituent, along with crocin, can reduce hydrogen peroxide-induced toxicity and the production of reactive oxygen species (Rahiman et al. 2018).

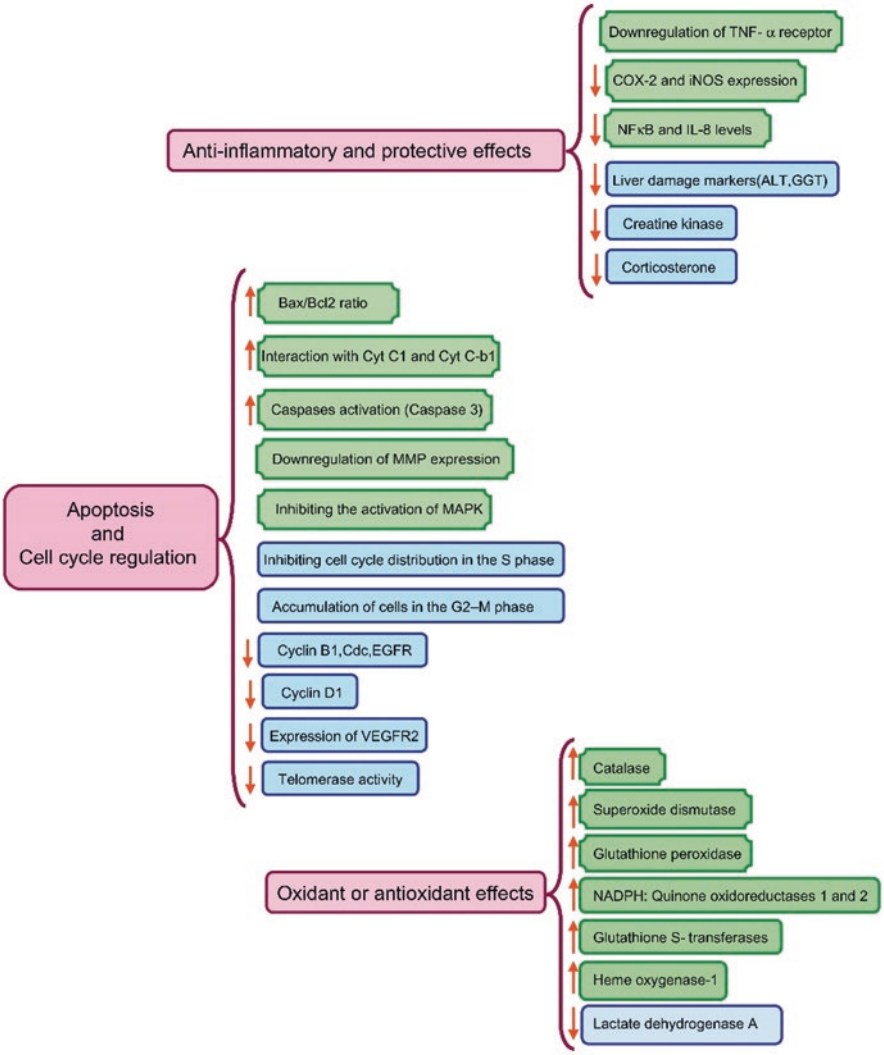

Fig. 13.2 The molecular anticancer mechanism of saffron main compounds. (Reproduced with permission Bathaie et al. 2014)

4.2 Apoptotic and Tumor-Suppressive Effects

Crocin also causes cell cycle arrest at the G0/G1 stage in the lung, head and neck, and colorectal cancer cells. It is also found to inhibit the expression of STAT3 (Signal transducer and activator of transcription 3), a STAT protein phosphorylated by receptor-associated Janus kinases (JAKs), that inhibits JAK1, JAK2, and c-terminal Src kinase. Transcription of several genes involved in cell proliferation

such as anti-apoptotic Bcl-2, pro-apoptotic BAX (Bcl-2-associated X protein), invasive CXCR4 (C-X-C chemokine receptor type 4), angiogenic vascular endothelial growth factor (VEGF) and cell cycle regulator cyclin D1 is thereby modulated, causing the arrest of the cell cycle in sub-G1 phase (Hoshyar and Mollaei 2017).

Safranal and crocin impose a suppressive effect on Bcr-Abl protein kinase through binding to the hydrophobic active site (Geromichalos et al. 2014). Cytotoxic properties of crocin are also associated with apoptosis and necrosis, leading to DNA fragmentation (Rezaee et al. 2013). Treatment of breast cancer cells with saffron and electromagnetic radiation can reduce the expression VEGF receptor and inhibit angiogenesis and tumor progression (Mousavi et al. 2014). A finding has also suggested that crocin inhibits the process of cellular mitosis by repressing the assembly of microtubules (Hire et al. 2017). Crocin suppresses tumor growth by downregulating cyclin D and p21 in breast cancer rat models (Ashrafi et al. 2015). Crocin nanoliposomes in the breast cancer cells downregulates the anti-apoptotic protein Bcl-2, enkephaline degrading aminopeptidase, and increase pro-apoptotic BAX protein expression (Sajjadi and Bathaie 2017). Similar findings are reported for the therapeutic effects against leukemia, bladder, lung, gastric, esophageal, and pancreatic cancer.

Crocetin upregulates p53 and p21 pathways along with the activation of FAS-associated death domain protein, hence causing cell death by imposing antiproliferative effects (Colapietro et al. 2019). In p53-Null colorectal cancer cells, crocin can cause autophagy-independent classical programmed cell death (Koch et al. 2015). Extracts of saffron also upregulate p21 and p27, thus activating of caspases 9 and as a result inducing apoptosis. Figure 13.3 shows the multiple pathways of

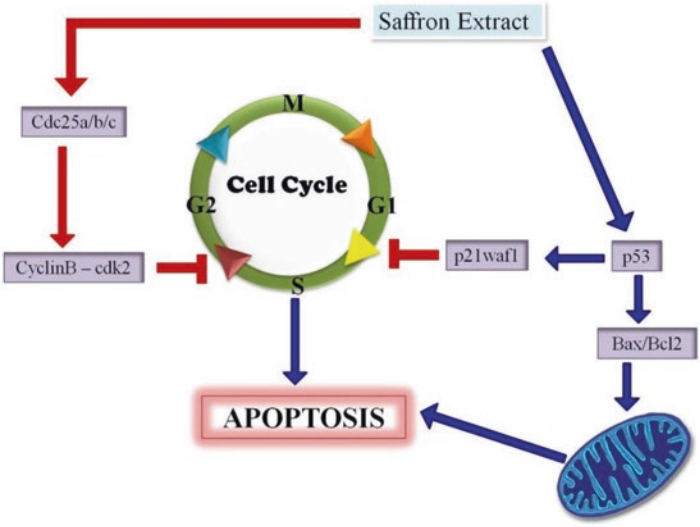

Fig. 13.3 Effects of saffron extract on multiple apoptosis pathways. (Reproduced with permission Patel et al. 2017)

apoptosis induced by saffron extract. Moreover, one of the interesting anticancer mechanisms of saffron included the reversal of epithelial to mesenchymal transition. EMT activates the Wnt biochemical pathway where the loss morphology of epithelial cells is one of the significant hallmarks of cancerous activity, and elevated mesenchymal cells mark the malignancy. Morphological alterations induced in cervical cancer cells as a result of saffron treatment led to cell death. It enhances the appearance of vacuole and pyknotic nuclei, shrinks the cells, and changes the shape (Colapietro et al. 2019).

In hepatocellular carcinoma cells, crocin exerts apoptotic effects by inducing poly ADP-ribose polymerase cleavage. Autophagy can be dependent on the inhibition of Akt/mTOR (mammalian target of rapamycin) and inactivation of associated proteins like p-Akt and p-mTOR (Che et al. 2016; Yao et al. 2018). Reduced expression of epidermal growth factor receptor, doublecortin calcium/calmodulin-dependent kinase-1, and Akt phosphorylation have also been reported in pancreatic cancer cells with the treatment of crocetin. Finally, it activates the Sonic hedgehog (Shh) pathway and mediates the activation of the Gli zinc finger transcription factor (Colapietro et al. 2019). Shh signaling is an essential signaling pathway for cell differentiation and proliferation. Two important downstream molecules, smoothened and Gli, are responsible for oncogenesis, and their inhibitors are used as anticancer drugs (Rimkus et al. 2016).

In human alveolar nonmalignant L929 cells, ethanolic extracts of saffron induce cytotoxicity, altering the morphology of cells and reduce cell viability (Samarghandian et al. 2010). It also increases the activity of caspases 3, 8, and 9 and induces apoptosis (Liu et al. 2014). Treatment of lung cancer cells with crocin has shown cell cycle arrest at the G0/G1 phase, increased P53, and BAX while reduced Bcl-2 activity (Chen et al. 2015a, b).

4.3 Anti-inflammatory Effects

Crocetin also reduces inflammatory cytokines like TNF-α and IL-1β morphological alterations induced in cervical cancer cells as a result of saffron treatment that led to cell death. It enhances the appearance of vacuole and pyknotic nuclei, shrinks the cells, and changes the shape. Additionally, crocin and crocetin enhance the activity of natural killer cells and reduce the production of prostaglandin-endoperoxide synthase two from cervical cancer cells (Colapietro et al. 2019). Additionally, crocin and crocetin enhance the activity of natural killer cells and reduce the production of prostaglandin-endoperoxide synthase two from cervical cancer cells (Colapietro et al. 2019).

In prostate cancer cell models, crocetin, in comparison to saffron and crocin, has the highest antitumor activity in this regard. It reduces the weight of tumor cells and angiogenesis and induces apoptosis. However, saffron and crocin have the highest potency against EMT marked by the reduced production of N-cadherin, β-catenin, and vimentin and increasing the differentiation of epithelial cells by upregulating

E-cadherin and K18. Crocetin suppresses metalloproteinases and urokinase expression, proteolytic enzymes involved in EMT (Zilaee et al. 2019).

4.4 Regulation of Topoisomerase Activity

Topoisomerase II is one of the significant markers of cell proliferation and is a target of breast cancer therapy (MacGrogan et al. 2003). Researchers have shown that crocin can inhibit DNA protein integration by inhibiting the action of topoisomerase (Festuccia et al. 2014).

5 Saffron and Cancer

Polyphenol components like safranal, crocin, crocetin, and alpha and beta carotene of saffron have antioxidant properties. These compounds reduce the load of free radicals and their adverse effects on various organs of the body. Antioxidant potential of crocin also implies the significance of this compound to treat cancer. These components also have anti-inflammatory and apoptotic properties (Siegel et al. 2019). It is effective against a many different cancers such as breast, ovarian, skin, colorectal, prostate, cervical, gastric, liver, leukemia, lung, osteosarcoma, pancreatic, and esophageal cancers (Colapietro et al. 2019).

5.1 Breast Cancer

A wide range of studies have assessed the effects of saffron and its active ingredients on breast cancer cell lines and animal models, with the involvement of multiple anticancer mechanisms. Mir et al. reported anti-proliferative effects on the MCF-7 cell line induced by the isolated crocetin (b-D-glucosyl) ester from the saffron leaf. Furthermore, crocetin (b-D-glucosyl) ester demonstrated a strong affinity for two breast cancer signaling receptors (histone deacetylase two and estrogen receptor alpha) (Mir et al. 2020). Ahmadabadi et al. studied the *in vivo* effects of saffron aqueous extract on breast cancer. The study found that saffron aqueous extract decreased Bax and caspase-3 levels and led to a significantly higher ratio of Bcl-2 to Bax. Moreover, the study observed no improvement of cancer-related muscle mass loss when augmentation saffron aqueous extract with high-intensity interval training was used (Ahmadabadi et al. 2020).

Hashemi et al. reported that crocetin significantly decreased catalase activity in the MCF-7 cell line, while crocin exhibited no effect on catalase activity. However, *in vivo* results from the breast cancer mice model showed that both crocin and crocetin improved catalase activity (Hashemi et al. 2020). Chen et al. studied the

anticancer and anti-angiogenesis effects of prolonged crocin treatment on MDA-MB-231 breast cancer cell line. The study reported that in a dose-dependent manner, crocin induced apoptosis and cell cycle arrest at the G2/M phase. Moreover, exposure to crocin led to a reduction of CD34 expression in tumor tissues (Chen et al. 2019).

In another study, it was reported that crocetin and saffron extracts inhibited proliferation in a time- and concentration-dependent manner in the MCF-7 breast cancer cell line. Moreover, pure crocetin showed to be a more potent anticancer agent when compared to saffron extract (Gezici 2019). In another study, crocetin derivatives exhibited anticancer effects on MCF-7 cell cultures (Chu et al. 2018). Arzi et al. reported that crocin improved survival rates, reduced tumor sizes, and prevented metastasis in triple-negative breast cancer female BALB/c mice. Moreover, Wnt/b-catenin target genes were downregulated by crocin (Arzi et al. 2018).

Results from another study reported the chemopreventive effects of saffron carotenoids where the tumor number and tumor volume in breast cancer rat models were significantly reduced in initiation and promotion stages after exposure to crocin and crocetin. Also, crocin and crocetin administration reduced breast cancer tumor incidence and enkephaline degrading aminopeptidase (EDA) in the ovaries (Sajjadi and Bathaie 2017).

Hire et al. reported that crocin targets microtubules which anti-proliferative effects on HCC1806, MCF-7, and HCC70 breast cancer cell lines were observed. Exposure to crocin caused the induction of multipolar spindle formation, inhibition of mitosis, and depolymerization of mitotic and interphase microtubules. Additionally, the assembly of microtubule-related protein-rich tubulin and tubulin was inhibited. Finally, the study revealed that crocin does not bind in the podophyllotoxin site but binds at the vinblastine site (Hire et al. 2017). Figure 13.4 illustrates the effects of crocin on the polymerization of tubulin.

Mostafavinia et al. (2016) reported that augmentation of crocin and hyperthermia led to anti-proliferative effects in a dose- and time-dependent manner with a degree of synergism in breast cancer cells. This combination resulted in up to 94% reduction in cancer cell colony formation. Moreover, the study revealed a higher Bax/Bcl-2 ratio and a reduction of heat-induced genes expression. Correspondingly, Hsp90 and Hsp70 proteins were reduced in cancer cells. (Bakshi et al. 2016) demonstrated exposing MCF-7 breast cancer cell lines to crocin and saffron extract will result in the upregulation of Bax, substantial DNA damage, and a decrease in anti-apoptotic Bcl-2 in breast cancer cells. Moreover, after 24 h of exposure to both treatments, downregulation of caspase 8 and caspase 9 and cleavage of caspase 3 occured. Ashrafi et al. (2015) reported that treatment with crocin inhibited the production of p21Cip1 and cyclin D1 in breast cancer tumors. The study showed that treatment with crocin resulted in the inhibition of tumor growth and induction of cell cycle arrest. Another study revealed that crocin significantly suppressed MCF-7 breast cancer cell proliferation. Moreover, crocin induced apoptosis via mitochondrial signaling pathways by upregulating Bax, releasing cytochrome c, activating caspase-8, and disrupting mitochondrial membrane potential (MMP) (Lu et al. 2015). Mousavi et al. (2014) evaluated the use of saffron extract in the

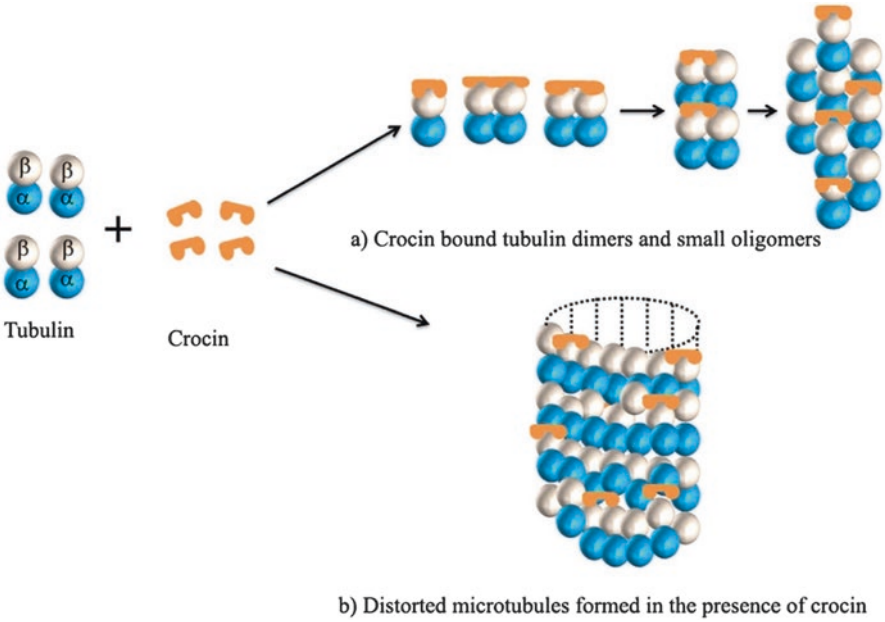

Fig. 13.4 Crocin effects on the polymerization of tubulin. (Reproduced from Hire et al. 2017)

electromagnetic field. The study revealed a significant VEGFR2 gene expression inhibition of saffron extract; however, a synergistic effect was detected when saffron extract was utilized with an electromagnetic field.

Chryssanthi et al. used MDA-MB-231, an invasive breast cancer cell line, to assess the crocetin cytotoxic effects. Crocetin showed to be effective in inhibiting cancer invasion and proliferation. Moreover, crocetin managed to decrease the production of MT1-MMP and MT2-MMP proteins and reducing the activity of MMP-9 protein and gelatinase (Chryssanthi et al. 2011). In another study, the saffron extract was employed for MCF-7 cell treatment. After 48 h of exposure to treatment, the study found that saffron extract increased cytotoxicity in a dose- and time-dependent manner with an IC50 value of 400 ± 18.5 mg/mL. Additionally, apoptosis was observed in MCF-7 cell cultures, and the expression of Bax was increased after treatment with saffron (Mousavi et al. 2009).

5.2 Bladder Cancer

Saffron aqueous extract has a dose-dependent *in vitro* suppressive effects on the cell proliferation of primary bladder carcinoma. The results were obtained from a study that was conducted on mouse fibroblast cell line (L929) and human transitional carcinoma cells (TCC 5637). Primary bladder carcinoma cells were exposed to

saffron aqueous extract with doses of 50, 100, and 200 μg/mL for 24 h, revealing a disruption of intercellular connections and reduction in cell growth compared to the control group. However, at a dose of 400 and 800 μg/mL, the study observed vacuolization and cellular detachment and an increase of pigmentation.

Furthermore, cell survival decreased to 10–15% when the concentration of 2000 μg/mL was used. After 48 h of exposure, 50, 100, and 200 μg/mL concentrations led to intercellular disruption and reduction in cellular viability with increased pigmentations. Moreover, 800 μg/mL concentration destroyed most cells. The quantitative assessment demonstrated a significant correlation between the decrease of cell viability and the increase of saffron extract concentration, with no survived cells were found after 120 h when the saffron extract was used at 400 μg/mL concentration (Feizzadeh et al. 2008).

5.3 Cervical Cancer

In a study involving cervical cancer cells, saffron extracts and crocetin reduced cell growth and cell viability in a dose- and time-dependent manner in the cervical cancer cells. It was also observed that the pure form of crocetin had better efficacy against cancer cells (Gezici 2019). Cheriyamundath et al. revealed that the survival rate and viability of the cervical carcinoma cell line were reduced after being exposed to crocin (Cheriyamundath et al. 2018). Jiang et al. revealed the *in vitro* effects of safranal on the HeLa cell line. The results demonstrated a concentration-dependent manner inhibition of cell viability was observed after treatment with safranal, with cellular microtubules exposed to negligible damage. Nevertheless, the recovery of the microtubule network was inhibited after cold-induced disassembly, which can propose the tubulin-targeted anticancer activity of safranal (Jiang et al. 2018).

Granchi et al. studied the effects of crocetin on lactate dehydrogenase inhibition. The results found that crocetin inhibited proliferation in the HeLa cervical cancer cell line with an IC50 of 113.0 ± 11.1 μM. The further biochemical investigation showed that the crocetin inhibited lactate production (Granchi et al. 2017). Mollaei et al. used MTT to show that crocin had anti-proliferative effects against sensitive (OV2008) and resistant (C13) human cervical cancer cell lines. Further assessments revealed induction of apoptosis, P53, and Bax were upregulated, miR-365 and Bcl2 were downregulated. Overall, the study demonstrated that crocin had better effects against OV2008 compared with C13 cervical cancer cell lines (Mollaei et al. 2017).

The evaluations done by Chen et al. showed that crocetin had chemopreventive properties after oral administration in cervical cancer mice models. The findings indicated that crocetin treatment induced a significant elevation in the plasma levels of TNF-alpha, MDA, PMN, nitrates, IL-1beta. Besides, in HeLa cells, the COX-2 mRNA levels were also increased (Chen et al. 2015a, b). Kim et al. assessed crocetin and crocin on several types of cancers in which cervical cancer cell line was

included. The results demonstrated that crocetin—in contrast to crocin—was able to induce cellular reactive oxygen species (ROS) at a significant level in HeLa cervical cancer cell line. Moreover, crocin and crocetin led an increase of 3.0- and 1.6-fold of nuclear factor erythroid 2-related factor 2 (Nrf2), respectively. Also, lactate dehydrogenase A (LDHA) was decreased by both of crocin and crocetin by 10.5% and 34.2%, respectively (Kim et al. 2014).

Tavakkol-Afshari et al. investigated the effects of the saffron extract on the HeLa cervical cancer cell line. The obtained results showed an increase of cytotoxicity with an IC50 of 800 μg/mL after 48 h of exposure, dependent of time and concentration. Additionally, flow cytometry histogram showed a sub-G1 peak was induced by saffron, suggesting the role of apoptosis in cancer cell death. The study also found the saffron extract effects were independent of ROS (Tavakkol-Afshari et al. 2008).

Abdullaev et al. discovered that crocin derivatives had the best effects against colony formation of cancer cells compared to other saffron carotenoids (Abdullaev et al. 2003). Escribano et al. reported the LD50 of saffron extract and its main ingredients on the HeLa human cervical cancer cell line. The results demonstrated that the ethanolic saffron extract, crocin, safranal, and picrocrocin had an LD50 of 2.3 mg/mL, 3 mM for, 0.8 mM, 3 mM, respectively. The study found no cytotoxic effects of crocetin (Escribano et al. 1996).

5.4 Colon Cancer

The use of main saffron ingredients as anti-colon cancer agents is not new in the literature. A study published in 1999 reported the use of crocin for the treatment of colon adenocarcinoma (García-Olmo et al. 1999). This publication showed that crocin treatment led to slowing down tumor growth and increased the life span in female rats. However, in male rats, the study did not find a significant antitumor effect. Moreover, kidney samples from rats treated with crocin showed acute tubular necrosis. The *in vitro* anticancer results in DHD/K12-PROb, and HT-29 cell lines showed the potent cytotoxic activity of crocin (Garc-Olmo et al. 1999).

In another study, HCT116 colon cancer proliferation was inhibited after treatment with crocin in a concentration-dependent manner. Further evaluations showed the role of crocin in increasing apoptosis and decreasing inflammatory factors and chemokines release. Also, the P-STAT3/STAT3 ratio was decreased, leading to a decrease in cytokines secretion in colon cancer cell lines (Wang et al. 2019). Another paper reported the anti-proliferative effects of safranal in colo-205 cells (IC50 of 20 μM). Further investigation showed the activation of ROS and the decrease of MMP, which caused the induction of apoptosis in colon cancer cells. Also, the use of safranal led to an increase in the production of Bax and decreased the production of Bcl-2. The study revealed the inhibition of PI3K/AKT/mTOR pathways and G2/M cell cycle arrest have resulted after treatment with safranal (Zhang et al. 2018a, b).

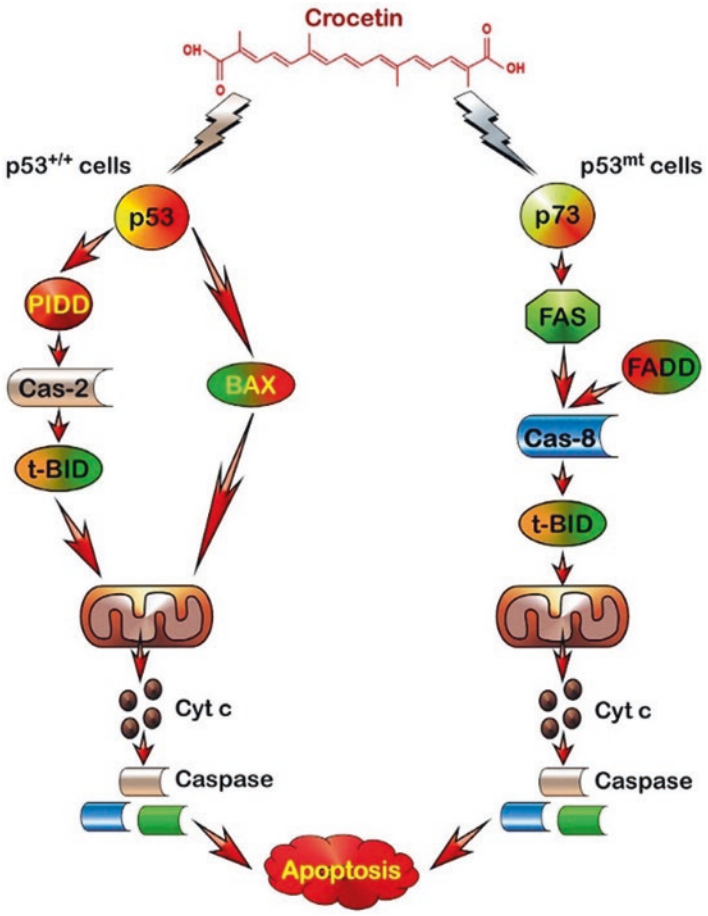

Fig. 13.5 An illustration representing the mechanism of colon cancer cell death after treatment with crocetin. (Reproduced from Ray et al. 2016)

Zhuang et al. (2018) reported a reduction in VEGF, MMP-9, and p65 in colon cancer cells after exposure to crocetin. Another study showed that crocin decreased the expression of GADD34 and GRP78, proposing the benefits of crocin in preventing lipid peroxidation and ER stress (Boussabbeh et al. 2016). Another paper reported the role of crocetin in inducing apoptosis in colorectal cancer through FAS-associated death domain (FADD) and p53-induced death domain (PIDD) proteins (Ray et al. 2016), as shown in Fig. 13.5.

Koch et al. reported the use of crocin on two colon cancer wild-type and p53−/− HCT116 cell lines. The authors reported the anti-proliferative effects of crocin with a concentration of 10 mM. Further evaluations showed that classical programmed cell death in an autophagy-independent pathway was induced by crocin (Koch et al. 2015). Kawabata et al. performed a study to assess the effect of crocin against

inflammation in colon cancer and colitis in mice. The obtained results from mice models revealed significant inhibition of growth of colonic adenocarcinomas, suppression of proliferation, decrease expression immunohistochemical of nuclear factor- (NF-) κB, and elevated production of NF-E2- related factor 2 (Nrf2). In another experiment, 4 weeks of crocin dietary feeding led to inhibition of colitis and decreased interferon γ, NF-B, cyclooxygenase-2, interleukin- (IL-) 1β, and expression of mRNA of tumor necrosis factor α. Moreover, the expression of Nrf2 mRNA was increased after dietary feeding. These findings demonstrate the potential of crocin in the prevention of inflammation-associated colon carcinogenesis and colitis (Kawabata et al. 2012).

The saffron extract was used by Bajbouj et al. to investigate the pro-apoptotic effects and anti-proliferative effects of the extract on HCT116 colorectal cancer cell lines (HCT p53−/− and HCT wild-type). The study showed a cell cycle distribution with a p53-dependent pattern and a full stop of G2/M in HCT116 p53 wild-type cell cultures. Further evaluations revealed a noticeable induction of apoptosis by the saffron extract in HCT116 p53 wild-type cells (Bajbouj et al. 2012). Additionally, the use of crocetin to treat SW480 cells was reported. Crocetin exhibited anti-proliferative activity in a dose-dependent manner. Furthermore, p53-independent mechanisms with the induction P21 was observed as the mechanism of cancer cell cycle arrest induced by crocetin therapy. Additional evaluations revealed the role of crocetin in enhancing apoptosis and reducing the repairing capacity of the DNA of cancer cells (Li et al. 2012).

5.5 Esophageal Cancer

Research performed on crocetin, a pharmacologically-active compound of saffron, showed that crocetin could be a promising therapeutic candidate for esophageal cancer treatment. Li et al. used esophageal squamous carcinoma cells (KYSE-150) to explore the mechanism of anticancer effects of crocetin on p53/p21, MAPK, and PI3K/AKT pathways. *In vitro* results on the esophageal squamous carcinoma cells demonstrated a time- and dose-dependent inhibitory cell proliferation effect. Moreover, the study observed cell apoptosis induced by crocetin. Crocetin showed inhibitory effects on an extracellular signal-regulated kinase-1/2 (ERK1/2), PI3K/AKT, and p38 and upregulation of the p53/p21 level. These effects activated the mitochondrial-mediated apoptosis pathway leading to MMP disruption, increased cleaved caspase-3 and Bax levels, and a decrease in Bcl-2 levels. These findings can demonstrate the anticancer potential of crocetin by interfering with multiple signaling pathways in esophageal squamous carcinoma cells (Li et al. 2019). These findings were aligned with a previous study conducted by the same research group on the KYSE-150 cell line, were crocetin showed anti-proliferation effects with an association with the S phase arrest and after 48 h of exposure to cancer cells (Li et al. 2015). Moreover, crocetin induced cell apoptosis and morphological changes in a concentration-dependent manner after increasing pro-apoptotic Bax expression

and activated caspase 3. Furthermore, crocetin inhibited cell migration of esophageal squamous cell carcinoma cell lines (Li et al. 2015).

5.6 Gastric Cancer

Several published papers reported the *in vitro* and *in vivo* therapeutic use of crocin, crocetin, and saffron extract for gastric cancer (Bathaie et al. 2013b; He et al. 2014; Luo et al. 2017). In a study, HGC-27 and AGS gastric cancer cells were treated with crocin. The study discovered a decrease in HIF-1α and KLF5 expression; in contrast, the expression of miR-320 was elevated. Moreover, crocin inhibited gastric cancer cell invasion, migration, and EMT. The study suggests the involvement of miR-320/KLF5/HIF-1α pathway in the observed anticancer properties (Zhou et al. 2019). Furthermore, Hoshyar et al. reported that crocin induces apoptosis in Human Gastric Adenocarcinoma (AGS) cell line by the activation of caspase and increasing the ratio of Bax/Bcl-2 (Hoshyar et al. 2013).

Another publication employed crocetin to treat gastric cancer in rats and AGS gastric cancer cells. The results showed significant suppression of cell proliferation in a dose- and time-dependent manner. Further investigations on AGS gastric cancer cells showed the induction of apoptosis, Bax expression up-regulation, inhibition of Bcl-2. The pathological evaluations in rats showed suppression of tumor progression in a dose-dependent manner (Bathaie et al. 2013a, b).

5.7 Glioblastoma

The extracted crocetin from saffron was proved to have anticancer properties in glioblastoma *in vitro* and *in vivo* models (Colapietro et al. 2020). Four glioma cell lines were evaluated *in vitro* (U87, U251, U373, and U138), after being treated with different concentrations of crocetin (250 and 500 μM). Crocetin inhibited cell proliferation and demonstrated pro-differentiative activity. These effects were because of the modulation of neuronal and mesenchymal markers, changing cell morphology, and reducing cell viability. Exposure to crocetin resulted in a decrease of Cluster of Differentiation CXCR4, CD90, CD44, and the mesenchymal marker OCT3/4.

On the other hand, crocetin led to an increase of βIII-Tubulin and the neurofilament neuronal lineage-related markers. Furthermore, downmodulation of HDAC3, HDAC1, and Histone Deacetylase was observed in U87 and U251 cell lines; however, the U3737 and U138 cells only demonstrated downmodulation of HDAC1 expression; this may suggest that epigenetic mechanisms could modulate these changes. Western blot analysis revealed inhibition of CD44, FASN, and Fatty Acid Synthase markers after treatment with crocetin, which was associated with apoptosis activation and a reduction in wound repair and cell movement of glioma cells.

The *in vivo* tests showed that crocetin had better anticancer properties compared to radiotherapy alone; however, crocetin anticancer activity was similar to temozolomide effects. Overall, crocetin treatment increased overall survival and disease-free survival rates (Colapietro et al. 2020). In another research paper, organic and inorganic saffron stigma were investigated and compared in terms of inducing apoptosis and inhibiting proliferation. The study demonstrated that organic stigma had more cytotoxic and antioxidant activity compared to the inorganic stigma. Where a significant decrease in cell viability was obtained with the organic stigma in a time- and dose-dependent manner. The induced apoptosis was associated with the increase of Bax and the decrease of Bcl2 gene expression, which increased the Bax/Bcl-2 ratio in a time-dependent manner (Behdani and Hoshyar 2016).

5.8 Head and Neck Cancer

A research study conducted on head and neck cancer cell lines (HN-5) assessed the cytotoxicity and the induction of radiation sensitivity of crocin. Different crocin concentrations were evaluated (12.5–1000 μg/mL) on HN-5 cell lines. The cell viability of head and neck cancer cell line was decreased after incubation with crocin in a concentration- and time-dependent manner. Flow cytometry histogram revealed a sub-G1 peak in the cells treated with crocin compared to control, proposing that the crocin toxicity was responsible for cell apoptosis. Furthermore, crocin was able to sensitize cancer cells to radiation; thus, combining the use of radiation and crocin will result in increased radiation sensitivity and cytotoxicity. As a result of anti-carcinogenic and anti-proliferation properties of crocin, these properties suggest the potential of crocin be implemented as an anticancer drug and radiotherapy sensitizer with low toxicity risks (Vazifedan et al. 2017).

5.9 Leukemia

The anti-leukemic impact of the saffron extract carotenoids has been broadly evaluated in the last three decades. In 1991, the first report described the antitumor activity of saffron on inhibition of DNA biosynthesis in Dalton's lymphoma ascites, Ehrlich Ascites Carcinoma (EAC), and sarcoma cells in mice. *In vitro* assay also indicated the cytotoxicity impact of the saffron extract on the same cancerous cells (Nair et al. 1991).

In 2013, Y Sun et al. designed an *in vitro* and *in vivo* study to evaluate the underlying antitumor mechanisms of crocin on human leukemia cells (HL-60). In the *in vitro* assay, the proliferation of cells and the rate of apoptosis were examined by MTT method and AO/EB staining, respectively. The authors also evaluated cell cycle profiles of the cells through flow cytometry assay. The obtained results demonstrated that crocin displayed a significant repressive effect on cancerous cell

proliferation and increased apoptosis rate of the target cells in a concentration and time-dependent manner. Furthermore, data indicated a remarkable decrease in the weight and size of the tumor in crocin treated HL-60 xenografts in mice, which were assumed to be in result of inhibition and decrease of Bcl-2 and Bax gene expression (Sun et al. 2013). They also designed an experiment to examine the inhibitory effect of crocin on Jurkat cell proliferation. Various concentrations of crocin were used to stimulate target cells. The cell proliferation first was detected through the cell counting and methyl thiazolyl tetrazolium (MTT) examinations. They employed the propidium iodide (PI) method to evaluate the apoptosis rate. Data from reverse transcription-polymerase chain reaction (RT-PCR) examination demonstrated that the expression of the Bax gene had been promoted while Bcl-2 gene expression has been significantly decreased. Data reported in this study indicated that Jurkat cell apoptosis increased after exposure to crocin; besides, cell growth was inhibited dependent on the dose and time of the exposure to the target agent (Sun et al. 2015).

In the more recent investigation, Zhang et.al aimed to study the impact of crocin on the cytotoxic function and proliferation of T lymphocytes separated from mononuclear cells of peripheral blood of ALL children with at least 6 months of complete remission. Crocin (final concentration 0.625–2.5 mg/mL) was used to stimulate cells. The level of different T lymphocyte-specific cytokines, as well as the CD4/CD8 ratio, were evaluated. Tail DNA%, Tail length, Tail moment, and sister chromatid exchange was considered as standard criteria to examine Ara-C induced DNA damage in crocin pretreated lymphocytes; they reported that DNA damage in these lymphocytes was significantly decreased. The result showed a considerable increase in lymphocyte proliferation, the level of IL-4 and IL-2 secretion, as well as CD4/CD8 ratio of T cells, dependent on crocin concentration (Zhang et al. 2018a, b). The cytotoxic consequence of crocin on the T lymphocyte leukemia cell line (MOLT-4) was also reported. This effect was assumed to be a result of the promotion of DNA fragmentation. Further results from studies conducted by Rezaee et.al showed that cells treated by crocin produced less amount of reactive oxygen species (ROS) compared to nontreated cells. They also reported apoptotic effects of the extract on tumor cells at all concentrations, while necrosis outcome only was shown at the highest dose (500 μM) (Rezaee et al. 2013).

In 2014, the study of the anticancer effect of saffron extracts, crocin and safranal was conducted on K-562 human chronic myelogenous leukemia (CML) cells to assess the effect of these components on the expression level of the Bcr-Abl protein. Although tumor cells treated with both substituents exhibited cytotoxicity activity, data from gene expression analysis indicated that Bcr-Abl gene expression was inhibited by treating cells with safranal; conversely, crocin-treated cells experienced an rise in the expression of the target gene (Geromichalos et al. 2014). In line with the previous studies, the anti-leukemic capability of saffron extract, crocetin, was comprehensively examined on primary acute promyelocytic leukemia (APL) cells, NB4, and HL60 cells. The results demonstrated that the proliferation of crocetin-treated tumor cells was considerably inhibited. The cells exhibited apoptotic consequences which might be mediated through the reduction of the multidrug resistance

(MDR) proteins, ATP-binding cassette membrane transporters (ABC) B1 and ABCC1, Akt and BCL2 prosurvival genes and the tyrosyl-DNA phosphodiesterase 1 (TDP1) suppression, whereas, a remarkable increase in the BAX/BCL2 ratio and the expression levels of the pro-apoptotic genes CASP3, CASP9 was reported. Additionally, the expression of histone deacetylase 1 (HDAC1) in all tumor cells was reduced significantly (Moradzadeh et al. 2019).

5.10 Liver Cancer

Despite the limited clinical evidence on the clinical use of saffron in cancer patients; however, a randomized double-blind clinical trial study reported the use of saffron on patients with liver metastases. Thirteen patients were enrolled in this study who either received standard chemotherapy in combination with a 50 mg saffron capsule twice daily or were treated with the standard chemotherapy regimen and placebo. Only seven patients were able to finish the trial to the end, where four patients and three patients were involved in saffron and placebo groups, respectively. One patient in the saffron group showed a complete response to the treatment, and another patient showed a partial response. The two other patients in the saffron group showed progressive (n = 1) and stable (n = 1) responses. Whereas, in the second group, the study did not observe complete or partial responses, two patients showed unchanged responses, and one showed progressive responses. This clinical trial reported one death in the saffron group and two deaths in the placebo group (Hosseini et al. 2015). Despite the small population included in this study, the results propose beneficial effects of saffron with patients suffering from liver metastases. Further clinical trials with larger study populations must be conducted to facilitate the pharmaceutical translation of saffron as an anticancer agent.

Alongside with the previous study, several preclinical studies were performed to evaluate the anticancer effects of saffron on liver cancer (Amin et al. 2011; Noureini and Wink 2012). A number studies reported the involvement of different mechanisms in the cytotoxic activity of saffron and its main phytochemicals; these mechanisms include inhibition of STAT3, induction of apoptosis progression, ER-stress-mediated cellular death, inhibition of telomerase, breakage of double-strand DNA, down-regulation of hTERT, and inhibition of Akt/mTOR activity (Al-Hrout et al. 2018; Kim and Park 2018; Noureini and Wink 2012; Yao et al. 2018). For instance, Fig. 13.6 illustrates the anticancer molecular mechanism of safranal in liver cancer cells. Moreover, another study reported the use *in vivo* animal models to confirm the therapeutic applications of crocin in hepatocellular carcinoma (Amin et al. 2015). These studies pave the way for conducting more research in clinical settings and animal models for saffron as a candidate for hepatocellular carcinoma.

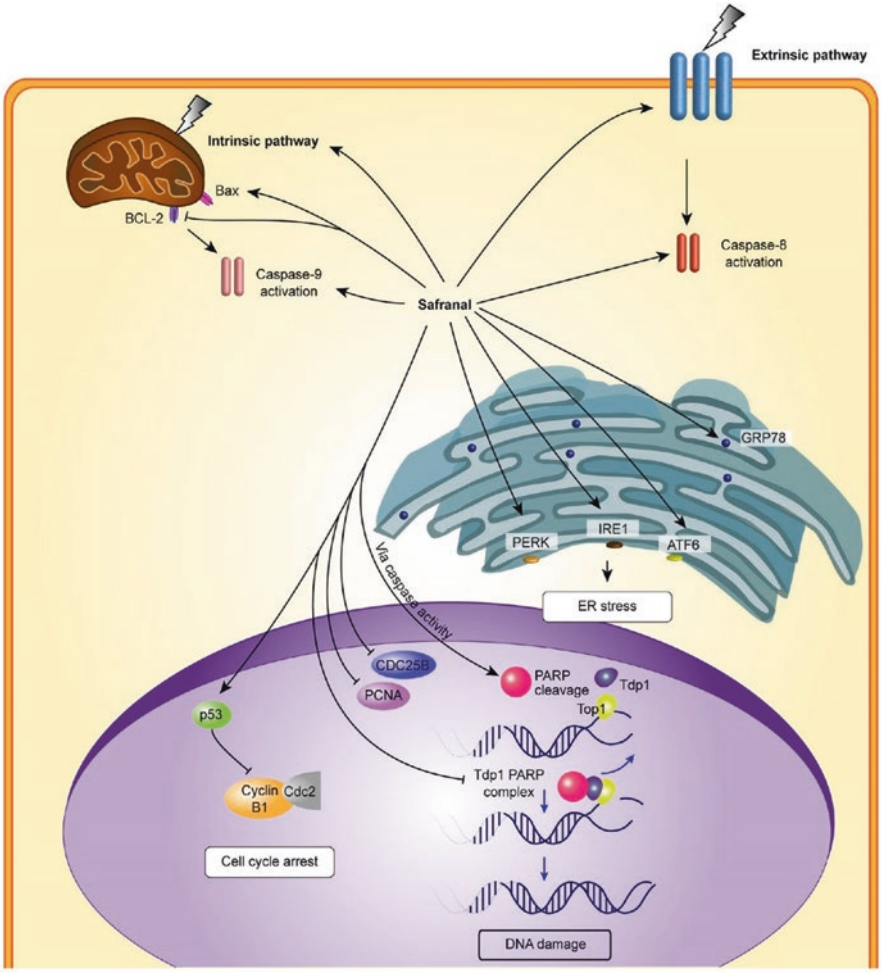

Fig. 13.6 An illustration represents the anticancer mechanism of safranal in liver cancer cells. (Reproduced from Al-Hrout et al. 2018)

5.11 Lung Cancer

Several studies reported the anticancer activity of saffron and its active ingredients where the role of caspase-mediated cell apoptosis was observed in lung cancer cells (Liu et al. 2014; Samarghandian et al. 2011, 2013). Other studies conducted on human non-small lung cancer cells (A549) confirmed the antiproliferative properties of saffron extract and its active compounds (Liu et al. 2014; Samarghandian et al. 2010, 2011). Another study investigated the administration of saffron and its active ingredients, which was followed by the administration of the E7-NT (gp96) DNA vaccine to combat tumors with E7 protein expression of human

papillomavirus for chemo-immunotherapy purposes. The study reported the induction of programmed cell death and inhibition of cell growth in murine TC-1 malignant (CRL-2785) cells by saffron treatments. Moreover, all of the crocin and treated mice were tumor-free (Khavari et al. 2014).

Another approach to achieve desired anticancer effects and tackle the low solubility of crocetin is synthesizing crocetin derivatives to attain higher solubility and improved activity. Where researchers prepared crocetin derivatives with enhanced solubility profile and improved inhibitory effects on human lung cancer cell line (Wang et al. 2020). Furthermore, *in vivo* evidence in mice showed that crocetin could reverse the pathological effects of lung cancer (Magesh et al. 2006).

5.12 Osteosarcoma

In a study, crocin and bicarbonate were loaded to hydroxyapatite disks to evaluate the *in vitro* effects on osteosarcoma cell lines (MG-63). Release studies demonstrated a plateaued release of bicarbonate and crocin in acidic and physiological environments over the course of 7 weeks, while the release of crocin was controlled by bicarbonate. After dose optimization tests were carried out, the dual loading of crocin and bicarbonate was able to reduce the cellular viability of osteosarcoma by over 50% compared to control by day 11 of the in vitro study, revealing changes in morphology and cell spreading. These findings propose that crocin and bicarbonate had a pro-apoptotic mechanism in osteosarcoma cell cultures, displaying the potential of crocin and bicarbonate to be used for osteosarcoma regulation (Koski et al. 2020). In another paper, the effects of hexane and dichloromethane saffron extracts were evaluated and showed anti-proliferation effects on osteosarcoma cancer cell cultures (U2-OS). Both extracts interfered with migration capabilities and colony-forming with the activation of the CDKN2B tumor suppressor gene. However, the main discussed phytochemicals of saffron—crocin and crocetin—were not present in these extracts, and only compounds like resveratrol, vanillic acid, 4-hydroxybenzoic acid, and caffeic acid were found in these extracts (Ege et al. 2020).

5.13 Ovarian Cancer

In a study, the mechanism and effects of crocin on ovarian cancer cell lines (HO-8910) were investigated. The study showed significant cell growth inhibition induced by crocin. Moreover, flow cytometry revealed an increased ovarian cancer cell proportion in the G0/G1 phase, with an observed increase in apoptosis. Additionally, the western blot analysis demonstrated the up-regulation of Fas/APO-1, p53, and Caspase-3 induced by crocin (Xia 2015). Also, another study assessed the therapeutic effects of crocin on the production and function of MRP1

and MRP2 in ovarian cancer cell culture (A2780) and ovarian cancer cisplatin-resistant cells (A2780/RCIS). This study demonstrated that crocin had anti-proliferative activity in a dose-dependent manner with a more obvious activity in A2780 cells compared to A2780/RCIS cells. Furthermore, the gene expression of MRP1 and MRP2 at the mRNA level was reduced in the A2780/RCIS cell culture. Also, crocin treatment led to an increase in cytotoxicity effects of doxorubicin on the resistant cells (A2780/RCIS) (Mahdizadeh et al. 2016).

On the other hand, human ovarian cisplatin-resistant cancer cell culture (A2780-RCIS) was used to investigate the effects of crocetin on MRP1 and MRP2. The results revealed that crocetin decreased cell proliferation in an ovarian cancer cell line (A2780, IC50: 183 ± 7 μM) and cisplatin-resistant ovarian cancer cell line (A2780-RCIS, IC50: 316 ± 9 μM). As well the expression rates of MRP1(22 ± 2%) and MRP2 (48 ± 8%) were decreased in the A2780-RCIS cell culture. Furthermore, crocetin directly blocked the function of MRP pumps in A2780-RCIS (88 ± 10%) and A2780 (44 ± 1%) and indirectly inhibited the MRP pumps in A2780-RCIS (48 ± 15%) and A2780 (32 ± 2%), respectively (Neyshaburinezhad et al. 2018). Thus, these findings propose that crocin and crocetin could be a potential candidate to combat drug-resistant ovarian cancer by modulation MRP transporters. On top of that, in two studies from the same research group, crocetin derivatives were synthesized, and their anticancer activity in human ovarian carcinoma cell cultures (SKOV3) was evaluated. The crocetin derivatives had significantly better solubility and anticancer effects compared to crocetin. These derivatives demonstrated high safety and promising therapeutic capabilities in cancer therapy (Chu et al. 2018; Wang et al. 2020).

5.14 Pancreatic Cancer

A series of tests were conducted to assess crocetin anticancer properties in animal models and cell lines. The human pancreatic cancer cell line (MIA-PaCa-2) were used for the *in vitro* experiments, whereas, crocetin showed anti-proliferative properties. Also, crocetin significantly altered cyclin-B1, Cdc-25C, Cdc-2, and epidermal growth factor receptor. Furthermore, other pancreatic cancer cell lines were used to confirm the results, where crocetin (200 μmol/L) was used on ASPC-1, Capan-1, and BxPC-3 pancreatic cancer cells showing the similar inhibition of proliferation. In the *in vivo* studies, crocetin was orally administrated to athymic nude mice; the results revealed significant suppression of tumor growth and inhibition of proliferation. In general, crocetin significantly induced apoptosis, which was shown in the Bax/Bcl-2 ratio. These effects demonstrate a clear potential of crocetin in pancreatic cancer therapy (Dhar et al. 2009). In another research paper, crocin anticancer efficacy was investigated in the human pancreatic cancer cell line (BxPC-3). Crocin promoted cell arrest at the G1-phase and apoptosis in pancreatic cancer cell culture, resulting in increased cytotoxicity in a dose- and time-dependent manner. Apoptotic morphology and reduced volume were observed in cells that were

exposed to 10 μg/L crocin. The DNA analysis confirmed apoptosis after 12 h of treatment with crocin. Thus, crocin appears to be a candidate for future studies investigating potential cancer treatments (Bakshi et al. 2010).

5.15 Prostate Cancer

A study evaluated the anticancer effects of crocin and crocetin after oral administration in male nude mice xenograft of two aggressive prostate cancer cell lines (22rv1 and PC3). The study found that crocetin had a higher anticancer efficacy compared to crocin, as elucidated after the increase of E-cadherin production and decrease in that of beta-catenin and N-cadherin. Moreover, crocin and crocetin led to inhibition in cell migration and invasion after downmodulating urokinase and metalloproteinase, proposing the role of crocin and crocetin in interfering with the metastatic processes (Festuccia et al. 2014). In another research study, the effects of crocin and saffron extract were investigated on seven prostate cancer cell cultures (C4–2B, LAPC-4, PC3, LnCaP, 22rv1, CWR22, and DU145) in which five of the cell lines were malignant whereas two of them were nonmalignant. The study revealed a decrease in cell proliferation in the malignant cell cultures with an IC50 range of 0.26–0.95 mM/mL for crocin and 0.4–4 mg/mL for saffron extract.

In contrast, crocin and saffron extract did not affect the two nonmalignant cell lines. Most cells have undergone through arrest at the G0/G1 phase with the simultaneous presence of the apoptotic cells, as it was shown by flow cytometry study. Also, the western blot analysis found that the expression of Bcl-2 was reduced, and that of Bax was increased. Additionally, the study proposed intrinsic pathway involvement after discovering a caspase-dependent pathway in which the activation of caspase-9 was involved (D'Alessandro et al. 2013). It is noteworthy that also safranal had cytotoxic effects on prostate cancer in a dose-dependent manner through the induction of apoptosis (Samarghandian and Shabestari 2013). Overall, these studies highly suggest that crocin and other saffron compounds can be considered for prostate cancer treatment.

5.16 Retinoblastoma

An *in vitro* study found that crocin demonstrated anticancer activity in human retinoblastoma cell lines (WERI-Rb-1 and Y-79). This study revealed that crocin had antiproliferation effects on retinoblastoma cell cultures. Crocin with a concentration of 10 μM ($P < 0.05$) inhibited cell proliferation, this effect increased rapidly once a 20 μM ($P < 0.01$) concentration of crocin was applied, these effects were in a time- and dose-dependent manner (every 24 h for 5 days with 0–80 μM of crocin). Also, crocin was able to induce apoptosis and inhibit clonogenicity in the WERI-Rb-1 and

Y-79 retinoblastoma cell lines. Furthermore, cell exposure to crocin caused a decrease in the stability and expression levels of MYCN compared to control. Moreover, crocin treatment significantly reduced the mRNA levels of MYCN. This study is a clear indication that crocin shows promising antitumor properties which can be applied for the prevention and therapy of retinoblastoma (Deng et al. 2019).

5.17 Rhabdomyosarcoma

In a study conducted on human rhabdomyosarcoma cells, crocetin was used with a concentration range of 5–20 μg/mL as a treatment. The study revealed that crocetin was able to induce cell damage in the malignant cells, while not damaging the normal cell lines (Jagadeeswaran et al. 2000). In another paper, the saffron extract showed anticancer effects in a dose-dependent manner in rhabdomyosarcoma cell cultures (A-204). The study revealed that rhabdomyosarcoma cells were more sensitive to saffron extract compared to hepatocellular carcinoma and cervix epithelioid carcinoma cell cultures (Abdullaev et al. 2003). Undoubtedly, these studies show that main saffron compounds have the potential to be implemented in rhabdomyosarcoma therapeutic strategies.

5.18 Skin Cancer

It has been a long time since the efficacy of saffron derived bioactive component crocetin, on the inhibition of the skin tumor initiation first has experimented. Gainer et al. in 1976 induced the skin tumors using 7,12-dimethyl benz(a)anthracene (DMBA) in mice, and the study reported that the initiation of the skin papillomas and Rous sarcomas had been delayed in the group was pretreated by crocetin. They also showed that crocetin could effectively promote oxygen transport in comparison to the other carotenoids (Gainer et al. 1976).

The inhibitory impact of saffron extracts on chemically induced skin papillomas and soft tissue sarcomas in albino mice (respectively by DMBA and 20-methylcholanthrene (MCA)) was evaluated (Salomi et al. 1991), which confirmed the results of the previous study (Gainer et al. 1976). Test groups were treated by oral administration of 100 mg/kg bodyweight of the saffron extract, and data reported in this study indicated that the onset of papilloma initiation was significantly delayed. The application of the extracts also exhibited a considerable potential to inhibit the tumor-initiating activity of MCA, which was subcutaneously administrated in mice to induce sarcoma. This effect is assumed to be probably in the result of the oxygen-scavenging acquisition of this natural substituent. Besides, crocetin as a potential anti-carcinogenic agent was used to examine its topical administration on 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced tumor promotion and edema in CD-I mice. This agent has also exhibited an inhibitory impact

on chemically-induced hyperplasia and ornithine decarboxylase (ODC) in mouse epidermis. The production of hydrogen peroxide and myeloperoxidase by TPA was remarkably inhibited in crocetin-pretreated mice in a dose-dependent manner (Wang et al. 1995).

Not until much later, saffron was drawn attention due to its promising properties for promotion of health, e.g., scavenging of radicals, anticarcinogenic and immunomodulatory effects were among the most useful properties of this natural agent. In 2004, Ila Das and his colleagues also used DMBA and croton oil to induce skin tumors and promote it in mice. The study investigated the inhibitory effects of an aqueous infusion of saffron, and data from their study indicated that papilloma initiation considerably decreased when saffron had been administrated before the tumor initiation period. This inhibitory impact of saffron also was reported while it was used after initiation time. However, the highest effect was exerted when saffron was administered both post and pre-formation of the tumor. Regulatory impact of the agent on glutathione-S-transferase (GST), glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD), which are all antioxidant enzymes, was considered as the main reason for the aforementioned inhibitory effect (Das et al. 2004).

In the next research was done by Ila Das et al., the previous results were confirmed entirely; however, the new experiment design followed a histopathological approach. DMBA-induced cancerous mice are divided into different groups to be evaluated in terms of the time of saffron application, pre, post, or both before and after DMBA administration. Both cancer initiation and tumor size in mice were positively affected by saffron. The activity of the detoxifying enzymes (mentioning in the biochemical assay of their previous research work, including GST, GPx, CAT, and SOD), considering as cellular defense systems, were analyzed as indications/parameters for oxidative stress in the samples of liver tissue taken from all groups. Saffron-treated groups exhibited different levels of enzyme activity elevation. The standard histological studies of skin showed that saffron was considerably effective, especially in pre and post-skin carcinogenesis applications (Das et al. 2010).

In another study, antitumor properties and the associated mechanisms of two different saffron extracted carotenoids, crocetin, which is a glycosylated based one, and a carboxylic component crocin were thoroughly examined *in vitro*. Various human cell lines, including adenocarcinomic alveolar basal epithelial cell (A549), liver cancer cell line HepG2, Hela cell line as cervical cancer cells, human colon cancer (HCT-116) and SK-OV-3 cell line as human ovarian cancer cells were used in this study. In comparison to crocin, crocetin exhibited significantly higher anticancer effects, which are assumed to be mediated through their different structural properties. Fluorescence-activated cell sorting (FACS) the method was utilized to evaluate the level of reactive oxygen species (ROS) production in cells induced by crocetin but not crocin. Nonetheless, nuclear factor erythroid-derived 2-like 2 (Nrf2) was activated in both crocin and crocetin-treated cells, while the effects of crocetin were at a higher level compared to crocin on Nrf2 activation. The expression of lactate dehydrogenase A (LDHA) protein in treated cells was decreased by both carotenoids (Kim et al. 2014).

The potency of crocin on melanoma tumor remission was investigated as well. Bakshi et al. investigated to examine the related parameters of tumor growth as well as tumor remission in a melanoma-transplanted *in vivo* model (C57BL/6 mice) treated with target agent at 2 mg/kg bodyweight for 21 days. They also assessed the profile of serum proteins in tumor-bearing mice dividing into crocin-treated (test) and normal saline-treated (control) groups. The results obtained from this study demonstrated that crocin is significantly able to alleviate tumor remission-related parameters including mean survival time, tumor growth delay, silent tumor period, and also decrease tumor volume doubling time. They also reported that crocin-treated ones exhibited almost normal serum protein profiles as compared to the control group (Bakshi et al. 2017).

In the next published research work of this scientific group, anti-metastatic, the potential of dietary crocin uptake was assessed through the development of a murine melanoma metastatic model. B16F10 cells were injected into the tail's vein of C57BL/6 mice. Then metastatic parameters and biomarkers, including inhibition of lung metastasis, mean survival time, lung hydroxypyroline, uronic acid as well as hexosamine levels were compared in crocin treated (at 250 and 500 μg/kg body weight) and control groups after a treatment period of 21 days. Data obtained here revealed that crocin has a remarkable potential to reduce melanoma cell invasion, migration, and adhesion, which is suggested to be attributed to the upregulated expression of E-cadherin in cells (Bakshi et al. 2018).

Evidence also demonstrates the anti-melanogenesis potential of glycosylated carotenoid of saffron. This effect of crocetin was studied by Hashemi and his colleagues on B16 murine melanoma cells. Spectrophotometry analysis was performed to evaluate the tyrosinase activity and melanin content, which significantly indicated that the agent was able to reduce the enzyme activity and the amount of melanin. Furthermore, a valid comparison of the expression levels of the targeted proteins, Microphthalmia-Associated Transcription Factor (MITF) and tyrosinase, in cells treated with crocetin and control group reported a decreased expression level after treatment. The data reported in this research work suggest that crocetin has a considerable effect on inhibiting melanogenesis in B16F10 cells (Hashemi-Shahri et al. 2018).

The antitumor potency of another saffron-derived bioactive component, picrocrocin, and its mechanism of action mediated by the inhibition of the JAK/ STAT5 signal transduction was confirmed in an *in vitro* study on SKMEL-2 cells, which was utilized in this study as a human melanoma cancer model. MTT method, along with ROS production assay and mitochondrial membrane potential (MMP), indicated antiproliferative the potential of picrocrocin, which was attributed to the down-regulation of the JAK/STAT5 protein expression (Yu et al. 2018).

In vitro cellular cytotoxicity and pro-apoptotic potential of crocin also was investigated on human skin cancer cells A431 and SCL-1. MTT the assay was done for cell viability evaluation, and data reported a negative correlation between the crocin concentrations and viability of cells, which confirmed the anti-proliferative impact of crocin in a dose-dependent manner. The expression of the apoptotic biomarkers, including procaspase-3 and Bid, was significantly increased in crocin treated cells

while anti-apoptotic proteins like Bcl-2 were reduced. The expression of Jak2 and Stat3 proteins was reduced, which was the account for the apoptotic potential of the crocin (Wang et al. 2018).

In more recent studies, new approaches to improve crocetin solubility have been developed. Studies reported that conjugation of the targeted agent with substituents, including diethylene, piperidyl, and benzylamine, could lead to a higher solubility of crocetin and, consequently, more appropriate pharmacological potentials of crocetin-derived novel compounds would be observed. For instance, anti-inflammatory capacity in macrophages, as well as antitumor bioactivity, was examined in several mammalian cell lines such as MCF-7 (human breast cancer) B16F10, SKOV3 and A549; as compared to limited potential of intact version, synthesized compounds of crocetin exerted extensively promoted potency (Chu et al. 2018).

This improved bioactivity effect also can be observed from the results obtained from the most recent study focusing on solubility-related strategies. Wang et al. showed that a chemical structure modification of crocetin through ethylamine and 4-fluorbenzylamine conjugation could exhibit a higher solubility, which consequently led to enhanced biological activities. Then, they examined the anti-proliferative effect of the synthesized compounds on different cancer cell lines such as human ovarian carcinoma, human lung cancer, and rat melanoma. Furthermore, data demonstrated that the solubility enhancement of the substituent could be account for a higher anti-inflammatory effect of crocetin on macrophages (Wang et al. 2020).

5.19 Tongue Squamous

The effects of crocin on cell proliferation and RNA and DNA content in human tongue squamous carcinoma cell line (Tca8113) were studied. In a concentration-dependent manner, crocin significantly increased cell cytotoxicity at 24, 48, 72, and 96 h after treatment ($P < 0.05$). Moreover, early and late apoptosis was significantly induced by a concentration of 0.4 mM of crocin. Also, the same concentration downregulated RNA and DNA content compared to control ($P < 0.01$) (Sun et al. 2011). In another publication, crocin and safranal were assessed in oral squamous cell carcinoma cell lines (KB cells). Crocin and safranal were used in a concentration range of 0.05–4 and 0.2–3.2 mM, respectively. The two phytochemicals caused a significant inhibition in cell proliferation. After 48 h of treatment, crocin had an IC50 value of 1.97 and 0.83 mM for safranal. Further results showed that the sub-G1 peak was induced, showing the role of apoptosis in cancer cell death. Moreover, cell cycle progression was not affected after treatment with crocin and safranal (Jabini et al. 2017). These discoveries state the substantial potential of main saffron compounds as therapeutic agents for human tongue squamous carcinoma.

6 Saffron Loaded-Nanoparticles for Cancer Therapy

Nanoparticles are a promising platform for saffron because of the unfavorable physicochemical properties of the main compounds of saffron, for instance, crocin main limitations are low absorption and bioavailability, rapid elimination, hydrolyzation by β-glucosidase, instability and losing functionality once these compounds are exposed to acids, higher temperatures, oxygen, and light (Asai et al. 2005; Tsimidou and Biliaderis 1997; Tsimidou and Tsatsaroni 1993). On the other hand, the main challenge of crocin delivery is low solubility due to its hydrophobic properties (Kanakis et al. 2007). Nanomedicine drug delivery systems can help overcome these challenges and improve the bioavailability and intestinal permeability of the loaded drugs (Bhia et al. 2021; Babadi et al. 2020; Santos et al. 2019; Siepmann et al. 2019).

Several nanoparticles have been used to encapsulate crocin, crocetin, and safranal, such as nanoliposomes, polymeric nanoparticles, nano-emulsions, dendrimers, and magnetite nanoparticles (Fig. 13.7) (El-Kharrag et al. 2017; Esfanjani et al. 2015; Faridi Esfanjani et al. 2017; Malaekeh-Nikouei et al. 2013; Mehrnia et al. 2016; Mousavi et al. 2011; Rahaiee et al. 2017; Rajabi et al. 2019; Soltani et al. 2017). The different delivery systems of saffron can help to achieve better stability and improve the overall quality of saffron products (Jafari et al. 2016, 2018; Mahdavee Khazaei et al. 2014; Rajabi et al. 2015). Additionally, the anticancer activity of saffron loaded-nanoparticles has been evaluated in different preclinical cancer cell lines and animal models, such as ovarian cancer, colon cancer, melanoma, and liver cancer (Puglia et al. 2019; Rastgoo et al. 2013). In a study, where

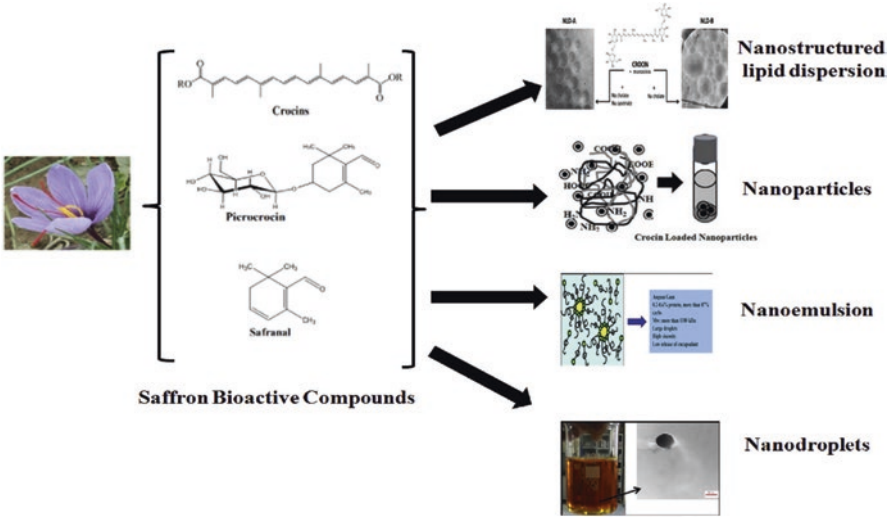

Fig. 13.7 Different methods for nano-encapsulation of saffron active phytochemicals. (Reproduced with permission from Garavand et al. 2019)

PLGA polymeric nanoparticles were used to encapsulate crocetin through emulsion/solvent evaporation technique. MTT assay in MCF-7 breast cancer cells revealed significant inhibition of proliferative activity. Where the IC50 of crocetin loaded-PLGA nanoparticles was significantly lower than pure crocetin with an IC50 of 84.73 ± 12.14 lM compared to an IC50 of 589.65 ± 5.72 lM for pure crocetin (Hafezi Ghahestani et al. 2017), these results clearly demonstrate the potential benefits of nanomedicine based drug delivery systems and what they can achieve to deliver the main saffron ingredients for improved therapeutic efficacy and overcoming the physicochemical barriers.

7 Saffron in Combination with Cancer Chemotherapeutics

Zhang et al. reported that crocetin had synergetic anticancer effects when combined with fluorouracil, where the growth of breast cancer cells was significantly inhibited. Also, the study suggests that in breast cancer cells treated with fluorouracil, autophagic cell death could be increased through combining crocetin and fluorouracil. Additionally, crocetin increased ATG1 levels and reduced Beclin-1 levels in breast cancer cells treated with fluorouracil (Zhang and Li 2017). Giakoumettis et al. conducted a research study to assess the anticancer effects of the extract of saffron (*Crocus sativus L.*) on C6 glioma rat cell cultures. Giakoumettis and colleagues evaluated cancer cell death mechanisms and synergistic effects caused by saffron extract alone or as augmentation with temozolomide. The obtained results revealed a decrease in cancer cell viability. Moreover, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) and flow cytometry tests showed that the apoptosis was not induced after treatment with the saffron extract. However, the augmentation of temozolomide with the extract led to a ten-fold increase in the ratio of dead/apoptotic cells. This study proposes a possible role of autophagy in cell death after finding a decrease in LC3-II protein levels after treatment with extract, where the tests showed a calpain-dependent programmed cellular death (Giakoumettis et al. 2019).

Amerizadeh et al. used a colitis-associated colon cancer mouse model to investigate the anticancer effects of augmentation of 5-fluorouracil and crocin. The study found anti-proliferative effects with the suppression of colon cancer cells invasive behavior after the modulation of E-cadherin and Wnt-pathway. Further assessments showed a significant decrease in tumor size and tumor number followed by the oral treatment with crocin alone or with 5-fluorouracil. Moreover, crocin managed to inhibit the inflammation of the colon through decreasing catalase and thiol levels and increasing malondialdehyde levels. Additional evaluations by crypt loss, inflammation score, histological assessments, and pathological changes confirmed the role of crocin in improving severe inflammation (Amerizadeh et al. 2018).

In a similar combination strategy, KYSE-150 esophageal squamous carcinoma cells were used to determine the anticancer potential and the synergistic effect of crocetin combined with cisplatin, with further evaluations of the involved cellular

pathways. The researchers found synergistic effects of cisplatin and crocetin combination that induced apoptosis and significantly inhibited cell proliferation. Further mechanistic tests showed that the expression of cleaved caspase-3 was upregulated and MMP was disrupted; however, Bcl-2 downregulation was observed in the augmentation of crocetin and cisplatin. Also, p21 and p53 had a higher expression when the augmentation of both agents was used (Li et al. 2017).

Another paper found that the combined use of crocin with cisplatin or pemetrexed can have additive effects after improving chemosensitivity in lung cancer cell lines. The results showed that treating lung cancer cells (A549 and SPC-A1) with crocin would induce apoptosis and anti-proliferative effect in a concentration-dependent manner. Additionally, crocin treatment led to an increase in G0/G1 arrest B-cell lymphoma 2-associated X protein (Bax) and p53, whereas Bcl-2 expression was reduced (Chen et al. 2015a, b).

In another study, crocin was implemented alongside with paclitaxel to induce synergetic anticancer effects on MCF-7 breast cancer cell line. The study showed that the combination therapy of crocin and paclitaxel had anticancer effects against breast cancer cells (Vali et al. 2015). In another paper, a combination of cisplatin and crocin was used to treat OS732 and MG63 osteosarcoma cells. The study revealed a significant decrease in cancer cell viability with combination treatment. The augmentation of cisplatin and crocin led to the suppression of osteosarcoma cells invasion and up-regulation of caspase-8 and cleaved-caspase-3 (Li et al. 2013). Another publication studied the effect of combining crocetin with vincristine on three types of cancers (non-small cell lung cancer cell line A549, cervical cancer cell line HeLa, and ovarian cancer cell line SKOV3). The study found that cytotoxicity of vincristine (1 μmol/L) was significantly improved after augmentation with crocetin (60 μmol/L), with an observed synergistic effect MCF-7/VCR vincristine-resistant breast cancer cell culture (Zhong et al. 2011).

8 Saffron Protective Effects Against Anticancer Drugs Toxicity

Treatment of cancers with approved drugs is usually associated with several devastating side effects. These adverse effects will lead to limiting the therapeutic potential of anticancer chemotherapeutic due to patient intolerance to the adverse effects the used dosage and treatment duration will be restricted. Thus, a new strategy has emerged to use saffron and its active compounds as protective agents against the toxicity of the anticancer chemotherapeutics. A wide range of studies had investigated these protective effects to ameliorate the damage caused by several anticancer drugs such as methotrexate, bleomycin, cisplatin, cyclophosphamide, and doxorubicin. These studies showed the protective effects of saffron in preventing and improving several drug-related toxicities such as renal toxicity, cardiotoxicity, pulmonary fibrosis, liver toxicity, ovarian toxicity, bladder toxicity, and genotoxicity (Table 13.1).

Table 13.1 A summary of studies investigating the role of saffron and its main active compounds in ameliorating anticancer drug-related toxicities

Chemotherapeutic agent	Toxicity	Saffron ingredient	Outcomes	Reference
Methotrexate	Renal toxicity	Crocin	Crocin was able to ameliorate damage in rats treated with methotrexate	Jalili et al. (2020)
Bleomycin	Pulmonary fibrosis	Crocin	Crocin exhibited antifibrotic activity	Mehrabani et al. (2020), Zaghloul et al. (2019)
Methotrexate	Liver injury	Crocin	Crocin showed hepatoprotective effects after increasing antioxidant defense and decreasing inflammation and oxidative stress	Kalantar et al. (2019)
Methotrexate	Liver toxicity	Saffron extract	The saffron extract reduced hepatotoxicity	Hoshyar et al. (2019)
Cisplatin	Nephrotoxicity	Safranal	Safranal could have nephron-protective potential against oxidative stress and cisplatin-induced toxicity in rat	Karafakioğlu et al. (2017)
Cyclophosphamide	Ovarian toxicity	Crocetin	Crocetin might have a promising potential in preserving fertility in patients treated with cyclophosphamide	Di Emidio et al. (2017)
Doxorubicin	Cardiotoxicity	Crocin	Crocin showed protective effects and improved the status of doxorubicin-induced heart damage. In vivo results revealed that crocin treatment led to a significant dose-dependent reduction of the toxicity, reestablishing normal cardiac architecture, attenuating ECG, ameliorating the biochemical parameters.	Razmaraii et al. (2016), Elsherbiny et al. (2016)
Cisplatin	Liver toxicity	Crocin	Serum alanine aminotransferase and aspartate aminotransferase levels indicated a significant improvement of the liver toxicity.	Sun et al. (2014)

(continued)

Table 13.1 (continued)

Chemotherapeutic agent	Toxicity	Saffron ingredient	Outcomes	Reference
Bleomycin	Scleroderma	Crocetin	In sclerotic mice, crocetin led to an improvement in skin and lung fibrosis after showing antiproliferative effects, inhibition of collagen production and differentiation, and decreasing ET-1 mRNA levels.	Song et al. (2013)
Cyclophosphamide	Liver toxicity	Crocin	Histological evaluations and biochemical markers showed hepatoprotective effects of crocin	Jnaneshwari et al. (2013)
Cisplatin	Renal toxicity	Crocin	Biochemical and histological analyses in rats propose crocin as a promising compound to ameliorate oxidative stress in cisplatin-induced kidney toxicity	Naghizadeh et al. (2008, 2010)
Cyclophosphamide	Bladder toxicity	Crocetin	Crocetin showed protective effects against cyclophosphamide-induced bladder toxicity without affecting the anticancer effects of cyclophosphamide	Nair et al. (1993)

In the case of cardiotoxicity, a number of published studies investigated the role of saffron extract to attenuate doxorubicin-induced cardiac toxicity (Chahine et al. 2013). One of these studies evaluated the cardioprotective effects of saffron extract in H9c2 cardiomyocytes. The study found that the extract inhibited the adverse effects of ischemia-reperfusion and doxorubicin treatment by reestablishing the expression of contractile proteins, activation of ERK1/2 and AKT/P70S6K, reducing caspase-3, inhibiting mitochondrial permeability transition pore, and reducing oxidative stress (Chahine et al. 2016). In another publication, isolated rabbit hearts were subjected to ischemia, followed by reperfusion and doxorubicin treatment. Saffron extract was administered afterward; the extract led to a decrease in oxidative myocardial damage. Western blot analysis showed that saffron treatment led to inhibition of the p38 MAPK pathway, preserved levels of cardiac troponin T proteins, and upregulation of the AKT/mTOR/4EBP1 pathway (Chahine et al. 2014).

Another application of saffron is to prevent cancer drugs induced-genotoxicity in preclinical studies. In fact, these studies are not new, for instance in a study published back in 2004, showed anti-genotoxic activity of saffron in combination with curcumin and garlic against cyclophosphamide (Premkumar et al. 2004). This study clearly aliens with another study that showed the protective activity of the saffron extract against genetic damage caused by three chemotherapeutics (cisplatin, cyclophosphamide, and mitomycin) (Premkumar et al. 2006).

Despite these results and efforts in preclinical studies, the clinical translation of the usage of saffron and its active ingredients as protective agents against anticancer drugs induced toxicity requires more studies in randomized controlled clinical trials settings.

9 Conclusions and Future Directions

Many herbal products have been known to have antitumorigenic properties. Preclinical data have suggested that carotenoids have chemopreventive effects. Intake of saffron and its extracts is effective against the prevention of several types of cancer. It has various effects on cancer cells, such as anti-inflammatory, apoptotic, and antioxidant. Crocin, a biologically active component of saffron, is one of the most significant carotenoid in this respect. A number of clinical trials are required to obtain human-based data regarding the effectiveness of saffron and its components against cancer. Scarcity and high price of saffron and its main bioactive components can cause a barrier to these investigations.

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Chapter 14
Olive Leaf (Oleuropein) and Its Role in Cancer: Therapeutic Updates

Bushra Ansari, Esra Küpeli Akkol, Haroon Khan, and Muhammad Ajmal Shah

Abstract *Oleaeuropea* L. commonly known as olive tree has immense health benefits. Traditionally it is used to treat different pathologies such as olive leaves used against coughing, cystitis and sore throat, cardiovascular diseases, mouth cleanser, dried leaves and fruits of olive tree in gastrointestinal problems (diarrhea) and urinary tract infections, hypertension, asthma, and most importantly cancer. The phytochemical analysis revealed rich phytochemical composition of the plant and led to the isolation of more than hundred different compounds, such as flavonoids, biophenols, and terpenoids such as iridoids, secoiridoids, triterpenoids, coumarin.

Similarly, olive leaf possessed a strong bioactive composition with a high concentration of oleuropein (glycosylated secoiridoid). Similarly, olive leaf possessed a strong bioactive composition with a high concentration of oleuropein (glycosylated secoiridoid). The leaf extracts and its derivative compounds have shown potent anticancer effects against different cell lines in different organs. These compounds include oleuropein, maslinic acid, erythrodiol, uvaol, oleanolic acid, hydroxytyrosol, tyrosol. Among others Oleuropein and its derivative compounds (hydroxytyrosol, tyrosol and others) were studied in a several types of cancer cells. In fact, many studies have demonstrated the oleuropein and its derivative have proved pharmacological activity against proliferation of cancer cells and several tumor cell lines by different mechanisms such arrest cell cycle and cause apoptosis in cancerous cells, by modulation of miRNA expression and upregulation and downregulation of several genes. This chapter deals with the anticancer effect exert by *O. europaea* leaf constituents and its possible anticancer mechanism.

Keywords Olive leaf · Anticancer activity · *Oleaeuropea*

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1 Introduction

Over thousands of years people rely on different plants and herbs for their therapeutic need based on their belief, experience and observation which further give rise to modern medicines. Most countries relied on these traditional plants for their health considered as integral part of the health care system. The knowledge of these plants transferred from generation to generation, and this knowledge support the discovery of modern medicines. *Oleaeuropaea* L. (Olive tree) is one of the oldest traditional medicinal plants native to tropical and warm temperate regions of the world (Özcan and Matthäus 2017). Large epidemiological studies demonstrate the reduced incidence of cancer and cardiac diseases in Mediterranean region which suggest the high intake of Mediterranean diet. Olive oil is one of the basic ingredient of the Mediterranean diet and many studies reported the healthy and unique characteristics of olives and olive oil (Wahle et al. 2004). Olive oil as important ingredient of diet contains high amount of monounsaturated fatty acids (MUFA) and other compounds in minor amount. MUFA are related with the preventive and curative effect of Mediterranean diet in different disorders/diseases. In MUFA oleic acid is also important fatty acid and consumed in western countries as a diet such as pork and poultry (Ferrari et al. 2002). The olive tree is considered traditionally as a sign of splendor and peace. Historically the leafy branches of olive tree were used to crown the triumphant in friendly games and wars.

Olive plant has raised its importance because of its high medicinal and potential values. Different parts of olive tree is considered having rich nutritional values and high medicinal importance (El and Karakaya 2009). Olive tree is commonly distributed in the eastern Mediterranean basin, southern Europe, northern Iran, Arabian Peninsula, southern Caspian sea, India, northern Africa, and western Asia (Özcan and Matthäus 2017). Mediterranean countries representing 65% of world surface area which is cultivated in olives, in total 74% olive harvested and 76% tree production by Mediterranean countries (Molina-Alcaide and Yáñez-Ruiz 2008). Commercially Mediterranean region is an important source for the production of olive oil in the world. Seventy-five percent of the world's olive oil is produced by the European Union in which Italy (31%), Spain (45%), and Greece (22%) are the main producers (Molina-Alcaide and Yáñez-Ruiz 2008). Olive has extremely bitter taste so was never used as natural fruit rather consumed as olive oil or table olives (Hashmi et al. 2015). Oil industry approximately used 90% of annually produced olives for oil processing. Industrialization of olive oil and table olives is very much important in countries like Italy, Spain, Palestine and others from Mediterranean area. The largest producer of olives is Spain followed by Greece and Italy (El and Karakaya 2009).Olive oil is considered as the main component extracted from olive tree, which is approximately produced globally around11 million tons per year (Vogel et al. 2015). Olive oil contains wide amount of phenolic compounds present in olive tree (Table 14.3), however olive leaves are also considered important source

of phenolic compounds such as the oleuropein, hydroxytyrosol, verbascoside, apigenin-7-glucoside and luteolin-7-glucoside (Benavente-Garcıa et al. 2000). Large number of residues and by-products are obtained from *O. europaea* tree cultivation and processing olives industry yearly, but mostly of them is not reused. The word olive leaf is the broadly refer to the leaves with branches of olive tree. The olive leaf earliest use comes from ancient Egyptian civilization. In their culture olive leaf was considered as a symbol of power of heaven. In Egypt olive oil was used for many purposes and olive leaves were used as a part of mummification rituals of their king. Numerous other cultures also used olive leaf for different medicinal and nutritional purposes specially Mediterranean cultures (Ritchason 1999). Extract of olive leaf is of special interest of research due to the presence of large amounts of phenolic compound and its proven therapeutic utilities (Table 14.2).

Olive tree (*O. europea* L.) is a small tree of Oleaceae family. Oleaceae family, a family of dicotyledons, has 30 genera [1, 2] and it includes 600 species of trees and shrubs [3]. Oleaceae is divided into numerous tribes, including, Fontanesieae, Oleeae, Jasmineae, Myxopyreae, and Forsythieae. Genus olea is derived from Greek word elaia and latin word oleum. Olea contains 30 different species but the most popular and commonly used is *O. europaea,* the only genus used as food in the Mediterranean region (El and Karakaya 2009)**.** Olive tree is a Mediterranean climatic evergreen woody tree, which grows natively in arid and rustic condition. Olive, small fruit of *O. europea*, is the source of olive oil, which is a key component of Mediterranean diet and source of and associated with numerous health benefits (Visioli et al. 2020). Trunk, branches, leaves, fruits, and roots of olive tree are very well adapted to drought Mediterranean native conditions because of xerophytic structural features, help reducing water loss and diagnostic characteristics of Oleaceae family are mostly related to its leaf and flower structure (Rapoport et al. 2016). An adult olive tree produces about 500,000 flowers a year. Flowers of olive tree are grouped in inflorescences known as panicles. It has small flowers with four yellowish white petal, four green fused sepals, two carpels and two stamens, with thick and short style and large stigma (Seifi et al. 2015). Olive tree has simple, elliptical to lanceolate leaves with smooth margins. The upper surface of the leaf is dark green in colour and looks glossy which is covered with a thick cuticle. Olive leaf is described as xeromorphic having two palisade parenchymal zones, palisade parenchyma zone I and palisade parenchyma II (Moreno-Alías et al. 2009).

Olive fruit is oval shape drupe having pulp with a size of 2–3 cm width and length. Olive fruit is mainly composed of three parts (1) skin or epicarp or exocarp; (2) pulp or mesocarp; (3) stone or endocarp. Olive oil extracted from epicarp of olivesis a main source of dietary fat. Skin of olive is enclosed in wax; from the growth period the color changes from lightish green to purple and brownish or blackish. Eighty-four to 90% of total fruit weight is mesocarp which is soft, pulpy flesh part. Mesocarp is the edible part of fruit and oil accumulation occur in mesocarp. The endocarp, containing seed or kernel, accounts for 13–30% of fruit mass (Blekas et al. 2002) (Table 14.1).

Table 14.1 Taxonomical classification of *O. europaea* L.

Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Lamiales
Family	Oleaceae
Genus	Olea
Species	*Olea europaea* L.

2 Traditional Uses of Olive

Olive tree a traditional plant of Mediterranean region, considered as a plant of peace and glory is important for the dietetic and economic benefits for the people of that region. Olive tree has been used traditionally as a remedy for different health problems in Mediterranean region countries like Israel, Tunisia, Turkey, Spain, Italy and France. Olive tree is a wild plant of Mediterranean island but also cultivated in countries like Asia, Indian subcontinent and Arabian Peninsula. Olive leaf extract as a herbal tea is the most common herbal treatment for different diseases (El and Karakaya 2009), including hypertension (Amel 2013).Olives, small fruits, are used to produce olive oil which is a key component of Mediterranean diet and related with numerous health benefits (Visioli et al. 2020)**.** Olive leaves and fruits have been used to reduce hypertension and hyperglycemia (Amel 2013), Mixture of olive oil and lemon juice are traditionally considered as good therapy for gall stones (Hashmi et al. 2015), decoction of olive leaves and fruits are used to treat diarrhea, urinary tract and respiratory infections (Bellakhdar et al. 1991). Freshly boiled Olive leaves extract are taken orally for treating asthma, massage of olive oil on fractured limbs is considered as best therapy. Olive leaf is potential anti-inflammatory agent and used to treat gout (Flemmig et al. 2011) and olive fruit is used as a skin cleanser (Fujita et al. 1995) Olive leaf has been reported to have cytotoxic activity against breast cancerous cells (Fu et al. 2010) and it has been used as vasodilator (Zarzuelo et al. 1991) (Table 14.2).

3 Pharmacological Study of Olive Plant

O. europaea (olive) is an essential plant of Mediteranean diet. *O. europea* has been broadly studied and a variety of pharmacological activities have been reported. *O. europaea* contains numerous phytochemical classes of compounds aiding to cure various pathologies. Olive oil is obtained by compressing, or other physical means, fruits of *O. europaea* (Bonvino et al. 2018) (Fig. 14.1). Extra virgin olive oil, one of the most beneficial food ingredients of Mediterranean diet, was associated with reduced risk of the progression of cardiovascular diseases (Nocella et al. 2018) .

Table 14.2 Traditional/folklore uses of *O. europaea* L.

Part used	Activity	Reference
Leaves and fruits (infusions and macerations)	Antidiabetic, antihypertensive	Amel (2013)
Fruits and leaves decoction	Hypoglycemic	Ali-Shtayeh et al. (2012)
Dried leaves/fruit decoctions or oral use	Diarrhea, respiratory tract infection, and urinary tract infections	Bellakhdar et al. (1991)
Fresh leaves taken orally or boiled leaves extract	To treat asthma	Lawrendiadis (1961)
Boiled leaves extract	Antihypertensive	Ribeiro et al. (1988)
Olive oil + lemon juice	Gallstones treatment	Patwardhan et al. (2005)
Infusions of leaves	Eye infections treatment	Guerin and Reveillere (1984)
Olive fruit	Skin cleanser	Fujita et al. (1995)
Olive leaf	Vasodilator	Zarzuelo et al. (1991)

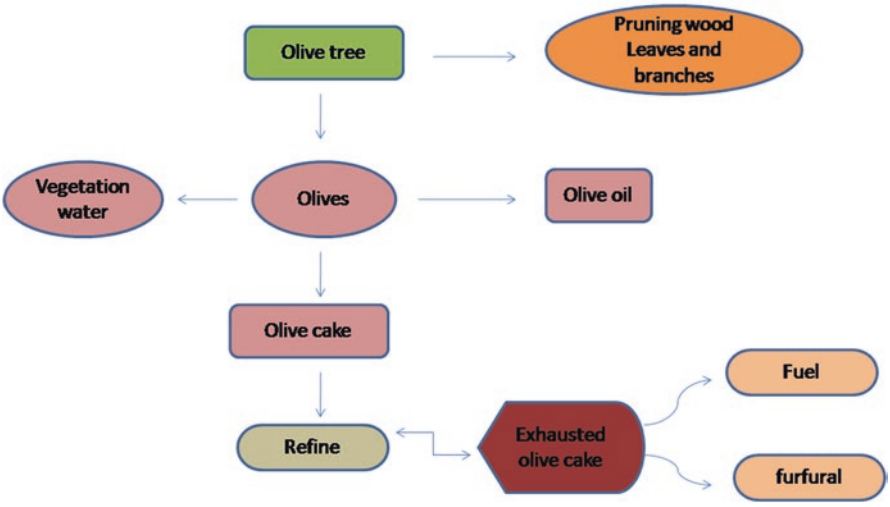


Fig. 14.1 By-products of olive tree and obtained from the processing of olives

Presence of large amount of MUFA like oleic acid and other minor components such as aliphatic and triterpenic alcohols, hydrocarbons (squalene), tocopherols, sitosterol, volatile compounds and pigments (carotenoids, chlorophylls), among others, contribute to nutritional and pharmacological effects of olive oil (Juan et al. 2008).

4 Pharmacological Effects of Olive Leaf

The medicinal properties of olive tree is referenced several times in Bible (Wren 1994) and olive has also been praised as a blessed tree and fruit in the Holy Quran (Quran, Chap. 24 Al-Noor, Verse 35). According to the reported literature olive leaf extract showed high potential for preventing or healing various diseases/disorders.

4.1 Hypoglycemic Effect

Olive tree leaves have been used for many years in Europe as a traditional remedy due to its antidiabetic effect (Gonzalez et al. 1992). Gonzalez et al., in 1992, studied the hypoglycemic effect of olive leaf extract and evaluated maximum significant effect of olive leaf and stated oleuropeside as a component responsible for this effect (Gonzalez et al. 1992). In another study Polyphenolic components from olive leaf have been identified as therapeutic ingredients accountable to delay the progression of glycation end products that are involved in inflammatory diseases like diabetes (Chandler et al. 2010). Oleuropein and tannins from olive leaf are demonstrated as a glucosidase inhibitors, decrease the carbohydrates absorption in GIT (Jemai et al. 2009). Further different research studies (Al-Azzawie and Alhamdi in 2006 (Al-Azzawie and Alhamdani 2006), Boaz et al., in 2011 (Boaz et al. 2011), Abu-zaitonan and Abu-albasal in 2012 (Abu-zaiton and Abu-Albasal 2012)) were carried out to demonstrate the hypoglycemic potential of olive tree.

4.2 Hypertensive Effect

The olive leaves have been used traditionally to treat high blood pressure and other cardiac diseases. Zarzuelo et al., in 1991, evaluated the antihypertensive effect of olive leaf decoction and stated that olive leaf effectively causes vasodilation and hypotensive effect on isolated aorta (Zarzuelo et al. 1991). Some other studies demonstrated hypotensive effect of oleuropein and reported that Oleuropein increased NO concentration which further enhances macrophages response to bacterial lipopolysaccharide and NO also exhibits vasorelaxant effect (Visioli et al. 1998). In another study the effect of olive leaf in blood pressure in L-NAME Induced Hypertension in Rats was evaluated and it was found a beneficial effect of olive extract on hypertension (Khayyal et al. 2002).

4.3 Cardiovascular Effect

Mediterranean diet is considered to reduce risk of cardiovascular diseases and indeed US food and Drug Administration permitted to take 2 tablespoon of olive oil which is approximately equal to 23 g (López-Miranda et al. 2010). In one study (Omar 2010) oleuropein, the major polyphenol from olive leaf, was suggested to have significant cardioprotective effect against cardiotoxicity by adriamycin and antiischemia effect. In this line, Poudyal et al. (2010) investigated olive leaf extract for its cardiovascular effect. They strongly suggested that olive leaf polyphenols have inhibitory effect on inflammation and oxidative stress that are responsible to initiate cardiovascular, metabolic and hepatic symptoms in rat model of diet induced obesity and diabetes.

4.4 Antioxidant Effect

Reactive oxygen species such as hydrogen peroxide, hydroxyl radical and superoxide anion causes oxidative stress which is damaging for proteins, nucleic acids, and cell membranes and also exposure to external oxidants may cause increased risk of cardiovascular diseases, diabetes, cancer or many other diseases, so antioxidant from natural sources in the form of drug or diet may reduce this risk (Dimitrios 2006). The antioxidant effect of oleuropein from olive leaf extract was evaluated by Visioli et al. (2002) and demonstrated powerful antioxidant effect.

4.5 Anti-inflammatory Effect

Oleuropein and hydroxytyrosol has been suggested to have marked anti-inflammatory response in preclinical experiments. Olive leaf has been investigated for its anti-inflammatory effect in *in vitro* and *in vivo* animal models and demonstrated significant anti-inflammatory activity by inhibiting tumour necrosis factor-α (TNFα), 12-O-tetradecanoylphorbol-13-acetate induced ear oedema, arachidonic acid-induced ear oedema and inhibiting carrageenan-induced rat paw oedema (Haloui et al. 2011; Qabaha et al. 2018).

4.6 Antihyperipidemic Effect

Atherosclerosis is an inflammatory disorder characterized by vascular wall thickness and infiltration of lymphocytes and macrophages. Wang et al. (2008) find out in their study the beneficial effect of olive leaf due to its antiatherosclerosis effect

by suppressing several inflammatory mediators. In another study oleuropein was claimed to reduce the total cholesterol and triglycerides level and lead to cardioprotective effect (Andreadou et al. 2006). Another report evaluated antihyperlipidemic effect of olive leaf and stated that administration of olive leaf extract effectively reduced the low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) and total cholesterol (TC) and increased level of high-density lipoprotein cholesterol (HDL-C) (Jemai et al. 2008).

4.7 Antiviral Effect

Micol et al. investigated olive leaf extract and its major bioactive compound such as oleuropein, and reported that olive leaf extract showed antiviral effect against both DNA and RNA virus and showed that extract of olive leaf effectively reduced the infectivity against salmonid rhabdovirus (Fredrickson 2000; Micol et al. 2005) (Fig. 14.2).

5 Phytochemical Profile of Olive Tree

Many epidemiological studies have manifest marked decrease in the pathological conditions related to cardiovascular system, nervous system, cancer and diabetes in Mediterranean population (López-Biedma et al. 2016). This favorable outcome is because of the consumption of large amount of Mediterranean diet which includes olive oil, table olives and olive processing wastewaters (Kok and Kromhout 2004). Olive oil by-products are harmful to environment but they are also good source of bioactive compounds (Fig. 14.1).Olive leaf extract is composed of large amounts and a wide variety of polyphenols (Table 14.5) with various Structural differences which may have important altering effect on health and improve outcomes (Hamdi and Castellon 2005). Over 8000 polyphenols have been recognized with structures ranging from simple monomeric units to complex oligomers and polymers (Ignat et al. 2011).

Phytochemical studies of olive products revealed the presence of large amounts of components in the different parts of olive tree (Table 14.3). Several phenolic compounds were found in olives including caffeic acid, luteolin, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, quercetin, and chryseriol, tyrosol, hydroxytyrosol, rutin and quercetin (Dekanski et al. 2009; Maalej et al. 2017; Essafi et al. 2019). Among these Hydroxytyrosol, tyrosol and oleuropein are therapeutically very important compounds of olive product. Hydroxytyrosol and tyrosol are very much related structurally, the only difference is that hydroxytyrosol has extra OH group present on *meta* position. Oleuropein is chemically an ester and consist of elenolic and hydroxytyrosol compounds, and it is the major compound present in olive leaf and olives while olive oil contains hydroxytyrosol as main phenolic compound (Hu et al. 2014).

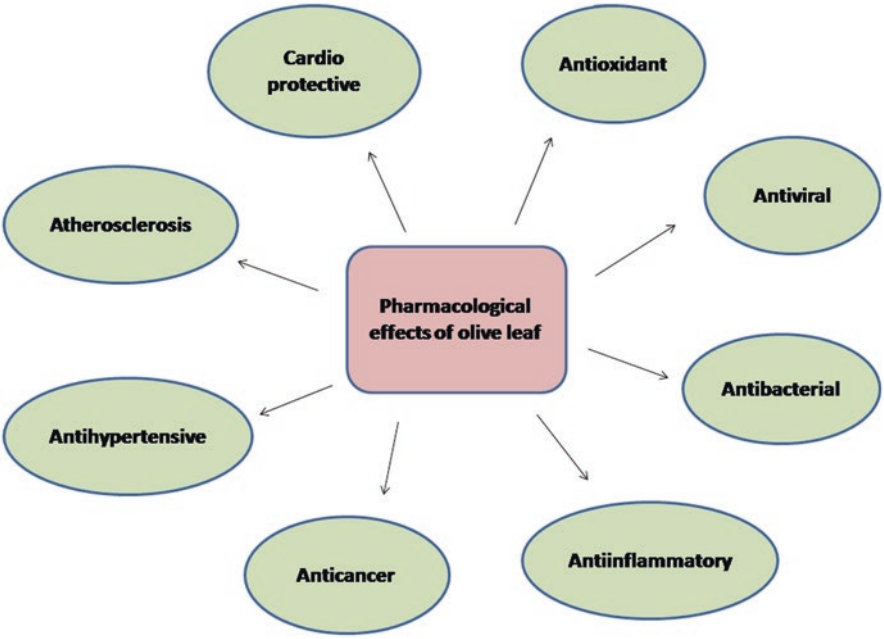


Fig. 14.2 Pharmacological effects of olive leaf

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of olive tree fruits (olives) identified oleic acid (30.00%), palmitic acid (11.65%), Stearic acid (5.30%), octadecadienoic acid (5.98%), palmitoleic acid (1.66%), Linoleic acid (6.50%) and tridecanoic acid (1.38%) (Ahmad et al. 2017). Another Phytochemical study revealed that ethanol, aqueous, ethyl acetate extracts of olive consist of saponins, alkaloids, flavonoids, steroids, phenols and tannins and glycosides except for ethyl acetate extract of olive leaf which does not contains glycosides while ethanol extract of the plant was found to have highest contents of alkaloids, flavonoids and phenols (Khan et al. 2019).

5.1 Olive Leaf Polyphenols

Olive tree prominently grows in Mediterranean region, and adapted to the characteristics of the region. To combat different drastic conditions such as prolong exposure of sunlight, large amount of pathogen and insect attack, olive plant has thick leaves that store considerable amounts of polyphenols (Erbay and Icier 2010) (Table 14.4). There are many factors such as geographical location, cultivar of olive tree and age of tree that influence the concentration and variety of polyphenols present in olive leaf (Elamin et al. 2013) (Table 14.5). Polyphenol consist of many phenolic groups as the name indicates, each phenol consist of aromatic ring with hydroxyl groups

Table 14.3 Phytochemical components isolated from different parts of olive plant

Part used	Constituents	References
Olive leaves	Oleuropein (232.0 mg) and other secoiridoids such as secologanoside, oleoside, 6′-*E*-*p*-coumaroyl-secologanoside, 6′-*O*-[(2*E*)-2,6-dimethyl-8-hydroxy-2-octenoyloxy]-secologanoside	Karioti et al. (2006)
	Oleoside	Karioti et al. (2006), Peralbo-Molina et al. (2012)
	Hydroxytyrosol-elenolate, elenolic acid methyl ester and ligstroside	Gariboldi et al. (1986)
	Secoiridoid glycosides oleuricine A and oleuricine B	Wang et al. (2009)
	Oleuroside and 3,4-DHPEA-EDA (oleacein)	Savournin et al. (2001), Savarese et al. (2007)
	Triterpenoids like *β*-amyrin, *β*-sitosterol, erythrodiol	Mussini et al. (1975)
	Oleanolic acid	Movsumov and Aliev (1985), Romero et al. (2010)
	Triterpene acids such as betulinic acid, uvaol, ursolic acid, and maslinic acid	Bianchi et al. (1992)
	Flavonoids like Apigenin-7-*O*-rutinoside, rutin, and luteolin-7-*O*-glucoside were isolated from the leaves of *O. europaea*	Meirinhos et al. (2005)
	Flavone glycosides, that is, luteolin-7,4′-*O*-diglucoside,diosmetin,and apigenin-7-*O*-glucoside	Savournin et al. (2001), Meirinhos et al. (2005)
	Lignan, 4′-*O*-*β*-D-glucosyl-9-*O*-(6″-deoxysaccharosyl) olivil	Schumacher et al. (2002)
	1,5-anhydroxylito	Campeol et al. (2004)
	3,4-dihydroxyphenylethanol-elenolic acid dialdehyde (3,4-DHPEA-EDA) and hydroxytyrosol-elenolate	Paiva-Martins and Gordon (2001)
	Hydrocarbons, esters, waxes, triglycerides, tocopherols, esterols, lineal, terpenic, alcohols, and terpenici alcohols have also been reported from the hexane extract of *O. europea* leaves.	Guinda et al. (2002)
Olive tree fruit and seed	Flavonoids, secoiridoids, secoiridoid glycosides	Bianco et al. (1993)
	Phenolics such as tyrosol, hydroxytyrosol	Owen et al. (2003)
	Oleuropein, demethyloleuropein and verbascoside, all parts of the fruit whereas nuzhenide etc.	Lo Scalzo and Scarpati (1993), Servili et al. (1999)

Olive tree bark	Lignans like (−)-olivil, (+)-cycloolivil, (+)-1-acetoxypinoresinol, (+)-1 hydroxypinoresinol, (+)-1-acetoxypinoresinol-4″-*O*-methyl ether, and (+)-1-hydroxypinoresinol-4″-*O*-methyl ether.	Pérez-Bonilla et al. (2006)
Olive wood	Ligstroside	Servili et al. (1999)
	Oleuropein-3″-methyl ether (**7**), 7″-*S*-hydroxyoleuropein, Oleuropein-3′-*O*-*β*-D-glucopyranoside, ligstroside-3′-*O*-*β*-D-glucopyranoside, jaspolyoside, jaspolyanoside, and isojaspolyoside A etc.	Pérez-Bonilla et al. (2011)
Olive oil	Hydroxytyrosol, hydroxytyrosol acetate	Perez-Trujillo et al. (2010)
	β-hydroxyacetamide, suspensaside, hellicoside, orbanchoside, acetoside, and wedelosin etc.	Rodríguez et al. (2009)

Table 14.5 Possible anticancer mechanisms of oleuropein and its derivatives

Cancer type	Anticancer mechanism	Reference
Breast cancer	Increased expression levels of miR-125b, miR-16, miR-34a, p53, p21, and TNFRS10B Decreased expression of bcl-2, mcl1, miR-221, miR-29a and miR-21	Asgharzade et al. (2020)
Leukemia	Arresting the cell cycle and inducing apoptosis in tumour cells	Fabiani et al. (2002)
Glioblastoma	Modulation of miRNA expression	Tunca et al. (2012)
Neoblastoma	Cycle arrest by down-regulating of CylinD1, CylinD2, CyclinD3,CDK4, CDK6 Up-regulating of p53 and CDKN2A,CDKN2B, CDKN1A gene expressions Induces apoptosis by inhibiting of Bcl-2 and activating of Bax, caspase-9 and caspase-3 gene expressions	Seçme et al. (2016)
Colorectal cancer	Decrease in HIF-1α protein and an upregulation of p53 protein expression Upregulation in peroxisome proliferator Activation of receptor gamma (PPARγ) expression	Cárdeno et al. (2013)
Cervical cancer	Increased in ATF-2, c-Jun NH2-terminal kinase (JNK) protein, p53, p21, Bax, and cytochrome *c* protein Cytochrome *c* and activation of caspase-9 and -3. SP600125 (JNK1/2 inhibitor) suppressed the formation of apoptotic bodies and JNK activation	Yao et al. (2014)

Table 14.4 Simple phenols, acids and other related compounds from olive leaf

SIMPLE PHENOLS, ACIDS AND OTHER RELATED COMPOUND
Simple phenols and acids
Hydroxytyrosol and glucosides, Tyrosol and glucosides, Benzoic acid (gallic, vanillic, syringic, salicylic, hydroxybenzoic, protocatechuic acids, vanillin), Cinnamic acids (cinnamic, caffeic, coumaric, ferulic, chlorogenic acids), Homovanillic acid
Other related compound Elenolic acid and derivatives, Verbascoside
SECOIRIDOIDS PRESENT IN OLIVE LEAVES
Demethyloleuropein, 3,4-DHPEA-EDA (3,4-dihydroxyphenylethyl 4-formyl-3-formylmethyl-4--hexenoate), Oleoside, Oleuropein, Oleuroside, Ligstroside, Ligstroside aglycone
FLAVONOIDS PRESENT IN OLIVE LEAVES
Apigenin Apigenin 7-O-glucoside, Apigenin 4-O-rutinoside, Apigenin 7-O-rutinoside **Hesperidin** Luteolin, Luteolin 4′-O-glucoside, Luteoline-7-O-glycoside, Luteoline-7-O-rutinoside **Quercetin** Quercitrin, Rutin

(Lockyer et al. 2012). Polyphenol are conjugates with one or more sugar moiety attached with hydroxyl group (Pandey and Rizvi 2009). The polyphenols are classified through the structure and numbers of phenol rings and this will determine its

bioactive properties. Major predominant phenolic compounds present in olive leaf are secoiridoids and flavonoids and have shown the ability to regulate metabolic and inflammatory biomarkers in both human and animal (Lockyer et al. 2017).

5.1.1 Oleuropein

Oleuropein belongs to coumarins like group, known as secoiridoids, abundantly found in oleaceae, gentianales, cornales and other plants. Oleuropein is an ester of 2′-(3′,4′-dihydroxyphenyl) ethanol (hydroxytyrosol). Iridoids and secoiridoids are those compounds which are formed from the secondary metabolism of terpenes, as precursor compounds of different indole alkaloids. Secoiridoids found in Oleaceae family is usually derived from oleoside (glucosides) which is exocyclic 8,9-olefinic functional group, combination of glucosidic residue and elenolic acid (Soler-Rivas et al. 2000). Secoiridoids make up mostly olive polyphenols (85% olive leaf polyphenols) (Bendini et al. 2007). In olive leaf extract oleuropein is the most abundantly found polyphenol and its derivatives such as the aglycones, oleoside and ligstroside are also present in different concentrations (Lockyer et al. 2012). Oleuropein is also found in other genus of *Oleaceae* family such as *Fraxinus excelsior*, *Syringa vulgaris*, *Phillyrea latifolia*, *Ligustrum ovalifolium* (Damtoft et al. 1993). Oleuropein has been intensively studied for its pharmacological effects on human health. Oleuropein suppressed pro-inflammatory mediators, and pro-inflammatory cytokines and thus inhibit inflammatory response (Park et al. 2017), Oleuropein reduced the development of heart failure (Janahmadi et al. 2017) and presented antimicrobial (Bisignano et al. 1999), antimycoplasmal (Furneri et al. 2002), anticancer (Seçme et al. 2016) and hepatoprotective effect (Kim et al. 2010).

5.1.2 Hydroxytyrosol (HT)

Oleuropein hydrolysis produces oleuropein aglycone, elenolic acid, HT, and glucose residue (Granados-Principal et al. 2010). Hydroxytyrosol is the second most abundant phenolic compound of olive leaf (Alipieva et al. 2014). Hydroxytyrosol is a polar phenolic compound present in olives, olive leaf and other parts of olive tree (gSeçme et al. 2016). Hydroxytyrosol is an amphipathic phenolic molecule having 154.16 g/mol molecular weight and chemical name of HT is 4-dihydroxyphenylethanol (DOPET) or 3,4-dihydroxyphenolethanol (3,4-DHPEA) or 4-(2-Hydroxyethyl)-1,2-benzenediol given by IUPAC system. HT is considered as minor soluble compound of extra virgin olive oil but abundantly present in leaves of olive tree (Robles-Almazan et al. 2018). HT is originated from the hydrolysis of oleuropein during the ripening season of olives, and also during storage and refining of table olives (Charoenprasert and Mitchell 2012).When the olive are crushed to obtain their oil, three layers of enriched polyphenols are obtained, olive oil, olive mill wastewater and pomace. As HT is amphipathic in nature, it is present in all the three layers in free form, or as acetate form or as a part of complex

compound such as oleuropein, oleacein, and verbascoside (Boskou 2008). The composition of HT in olive oil or extract depends greatly on the type of olive tree, its location of the cultivation, olive oil extraction method and the olive quality (Romero et al. 2004). As HT is present in large amounts in olive leaves in free form and it is also involved in the complex structure of different elements (Fabiani et al. 2006). HT and related polyphenols are commonly extracted by methanol, ethanol or both and stimulate enzymatic activity of galactosides to produce HT from oleuropein (Briante et al. 2002). HT has been reported for its pharmacological activities especially in cancer. HT from olive leaves extract inhibit cell cycle progression in MCF-7 breast cancer cells (Bouallagui et al. 2011) and it has strong antioxidant effect (Yvonne et al. 2004), anti-inflammatory effect (Richard et al. 2011), among others.

5.1.3 Hdroxytyrosol Derivatives

HT in the form of acetate has higher antioxidant effect then HT while nitro-ester derivatives of HT have shown significant effect in Parkinson's disease characterize by their antioxidant capacity (Trujillo et al. 2014). Lipophilic nature of alkyl ether derivative of HT has high anticancer activity against A549 and MRC5 lung cancer cells (Calderón-Montaño et al. 2013). Other derivatives of HT are HT containing thioacetate, thiol and disulphide (Robles-Almazan et al. 2018)

5.2 In Vivo *Absorption, Metabolization and Excretion of Oleuropein and Its Derivatives*

The absorption studies of polyphenols present in olive oil or leaf extract showed that only hydroxytyrosol has better absorption while all other phenolics compounds metabolize in small intestine and large intestine. Oleuropein is not absorbed in small intestine and rapidly reaches large intestine where oleuropein is rapidly degraded i to hydroxytyrosol metabolite (Fig. 14.3).

Hydroxytyrosol mainly absorbed in small intestine and colon by passive transport. Its absorption majorly depends on the type of vehicle employed, such as in the form of olive oil, hydroxytyrosol is effectively absorbed (Tuck et al. 2001). Further studies demonstrated that hydroxytyrosol when administered in the form of red wine has highest level in urine, this may be due to dopaminergic pathway interaction with ethanol. Hydroxytyrosol is a dopamine metabolite and its higher level in urine may be due to increase metabolism of dopamine (de la Torre et al. 2006) (Fig. 14.4). HT is rapidly metabolized firstly in enterocytes and then in the liver. Gut microflora is an important modulator of absorption of HT and its metabolites. Microflora plays a transforming part of HT, HT acetate and tyrosol as it causes release of HT from its complex forms. HT metabolism by microflora firstly occur by oxidation and then is followed by transformation of HT into hydroxylated

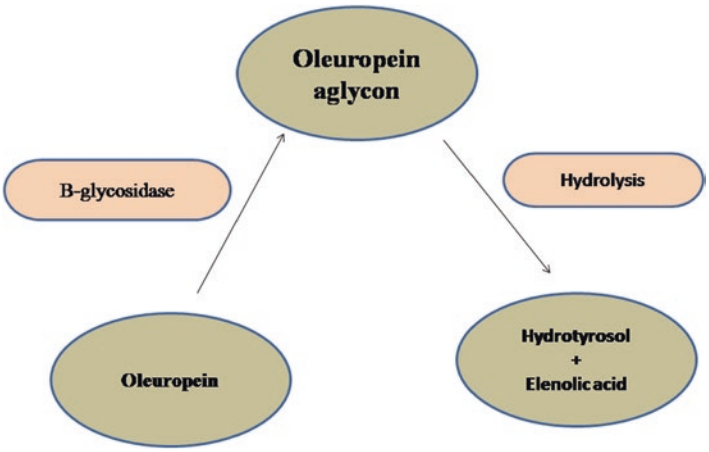


Fig. 14.3 External metabolism of oleuropein into hydroxytyrosol during ripening of fruit

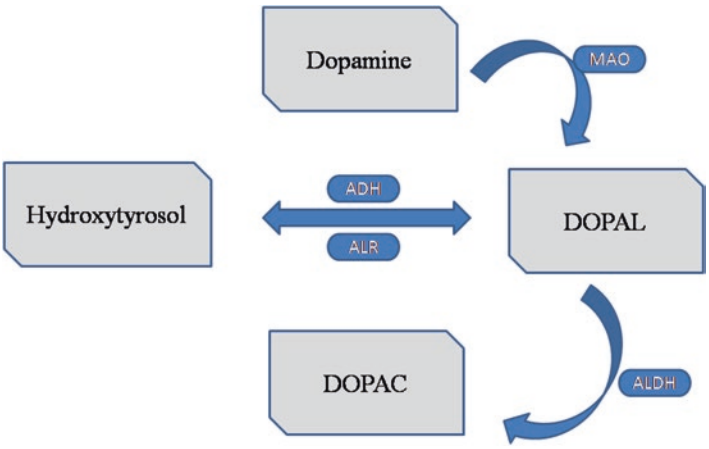
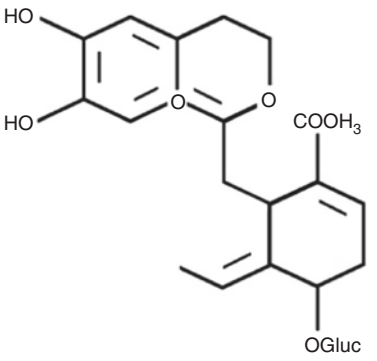


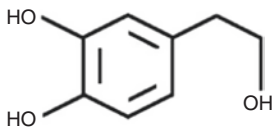
Fig. 14.4 Internal metabolism of hydroxytyrosol: DOPET is hydroxytyrosol; DOPAL is 3,4-dihy droxy-phenylacetaldeyde; DOPAC is 3,4-dihydroxy-phenylacetic acid, *MAO* monoamine oxidase, *ALDH* aldehyde dehydrogenase

phenylacetic acids (Mosele et al. 2014). HT is present in very low concentration in plasma, however mostly it is found in orthomethyl products of HT (homovanillic alcohol and acid), gluthathionyl conjugates, sulfate derivatives and glucuronide derivatives (Rodríguez-Morató et al. 2016). HT undergoes methylation and oxidation reaction and it is enzymatically converted into 3,4-dihydroxyphenylacetaldehy de, 3,4-dihydroxyphenylacetic acid, 4-hydroxy-3-methoxyphenylacetic acid and 4-hydroxy-3-methoxyphenylethanol. 3,4-Dihydroxyphenylacetaldehyde was produced by alcohol and aldehyde dehydrogenase in oxidation reaction and then subsequently 3,4-dihydroxyphenylacetic acid. In the methylation reaction, catechol

ortho methyl transferase produces homovanillic acid (4-hydroxy-3-methoxyphenylethanol). And finally converted these compounds to sulfo conjugates with the help of sulfotransferase enzyme (D'Angelo et al. 2001). D′ Angelo et al., in 2001, evaluated the hydroxytyrosol toxicity and its pharmacokinetics. The oral administration of hydroxytyrosol to rats at dose upto 2 g/kg does not show toxicity but it is administered intravenously to understand the identification and quantification of hydroxytyrosol metabolites. Hydroxytyrosol is readily taken up by the organ and tissue preferentially by renal uptake. Ninety percent of the drug is readily excreted in urine after 5 h of administration and 5% in feces and gastrointestinal tract. Metabolites extracted from body and different organs showed that hydroxytyrosol is enzymatically converted in four oxidized or methylated derivatives (D'Angelo et al. 2001). The major metabolites of hydroxytyrosol were in glucuronides form and one of the novel compounds of hydroxytyrosol was glutathionylated conjugate. In contrast, oleuropein has not any absorption model as oleuropein was readily metabolize by normal flora in large intestine and converted into hydroxytyrosol (Corona et al. 2006). Hydroxtyrosol excretion is different in rats and humans as studied by Visioli et al. (2003). They reported that the hydroxytyrosol urinary excretion was greater than excretion in rats, which suggested that rat model is inappropriate for evaluation of hydroxytyrosol excretion and preparation or food containing hydroxytyrosol should be carefully formulated.



Oleuropein



Hydroxytyrosol

6 Anticancer Activity of Olive Leaf Bioactive Compounds (Oleuropein and Related Compounds)

Recent studies have focused on the compounds of the olive leaves reporting the mechanism responsible for inhibiting the polymerization of human chromosomes and hence the highest potential for constrain of cancer cell formation (Salama et al. 2020).

Secoiridoids in oleaceae (derived from glycosides oleosides) are characteristically an exocyclic 8, 9-olefinic compounds, which is combination of elenolic acid with glycosidic residue. As demethyloleuropein is not present in all varieties of olives so it could be used as marker for the identification of specific variety (Esti et al. 1998). Many studies have demonstrated the oleuropein and its derivative present in leaves have the ability to inhibit the proliferation of cancer cells and several tumor cell lines. Previous studies evaluated the antioxidant and anticancerous effects of oleuropein isolated from olive oil and inter-relation between reactive oxygen species and carcinogenesis (Owen et al. 2000). Further studies have been preceded on the oleuropein activity from olive leaf on cancer cell line.

Breast cancer is considered the most common cause of death in women worldwide. The rate of breast cancer elevated rapidly with age specially within the reproductive years and after 50 years the incident of breast cancer increases at slower rate at average age of menopause (Key et al. 2001). Recent studies suggested phytochemicals obtained from different medicinal plants, herbs, fruits vegetables such as polyphenols such as flavonoids and carotenoids such as terpenoids are considered as promising suppressants in breast cancer chemoprevention (Rabi and Bishayee 2009)**.** Han et al. (2009) demonstrated that oleuropein in dose of 200 μg/mL and hydroxytyrosol in dose of 50 μg/mL decreases MCF-7 cells by MTT assay (Han et al. 2009). Oleuropein and hydroxytyrosol reduce the MCF-7 cells number by initiating cell apoptosis and inhibiting cell proliferation. They also reported that oleuropein significantly block the G1 to S phase transition by increasing the number of cells in G0/G1 phase (Han et al. 2009).Olive leaf extract exhibited significant antiproliferative response at concentration of (100, 50, 25, 12.5, 6.25, 3.125 and 1.56 mg GAE L^{-1}) against breast, colon, hepatocellular and cervical carcinoma cells (MCF-7, HCT, HEPG-2 and HELA cancer cell lines) and IC50 values (50% of growth inhibition) of olive leaf methanolic extract showed (breast cell cancer) MCF-7 is 81.6 mg GAE L^{-1}, (colon cancer cells) HCT 43 mg GAE L^{-1}, (hepatocellular cancer cells) HEPG-2 is 21.5 mg GAE L^{-1} and (cervical carcinoma cells) HELA is 77.9 mg GAE L^{-1}(Nashwa and Abdel-Aziz 2014). Olive leaf extract demonstrated inhibitory effect on the metabolic pathway of SKBr3 and MCF-7 and JIMT-1 breast cancer cells (Fu et al. 2010; Taamalli et al. 2012).

6.1 Prostate Cancer

Prostate cancer is the sixth most common type of cancer worldwide and second primary cause of cancer related mortality in men (Lassi and Dawson 2009). The activity of oleuropein was evaluated on two different prostate cancer cells in human LNCaP and DU145 which showed two different disease stages and one BPH-1 non-malignant cells. It is demonstrated that oleuropein at concentrations of 100–500 μM significantly decreases prostate cancer cell proliferation and initiate necrotic cell death. Oleuropein decrease cancer cell viability and induced modification of thiol group, reactive oxygen species, γ-glutamyl cysteine synthetase, pAkt and heme

oxygenase-1. It is reported that oleuropein showed antioxidant effect in BPH-1 cells because of its ability to stimulate the expression of HO-1 and increase RSH levels (Acquaviva et al. 2012).

6.2 Cervical Cancer

Cervical cancer is a major health problem, almost effected 500,000 women from developing countries in world. Cervical cancer gets worst mostly in under developing countries where there is no effective screening and chemotherapy available (Waggoner 2003). Study by Yao et al. (2014) showed that oleuropein at 150–200 mM concentration arrested HeLa cell at G2/M phase (Yao et al. 2014). Oleuropein treatment significantly and dose dependently increases phosphorylated ATF-2, p53, p21, Bax, c-Jun NH2-terminal kinase (JNK) protein, cytochrome *c* protein in the cytoplasm. They also demonstrated that oleuropein at 200 mM suppressed the apoptotic bodies formation and induced JNK activation (Yao et al. 2014).

6.3 Lung Cancer

Oleuropein was reported to inhibit proliferation and migration of lung carcinoma A549 cells. The IC50 of oleuropein was calculated at 59.96 μM and it showed high levels of ROS with relative values of migrated cell are $r^2 = 0.9578$ and MDA 0.9972. Cells arrest in G1 cycle phase was observed with increases in oleuropein concentration (Mao et al. 2012).

6.4 Thyroid Cancer

Thyroid cancer cells TPC-1 and BCPAP was treated with oleuropein and its peracetylated derivative (Ac-OLE) at concentration of 10, 50, and 100 mM and their anticancer effect was evaluated by cell counting, and trypan blue exclusion and tetrazolium assays. It was found that oleuropein and acetylated oleuropein inhibit proliferation of both TPC-1 and BCPAP cancer cells. Oleuropein as strong antioxidant also inhibited *in vitro* cancer cell growth and cell proliferation exerting its effect mainly by acting on growth-promoting signal pathways (Bulotta et al. 2013).

6.5 Gloiblastoma

Olive leaf extract (OLE) and temozolomide (TMZ) in combination at dose of 3 mg/mL, 2 mg/mL, 1 mg/mL, 500 μg/mL, 250 μg/mL, 100 μg/mL, 50 μg/mL, 30 μg/mL, 10 μg/mL, and 5 μg/mL and 300–500 μM respectively was tested in human glioblastoma cell line T98G. Results showed that OLE and TMZ both have synergistic effect with toxicity of TMZ. OLE and TMZ collectively upregulated miR genes expression specifically miR-181b, miR-153, miR-145, miR-137, and let-7d in human glioblastoma cell line T98G and effectively inhibit the proliferation of T98G cells (Tunca et al. 2012).

6.6 Neuroblastoma

Seçme et al. treated SH-SY5Y neuroblastoma cell line with oleuropein at concentration of 25, 40, 50, 75, 100, 150, 175, 200, 250, 300, 350, 400, 500, 700 and 800 μM during 72 h and oleuropein IC50 value in SH-SY5Y cells was detected as 350 μM at 48 h. They further demonstrated that oleuropein induced apoptosis by Bcl-2 and activation of Bax,caspase-3 and caspase-9 gene expressions and causing cycle arrest by down-regulating of CylinD1, CylinD2, CyclinD3, CDK4,CDK6 and up-regulating of p53 and CDKN2A,CDKN2B, CDKN1A genes expressions (Seçme et al. 2016).

6.7 Skin Cancer

Effect of olive leaf whole extract and oleuropein was studied on mice skin damaged caused by chronic UVB at doses of 300 and 1000 mg/kg for olive leaf extract and at 10 and 25 mg/kg for oleuropein. The data showed significant inhibition of carcinogenesis and tumor growth by inhibiting the VEGF, MMP-2, MMP-9, and MMP-13 genes expression by reduction in COX-2 level (Kimura and Sumiyoshi 2009). Mijatovic et al. (2011) demonstrated the effect of dry olive leaf extract (DOLE) at a dose of 0.6 mg against malignant, immune- and chemo resistant skin cancer melanoma. DOLE significantly stop the cell proliferation and inhibit mouse B16 melanoma cells clonogenicity and reduced tumor volume. DOLE successfully caused cancer cell death by disruption of cell membrane and caspase independent genetic material fragmentation (Mijatovic et al. 2011).

6.8 Antitumor Agent

Hamdi et al., evaluated the antitumor activity of oleuropein at dose of 0.005–0.025% solution and reported that oleuropein has strong antitumor activity and anti-cancer effect on cells by directly disrupting actin filaments in tumor cells. Oleuropein inhibits proliferation and migration of advance grade tumor cells and prevents invasiveness, mortality and replication by irreversibly rounded cancer cells which is linked to the disruption of the actin cytoskeleton (Hamdi and Castellon 2005).

6.9 Leukemia

Samet et al., in 2014 reported the anticancer activity of olive leaf against human leukemic K562 cells. This study revealed that chemlali olive (type of olive in tunisia) leaf extract at dose of 50, 75, 100, 125, and 150 μg/mL significantly inhibits leukemic cell proliferation and arrest cell cycle firstly at G0/G1 and then at G2/M phase in treatment time. Chemlali Olive leaf extract induce apoptosis and differentiation of K562 cancerous cells.

In another study ethanolic extract of olive leaf was used against HL-60 cells. Olive leaf extract significantly inhibited the proliferation of HL-60 cells. The inhibition of HL-60 cells is directly related to cellular differentiation. Oleuropein was used in concentration of 2.4×10^{-6} μM which showed 38% differentiation while this differentiation percentage was decreased to 14% when the dose of oleuropein become doubled on the other hand cells treated with apigenin 7-glucoside at dose of $2.7–5.4 \times 10^{-6}$ μM showed 77% result. When both of these oleuropein and apigenin 7-glucoside were used in combination at concentration of 2.4×10^{-6} and 5.4×10^{-6} μM respectively, the differentiation concentration becomes 88% but when the oleuropein concentration was doubled in this concentration the differentiation percentage reduced to 60%. This results showed the apigenin 7-glucoside is responsible for differentiation and oleuropein influenced the differentiation because of the oleuropein inherent cytotoxicity at high concentrations (Abaza et al. 2007).

6.10 Pancreatic Cancer

Pancreatic cancer is one of the most lethal and devastating disease with a poor prognosis and kills almost 250,000 people per year in a world (Hart et al. 2008). Pancreatic cancer showed significant resistant to the conventional treatment and highly toxic effects of the current chemotherapy. Thus, it is essential to develop a safe and convenient therapy of pancreatic cancer. One of the reported data demonstrated olive leaf extract at dose of 100 and 200 μg/mL was effectively reduced the

growth of pancreatic cancer cells with comparison standard anticancer drug gemcitabine at its IC50 (Scarlett et al. 2006).

7 Mechanisms Involved in Cancer Treatment by Olive Leaves (Oleuropein and Its Derivatives)

Mechanisms of oleuropein and its derivatives as cancer therapeutic agents or chemopreventive agents are not clear yet however different approaches and investigations has been devoted to elucidate their underlying cellular and molecular mechanisms in cancer treatment.

Energy produced in the mitochondria of eukaryotic cells results in oxidative metabolism. This oxidative metabolism further produces several beneficial and less toxic (5%) compounds. These toxic compounds normally in low concentration are necessary for some cellular processes like signal transduction, enzyme activation, disulfide bond formation during new protein folding in ER, gene expression, and caspase enzyme regulation during apoptosis. Internal oxidative stress may be caused by peroxisomes and enzymes (detoxifying enzymes from p450 family), xanthine oxidase and nicotinamide adenine dinucleotide oxidase complexes (NADPH), among others and sources of external oxidative stress include UV radiation, chemical compound (like pollutants, smoking and alcohol) and exercise (Sosa et al. 2013). Reactive species include ROS (reactive oxygen species), RNS (reactive nitrogen species), RSS (reactive sulfur species), RCS (reactive chloride species) (Halliwell and Gutteridge 2015). From all of these four, ROS are the most abundantly produced. ROS caused damage to the cell depends mainly on their concentration in the cell and the equilibrium between endogenous antioxidant species and ROS. When this equilibrium becomes disturbed, oxidative stress is generated which then cause damage to DNA, RNA, lipids and proteins (Veskoukis et al. 2012). These species cause nicks in DNA and thus damage the DNA repairing mechanism and thus DNA oxidation produced 8-hydroxy-2-deoxyguanosine, and this product generates DNA mutation by a process that initiate and enhance carcinogenesis and aging (Matsui et al. 2000). Also, cellular membrane is made up of polyunsaturated lipids which are highly susceptible to oxidation by these species. Reactive species initiate lipid peroxidation reaction and as a result increase permeability of cell membrane, that could cause cell death (Halliwell and Chirico 1993). Cellular proteins are also affected by these reactive species. High concentration of these species generation and aggregation of carbonyl groups like aldehyde and ketones and thiol groups (that may be changed to reactive sulfur species) and this oxidative induced modification altered protein structure and loss of its function (Levine 2002). Oxidative stress is associated with variety of pathological diseases such as Parkinson's and Alzheimer's disease, inflammatory diseases like rheumatoid arthritis, cardiovascular disease, cancer, immune system dysfunctions, diabetes, and allergies.

Oxidative stress effects cells and promote development and progression of tumor by mechanisms such as activation of ligand dependent RTK and ERK1/2 (extracellular regulated kinase 1/2), evasion of apoptosis, tissue evasion and metastasis, Met overexpression, Rho-Rac interaction, angiogenesis. Oxidative stress affects various pathways in cellular proliferation including epidermal growth factor receptor, mTOR that involve main signaling protein e.g. nuclear factor erythroid 2-related factor 2 (Nrf2), c-Jun N-terminal kinase (JNK), mitogen activated protein kinases (MAPK) such as ERK1/2, MEK, p38, Ras, Raf, kelch-like protein 19 (Keap1), p53, c-myc and PKC (Matsuzawa and Ichijo 2008).Oxidative stress plays very important role in developing cancer and cancer treatment is associated with ROS production as most of the chemotherapeutic agents inhibit DNA replication and damage DNA. For instance, the over expression of tumor suppressor genes with radiation is considered to resume apoptotic program and thus initiate enormous cancer cells killing and antioxidant blocked this process. Antioxidants are the agents that constitute cellular defense mechanism against increase concentration of reactive species especially reactive oxygen species (Oh et al. 2008). Antioxidants prevent tumor progression and increase life span and this is evidently proved (Kovacic and Jacintho 2001). They also play an important role in prevention of tumor. Moreover, antioxidant supplements decrease dose limiting adverse reaction or toxicity in patient with cancer (Block et al. 2008). It is reported that oleuropein and luteolin glucosides as main constituents of olive leaf extract exhibited strong antioxidant activity and are an easily available supplementation for chemotherapy patients (Kontogianni and Gerothanassis 2012).

Apoptosis is a very important natural physiological pathway of programmed cell death evokes by inflammation, oxidative stress and other factors, and this process is mainly controlled by proapoptotic and antiapoptotic Bcl-2 gene family. Apoptosis help to remove any damaged or unnecessary cells from the body. The process of apoptosis includes nuclear fragmentation, blebbing and shrinkage of irreparably damaged cell. Apoptosis is an important approach in the cancer therapies as deregulation of apoptosis leads to cancer pathogenesis. Many natural products or plant biochemicals target apoptosis (Li-Weber 2013). The p53 tumor-suppressor gene acts as a conduit between detection of impaired DNA and beginning of apoptosis. P53 gene is frequently inactivated by mutation in cells. P53 inactivation leads to bypass intrinsic apoptotic response and results in uncontrolled proliferation of cells (Lee and Bernstein 1995). The apoptotic process of cells involves many transduction pathways. Apoptotic mechanism of the cell is complex and occur through intrinsic and extrinsic pathways (Igney and Krammer 2002). The proapoptotic ability of p53 is due the transactivation of the apoptosis related genes which directly activates transcription of apoptotic genes (Elamin et al. 2013). P53 may induce 2 types of genes on stress signal one set is activation of p21/waf-1 and GADD45, functions in cell growth control and other set is Bax and Bcl2 genes (Haupt et al. 2003).

P53 gene mediates apoptosis via the ability to control transcription of proapoptotic members of Bcl-2 family members (Fridman and Lowe 2003). One possible mechanism explained in one study suggests that oleuropein from olive leaves

increased mRNA expression of p53 genes and Bax, Bid and Bad mRNA expression and decreased Bcl-2 antiapoptotic gene mRNA expression. Increased in caspase-9 and caspase-3 genes expression was also seen in oleuropein treated cells. So this study showed that oleuropein is inducing apoptosis in SH-SY5Y cells via intrinsic or mitochondrial pathway and ratio of apoptotic cell elevated approximately 30% in SH-SY5Y cells with treatment of oleuropein (Elamin et al. 2013; Seçme et al. 2016). In another study it is reported that oleuropein and hydroxytyrosol induce cancer cell apoptosis by mechanisms like decreasing HIF-1a, upregulating p53 and cyclin-dependent inhibitors p21 and down regulation of NF-kappa B and cyclin D1 (Barbaro et al. 2014; Samara et al. 2017). In another study by Berrin et al. reports that oleuropein target miR-153 in GBM (glioblastoma cells) and upregulated expression of miR-153 and thus increases cell apoptosis mechanism by targeting antiapoptosis members Bcl-2 (B-cell lymphoma) and Mcl-1(myeloid cell leukemia sequence 1). Some literature reported that oleuropein induces G1 phase arrest of cell cycle in several cells such as A549 (Mao et al. 2012). However some data data suggested that oleuropein cause accumulation of cells in G2/M phase in the cervical cancer cells (Yao et al. 2014). Elamin et al., reported that oleuropein inhibits breast cancer cell proliferation by retarding the S phase of cell cycle and downregulation of NF-kB and cyclin D1 but activating p21(CDKN1A) (Elamin et al. 2013).

Cycline dependent kinases and inhibitors of cyclin kinases play an important role in the cancer cells development. CCND1(cyclin D1) is an important check point regulator of G1 and S phase of cell cycle and support various cancer cell growth by overexpression or amplification (Musgrove et al. 2011). In one study it is demonstrated that CCND1, CDK4 and CDK6 genes are over expressed in neuroblastoma as compared to other cancer type cells. Furthermore it is observed that CCND1 and CDK4 activity help to the undifferentiated phenotype in neuroblastoma (Rader et al. 2013). Oleuropein treatment of neuroblastoma showed downregulation of CCND1, CCND2, CCND3, CDK4, CDK6 mRNA expressions upregulation of cyclin-dependent kinase inhibitors CDKNA2A, CDKN2B, CDKN1A (Seçme et al. 2016). In some reported data of breast cancer has stated that cyclin D1 is the major marker which is found to be over expressed in 50% cases of breast cancer. It is found that Pin1 was involved in the regulation of cyclin D which might be at transcriptional level or by post transcriptional level. At transcriptional level it is considered to effect transcriptional factor Jun, and post transcriptional it is involved in controlling the stability of target protein. So in this mechanism by targeting either inhibition of Pin1 leads to lower the risk or susceptibility of cancer and increasing Pin 1 level lead to increase tumorigenesis (Dong et al. 2010). Bouallagui et al., explains the effect of hydroxytyrosol from olive leaf on breast cancer cells and proposed a preliminary mechanism of hydroxytyrosol exhibiting or blocking G1 phase in cell cycle of MCF-7 human breast cells. They demonstrated that hydroxytyrosol down the expression of Pin 1 and cyclin D1 and upregulated the expression of c-Jun (Bouallagui et al. 2011).

Cyclooxygenases are bi-functional membrane bounded enzymes formed from prostaglandins compounds which are oxygenated carbon 18 and carbon 22 compounds (Garavito and Mulichak 2003). COX1 enzymes are generally involved in

housekeeping functions and expressed in cells and tissues of the body while COX3 only appear in some specific tissues like brain and spinal cord (Kis et al. 2003). COX2 is mostly found in low amount in cells but their level increased although 80–90% of colon or other cancers (Wang and DuBois 2010). This might be due to cross talking between several mediators of inflammation for instance cytokines and interleukins. And this explains why increased expression of COX-2 in colorectal cancer is associated with large size of tumor and poor survival rate (Sheehan et al. 1999). And COX-2 is considered as a target for cancer preventive therapies (Romagnolo et al. 2010). One study of polyphenols from olive oil proposed cancer cell cycle blockage in G2/M phase in colon cancer cells and this inhibition of cancer cells proliferation may be due to inhibition of COX-2 (Corona et al. 2007). COX-2 mRNA level is decreased when accumulation of cells occur in G2/M phase and its expression is regulated in cell cycle dependent manner. The transcription of COX-2 is controlled by activation of different transcription factors such as cyclic AMP response element binding protein (CREB), C/EBP, NFAT or AP-1 and this activation is regulated by p38 and other signaling pathway (Arbabi et al. 2001). So polyphenols are able to down regulate the COX-2 genes expression in colorectal cancer (Corona et al. 2007).

Hypoxia-inducible factor alpha (HIF-1α) is a transcriptional activator that activates in response to hypoxia. In human cancer HIF-1α activity elevated as a result of genetic alteration or intratumor hypoxia. HIF-1α stimulates transcription genes that increased oxygen availability via stimulation of angiogenesis and as a result induced expression of endothelial growth factor, which is closely associated with the initiation of neovasculature in human cancer or reprogrammed the cell metabolism so to adopt to low oxygen concentration (Semenza 2010). Cardeno et al. explained the mechanism of oleuropein and hydroxytyrosol on HIF-1α protein expression. Oleuropein causes downregulation of HIF-1α. and consider as the major mechanism responsible for the shrinkage of tumor (Cárdeno et al. 2013). In another study it is demonstrated that oleuropein inhibits cell migration and invasion in LN-18, RPMI-7951, T-47D tumor cell (Hamdi and Castellon 2005).

Samet et al., reported the inhibition cell proliferation in K562 cells leukemic cell by the increase expression of CHECK2 Gene. The encoded protein causes inhibition of CDC25A, CDC25B, and CDC25C and causes cell cycle arrest. As CD25 proteins are responsible for triggering the entry into the mitosis stage at different phases of cell cycle by activating the Cdk-cyclin complexes. CDC25A acts in the initial stage of cell cycle and regulating G1/S transition, while CD25B and CD25C act at G2/M stage of cell cycle. And olive leaf extract showed decrease CDC25C genes expression and increase the expression of CDC25A, it may demonstrate the arrest of cell cycle at G2/M phase (Samet et al. 2014).

Hematopoiesis is the production and replacement of blood cells. During early stage of embryo formation hematopoiesis occur in yolksac, liver, spleen and lymph nodes while in adult stage it take place in bone marrow, femoral head and iliac crest. Some proportion of bone marrow cells that is hematopoetic stem cells produces undifferentiated and multipotent hematopoetic cells in adults. These cells have high capability of self-renewal, differentiation and proliferation. Mostly of the blood

cells are highly differentiated and their life time varies from hours (neutrophil) to days (platelets) to weeks (red blood cells). Blood cells are functional and terminal elements associated with two major hematopoietic linkages such lymphoid and myeloid. These hematopoietic cells proliferate, differentiate and become mature before entering to blood circulation. These proliferation, self-renewal and differentiation involve several cells and molecules such as stromal cells and molecules like chemokines, transcription factor, and cytokines (Broxmeyer et al. 2005; Wickrema and Crispino 2007) and several miRNAs (Mathieu and Ruohola-Baker 2013). There are transcription factors that regulate the process of hematopoiesis and deregulation of hematopoiesis leads to blood disorder like leukemia. Differentiation is an important process of cell (Reiss 1986). Despite of cell proliferation and survival, the absence of normal differentiation characterizes malignancy of cell. Therefore, in recent strategy for cancer treatment molecular mechanism associated with the differentiation process is considered as a very important therapeutic target for cancer cells. Therapies that induced cell proliferation arrest and cell differentiation concomitantly has considered as alternative treatment to cytotoxic chemotherapies. The aim of differentiation induction is to develop a pathway of expression of certain genes that leads tumor cell to differentiation stages and invert the cancer cell growth plot. This therapy mainly act to reprogram the malignant cells into functional and properly differentiated cells rather than killing them (Leszczyniecka et al. 2001). Treatment of leukemic cells K562 with olive leaf extract up regulated the expression of CD14 on the surface of cells indicating the differentiation of K562 cells toward the monocyte/macrophage lineage. This hypothesis is supported by the higher percentage of cells positive for CD11b because CD11b is expressed in both monocyte and granulocytes. Thus, the increase of CD14 and CD11b promotes the granulocytic differentiation of K562 leukemic cells into monocyte/macrophage lineage (Samet et al. 2014).

8 Conclusion

Olive plant is a very essential medicinal plant considering for the cure of hypertension, cardiovascular diseases, antidiabetic, antibacterial, respiratory tract infection, respiratory tract infection, viral infection, rheumatism, bacterial diseases, skin diseases, asthma and cancer. In this chapter most of the reported data suggested strong anticancer activity of oleuropein and its derivatives from olive leaf. Many mechanisms have been proposed that involved in killing of cancer cell by apoptosis, DNA damage, up regulation and down regulation of different genes expression but still exact mechanism of action is unknown. Further studies are required to investigate its potential mechanism and safety profile of olive leaf extract.

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Chapter 15
Honey Against Cancer

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Abstract Honey, a natural sweetener, is used worldwide for different purposes. Most importantly it is used for nutrition purposes and hence it is in use in almost every society of the world where the people consume honey as a raw food or can be used as sweetening agent in different kinds of food. Apart from its natural sweetening property, honey also possesses therapeutic role in the treatment of various health ailments. Among these, chemoprotective properties are more evident. Honey is thought to have chemopreventive effect against different types of cancers since long time. In fact, tt has been widely used for its antioxidant activity, anti-apoptotic, anti-proliferative, anti- anti-mutagenic, inflammatory and immunomodulatory activities and as a Tumor Necrosis Factor (TNF). The role of honey in each of these activities is well established and is used extensively worldwide in different settings of the health care.

Keywords Honey · Antioxidant · Anti-apoptotic · Anti-proliferative · Tumor necrosis factor (TNF) · Anti-inflammatory and immunomodulatory · Anti-mutagenic activity

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1 Introduction

Honey has been used as a food and as well as a medicine since ancient times. Natural honey has been considered as the most ancient sweetening agent used in different nutraceutical products (Ajibola et al. 2012). The Natural honey is one of the major nutraceutical agent with full of flavors (Bogdanov et al. 2008). This is considered as product having huge benefits for Human health. Natural honey is produced as blossom honey by honey bees which are secreting nectars of flowers whereas the forest honey is produced as exudates of plant sucking insects. Natural honey is used by people of all ages and in every culture and ethnicity and by people of all beliefs which considered honey as diversely used food and medicine for different purposes (Ajibola et al. 2012).

Natural honey has been used in all times and by all generations, this shows the significance of honey in every age (Eteraf-Oskouei and Najafi 2013). In Islam even the significance of honey has been recommended by the Holy Quran and also there is a complete chapter on use and significance of honey named as Surah al-Nahl. So, there is emphasis on the use of honey as a food and also for the treatment of various types of diseases as well. The Holy Prophet Muhammad (PBUH) also recommended the use of honey for different ailment conditions on various occasions (Purbafrani et al. 2014). Most of the ancient populations like Egyptians, Greeks, Babylonians, Romans, Chinese and Mayans used honey for nutritional and medicinal purposes (Dashora et al. 2011).

Different studies suggested that honey has the ability to combat cancer through different mechanisms although the full mechanism of action of honey is not fully understood yet but numerous studies reveal that honey exhibits its action by inferring with different cell signaling pathways (Ahmed and Othman 2013). Honey also has the ability to modulate the immune system of the body (Ahmed et al. 2018).

Beside nutritional importance, honey also has a very potent anticancer activity (Othman 2012). Cancer is considering to be the second leading cause of death globally and there is approximately 18 million cancer cases have been reported around the globe, comprises of 8.5 million in women and 9.5 million in men (WHO 2020). Cancer involves many steps starting from the transformation of a single cell which is then characterized by rapid proliferation followed by invasion and then metastasis. The cause for this process includes different types of carcinogens, tumor promoters, and other inflammatory agents. This whole process is regulated through different factors including transcription factors different proteins which include proapoptotic proteins, antiapoptotic proteins, protein kinases, cell cycle proteins, cell-adhesion molecules, COX-2, among others (Martin et al. 2013).

The treatment for cancer mainly includes removal of the tumor by surgery, followed by radio therapy and chemotherapy (Baskar et al. 2012). But this type of treatment has serious side effects and normally the patient is passing from a very hard time due to which many of the patients leave the treatment before the end. Recent advances in cancer treatment mainly focus on the specific target molecules that are involved in carcinogenesis (Ajibola et al. 2012). Natural honey is thought to

have anticancer activity reported in many research papers. Honey is a blend of various natural compounds comprising of flavonoids, amino acids, proteins, different types of enzymes, phenolic acids and many more compounds (Cianciosi et al. 2018). The ingredients change depending on the floral sources as well as the origin of the honey (Kaškonienė and Venskutonis 2010). Apart from many useful effects of honey it has also an anticancer activity against different types of carcinomas. The phenolic compounds like quercetin, kaempferol, galangin, apigenin, acacetin present in the honey are thought to have antileukemic activity against various types of leukemic cell lines (Abubakar et al. 2012). Also, the anticancer activity of the honey is found against other cancer types like endometrial, prostate, renal, breasts, cervical, colorectal, and oral cancer (Abubakar et al. 2012).

Honey also has the ability to potentiate the anticancer activity of the anticancer drug cyclophosphamide and 5-fluorouracil (Ghramh et al. 2020). Anticancer potentials of honey against tissue culture and animal models has been studied and also clinical trials have been performed. Polyphenols like caffeic acid, caffeic acid phenyl esters, chrysin, galangin, quercetin, kaempferol, acacetin, pinocembrin, pinobanksin, and apigenin found in honey are considered to be responsible for the antitumor activity (Jaganathan and Mandal 2009).

2 Chemical Composition of Honey

Honey is mostly composed of water and sugars. It also consists of different vitamins and minerals as well. Among vitamins the most important are those from complex B (Ajibola et al. 2012). Apart from these ingredients there are also different other types of constituents which are listed in Tables 15.1 and 15.2. Because of these vitamins and minerals honey is considered as a rich food from the nutritional point of view. The sugars found in honey are considered as sweeteners and are capable of giving much more energy as compare to artificial sweeteners. Fructose is the most abundant sugar found in honey (Ahmed and Othman 2013). Honey comprises a vast range of vitamins including ascorbic acid, niacin, pantothenic acid and riboflavin as well as different minerals including calcium, potassium, iron, zinc, magnesium, manganese, phosphorus. The detailed list of these vitamins and minerals along with its quantity per 100 g is available in Table 15.2. The ingredients found in honey have a vast nutritional and health importance (Ajibola et al. 2012). Apart from this, some other elements are also found in honey that include aluminum, arsenic, barium, boron, bromine, cadmium, chlorine, cobalt, fluoride, iodide, lead, lithium, nickel, rubidium, silicon, strontium, sulphur, vanadium, zirconium and molybdenum. The detailed amount of these elements in honey (per 100 g) is available in Table 15.3.

The rich nutrition value of honey and its vast range of nutrients encourages the honey use as a food. Because honey presents low quantities of some ingredients honey, it is sometimes recommended for the adults to take honey in larger amounts in order to fulfill the daily requirements and also to get the more desirable results (Ahmed and Othman 2013).

Table 15.1 Nutritional composition of honey

	Blossom honey		Forest honey	
	Range	Mean	Range	Mean
Water	15–20	17.2	15–20	16.3
Total sugars		79.7		80.5
Monosaccharide				
Fructose	30–45	38.2	28–40	31.8
Glucose	24–40	31.3	19–32	26.1
Disaccharides				
Sucrose	0.1–4.8	0.7	0.1–4.7	0.5
Others	2–8	5	1–6	4
Trisaccharides				
Oligosaccharides		3.1		10.1
Erlose	0.5–6	0.8	0.1–6	0.1
Melezitose		˂ 1	0.3–22	4
Others	0.5–1	0.5	0.1–6	3
Minerals	0.1–0.5	0.2	0.6–2.0	0.9
Amino acids	0.2–0.4	0.3	0.4–0.7	0.6
Acids	0.2–0.8	0.5	0.8–1.5	1.1
pH value	3.2–4.5	3.9	4.5–6.5	5.2

Data in g/100 g of honey as adopted from Ahmed and Othman (2013)

Table 15.2 Chemical elements found in honey

Minerals	Amount (mg/100 g)	Vitamins	Amount (mg/100 g)
Sodium (Na)	1.6–17	Thiamine (B1)	0.00–0.01
Calcium (ca)	3–31	Riboflavin (B2)	0.01–0.02
Potassium (K)	40–3500	Niacin (B3)	0.10–0.20
Magnesium (mg)	0.7–13	Pantothenic acid (B5)	0.02–0.11
Phosphorus (P)	2–15	Pyridoxine (B6)	0.01–0.32
Selenium (se)	0.002–0.01	Folic acid (B9)	0.002–0.01
Copper (cu)	0.02–0.6	Ascorbic acid (C)	2.2–2.5
Iron (Fe)	0.03–4	Phyllochinon (K)	0.025
Manganese (Mn)	0.02–2		
Chromium (Cr)	0.01–0.3		
Zinc (Zn)	0.05–2		

3 Types of Honey and Their Synthetic Analogues

Honey is available in wide varieties throughout the world, each of them having a very unique flavor and color which depends on blossoms produced by honey bees (Al-Mamary et al. 2002). The same variety of honey is produced when the honey bees collect nectar from flowers of the same type. This procedure is supported by beekeepers who put their hives in an orchard or adjacent to a single type of flower and then very carefully examine the collection of the honey. The percentage of

Table 15.3 Some other elements found in honey

Elements	Amount (mg/100 g)	elements	Amount (mg/100 g)
Aluminium	0.01–2.4	Lead	0.001–0.03
Arsenic	0.014–0.026	Lithium	0.225–1.56
Barium	0.01–0.08	Molybdenum	0–0.004
Boron	0.05–0.3	Nickel	0–0.051
Bromine	0.4–1.3	Rubidium	0.040–3.5
Cadmium	0–0.001	Silicon	0.05–24
Chlorine	0.4–56	Strontium	0.04–0.35
Cobalt	0.1–0.35	Sulphur	0.7–26
Fluoride	0.4–1.34	Vanadium	0–0.013
Iodide	10–100	Zirconium	0.05–0.08

Ajibola et al. (2012)

fructose, glucose, type and amount of amino acids and organic acids differ by floral source of honey that eventually determines the flavor of honey (Ball 2007).

3.1 Acacia Honey

This variety of the honey also popular as light and clear honey has mild floral taste of the blossoms of Robinia pseudo acacia also known as Black Locust in North America and Europe. The fructose being a major constituent of the honey maintain its liquid sate for long period. Furthermore, low sucrose content makes it popular among the diabetics and with as a rich source of anti-inflammatory properties, and also used to treat respiratory disorders (Muhammad et al. 2016).

3.2 Alfalfa Honey

Alfalfa honey has a mild floral taste and are widely produced in Canada and in the United States. It is made from the blue or purple blossoms.

3.3 Avocado Honey

Avocado Honey is obtained from the California avocado blossoms having a dark color with buttery flavor which makes it good for salad dressing or condiment when mixed with other ingredients.

3.4 Basswood Honey

The Basswood Honey is produced from the cream-colored blossoms in North America, and due to its biting taste, distinctive white color and exceptional malleability quality it is very popular and favorable with any food item.

3.5 Blueberry Honey

Having a very pleasant flavor produced in England and in Michigan, it is extracted from the white flowers of the blueberry bush. Light amber in color and having a hint of tanginess from the Blueberry.

3.6 Buckwheat Honey

The *Buckwheat Honey is* mostly produced in Minnesota, Ohio, Pennsylvania and some parts of Canada. It is the strongest and darkest of all honey varieties, and is a rich source of iron and other essential nutrients.

3.7 Clover Honey

Clover Honey is one of the most widely available and popular honey varieties in the world. It is majorly produced across Canada and New Zealand. It has sweet flowery flavor and pleasant taste.

3.8 Eucalyptus Honey

The Eucalyptus Honey is a great medicinal honey variety, first originated in Australia, and now extracted in California as well. Traditionally, it is used in protection against cold and headaches by people across the world. It differs in taste and flavor but having a characteristic herbal flavor and a slight aftertaste of menthol.

3.9 Fireweed Honey

Fireweed Honey being light in color and having smooth, delicate and buttery taste. It is found mainly in north-west US. It is a great option for gourmet cooking, baking etc.

3.10 Manuka Honey

Manuka Honey is collected from the flower of the Tea Tree bush and Native to New Zealand's coastal areas.it has antibacterial property that aids in effectively treating stomach ulcers, sore throat, cold, indigestion and acne and pimples among others. Manuka Honey's taste varies due to the difference in source but usually boasts of a robust aftertaste that stays for a while.

3.11 Orangeblossom Honey

Orange blossom Honey, having a light color and mild flavor with a fresh fruity scent, and a fragrant citrusy taste. It is found originally in Spain/ Mexico, but now produced in many countries like Florida, Southern California and Texas etc.

3.12 Sourwood Honey

It is a light-colored, delicate honey type of Honey with a caramel kind of taste. While many thinks its taste to be sour, its lover always finds it to be as sweet as any other type of Honey available.

3.13 Sage Honey

The light-colored honey is mostly found in California. Sage Honey is packed with a property to granulate very slowly and is commonly used to mix with other types of honeys to reduce the process of granulation.

3.14 Tupelo Honey

One of the most premium honeys ever produced, Tupelo Honey is also known as "Southern Gold" is produced in the Southeastern U.S. It is usually light golden or amber in color having a very faint greenish glow and a mild and distinctive taste. Tupelo honey is considered as one of the sweetest honey varieties, because of its high fructose content and its ability of not granulating like most other types of honey.

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sweetest honey varieties, because of its high fructose content and its ability of not granulating like most other types of honey.

4 Synthetic Analogues

As the primary use of honey as the sweetener in different product (Figs. 15.1 and 15.2), therefore the possible analogs of honey as sweetener according to FDA are:

- Aspartame
- Saccharine
- Sucralose
- Neotame
- Sodium cyclamate
- Stevia (Rosenbaum 2002).

5 Anticancer Activity of Honey

Honey exhibits a potent anticancer activity (Goldman 2003) and it is using since ages for different types of diseases. Different studies also prove that honey has a very potent anticancer activity against different types of cancer cells. Honey produces its effect against cancer cells through the mechanisms indicated in Fig. 15.3. In brief.

5.1 Honey and Its Antioxidant Activity

The oxidative stress having free radicals is responsible for carcinogenic process according to many literatures. Reactive oxygen species as well as reactive nitrogen species like hydroxyl radical (OH), nitric oxide, hydrogen peroxide, superoxide, peroxynitrite as well as many other agents are considered as stress agents which is responsible for the destruction of lipids, proteins and DNA in the target cells. These cells have a defense system against these oxidative damages. This defense system includes antioxidative agents like catalases, peroxidases, ascorbic acid, superoxide

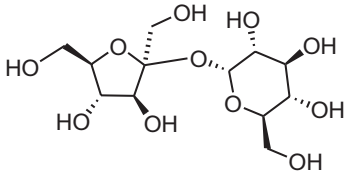

Fig. 15.1 Chemical structure of sucrose

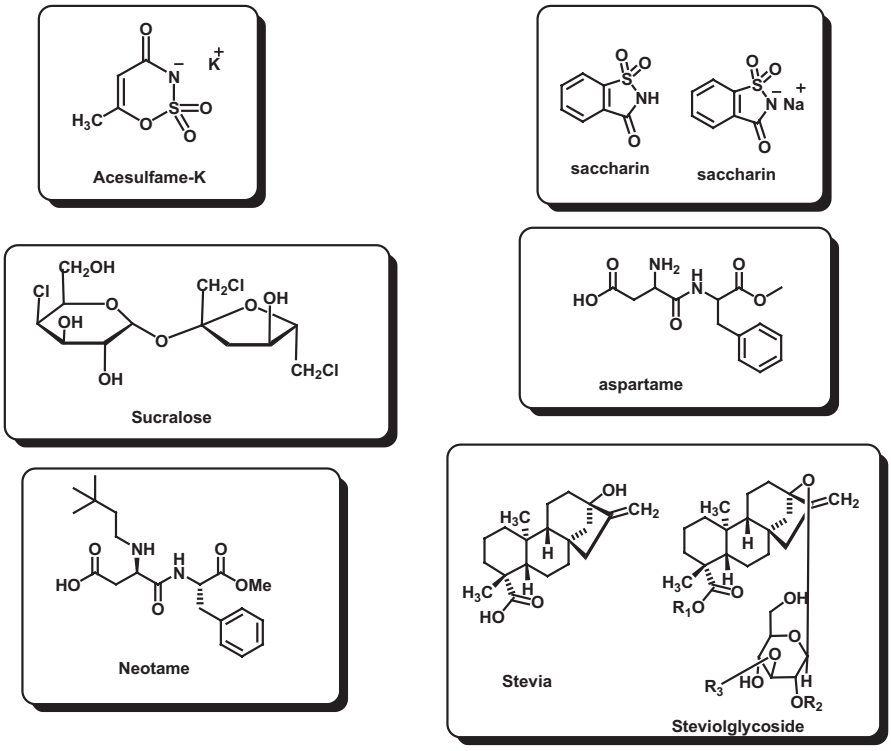

Fig. 15.2 Chemical structure of synthetic analogs of honey

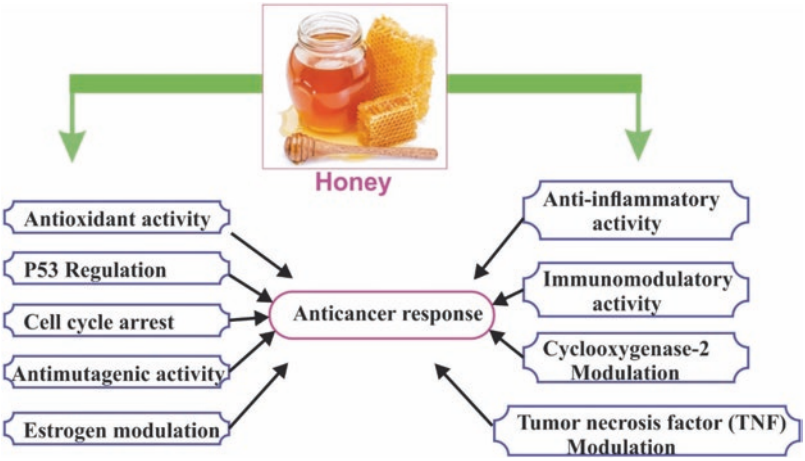

Fig. 15.3 Anticancer properties of honey

dismutase, polyphenols and tocopherols. The role of these agents is to inhibit or scavenge these free radicals therefore inhibit cancer development. The exact mechanism is unknown but it is thought that this antioxidant property is because of hydrogen donation, metallic ion chelation, free radical sequestration, flavonoids substrates for hydroxyl and superoxide radical actions. Due to its antioxidant properties, honey has an important role in the prevention of different types of acute and chronic disorders (Ahmed et al. 2018).

5.2 Honey and Its Apoptotic Activity

The two major characteristics of cancerous cells are proliferation as well as inappropriate apoptotic turnover (Lowe and Lin 2000). Cancer treatment commonly involves the drugs that are apoptosis inducers. Apoptosis is generally a three-stage process involving an induction phase followed by an effector phase and lastly the degradation phase. The induction phase involves stimulation of proapoptotic signal transduction cascades by producing death inducing signal (Ahmed and Othman 2013).

Effector phase starts by causing cell death through a regulator known as mitochondria while the last phase consisting of different nuclear and cytoplasmic reactions. The different changes in nucleus involves chromatin condensation, shrinkage of the cell, fragmentation of DNA and membrane blebbing while in the cytoplasm protein cleaving enzymes known as caspases are activated, leading to fragmentized apoptotic bodies formation which are subsequently phagocytosed by different macrophages and other surrounding cells (Elmore 2007).

The apoptosis normally consisting of two pathways namely caspase 8 also called as death receptor pathway and caspase 9 called as mitochondrial pathway. Honey is thought to start apoptosis by causing depolarization of mitochondrial membrane. Honey also causes increase in level of different enzymes in different types of cancers. Honey also has the role in up regulating of proteins which has pro and anti-apoptotic role. So basically, honey has the role in increasing the expression of pro and anti-apoptotic enzymes and proteins which are responsible for the apoptosis of cancerous cells. The apoptotic property makes honey a very good agent as anticancer agent like many chemotherapeutic agents currently in used (Elmore 2007).

5.3 Honey and Its Anti-proliferative Activity

Epithelial cell has the ability to divide throughout the life (Ragkousi and Gibson 2014). The cycle of the cell generally consists of different phases known as G0, G1, S, G2 and M. All these stages are controlled and regulated by different types of proteins (Bertoli et al. 2013). Among these proteins the most important ones are cyclins and cyclin dependent kinases (Malumbres 2014). Out of these phases G1/S

phase is the most important one where all important steps like apoptosis, differentiation, proliferation and quiescence occur. If there is over expression and dysregulation of these growth factors, then it will lead to cancer. Recently a protein called as Ki-67 is a new biomarker seen in the proliferation phase in G1, S, G2 and mitosis while it is absent in the resting phase of the cell cycle (Neganova and Lako 2008).

Honey has been associated to arrest the cell cycle (Fauzi and Yaacob 2016). In combination with Honey along with *Aloe vera* solution, is responsible for a marked decrease in the expression of proteins Ki-67 in tumor cells in rats (Tomasin and Cintra Gomes-Marcondes 2011). The probable mechanism is that honey lowers proliferation of cancer cells by cessation of cell cycle. The substances responsible for cell cycle stoppage are phenolic compounds in honey like quercetin, apigenin (Abubakar et al. 2012). The inhibitory effect is then followed by the down regulation of many cellular pathways (Tomasin and Cintra Gomes-Marcondes 2011).

5.4 Tumor Necrosis Factor (TNF)

It has been shown that tumor necrosis factor is responsible for the initiation of tumor, its promotion as well as progression. It also has been shown that due to the proinflammatory role of tumor necrosis factor is responsible for many diseases because of its property to activate NF-kb. TNF causes activation of NF-kb which is then responsible for the expression of different types of inflammatory genes which ultimately plays its part in causing of cancer. It also serves as growth factor for different types of tumor cells. However, TNF-α has been associated with the defensive role of the host cell as a primary cytokine (Ismail et al. 2006).

Honey has antitumor properties and the proteins responsible for that include Royal jelly (RJ) proteins i.e. apalbumin-1 and apalbumin-2. These proteins have the ability to stimulate macrophages which are then responsible for the production of TNFα, IL1 and IL-6. Different honey types like Manuka, Pasture, jelly bush (at 1% w/v concentration) cause monocytes to produce TNF-α, interleukin- (IL-)1β and IL-6. The propose mechanism involves binding of TNF-α and adaptor protein which involves TNF receptor associated factor (TRAF), TNFR associated death domain protein and receptor-interacting protein cause the regulation of apoptosis as well as inflammation by these cytokines. Thus TNF-α release has a very important role as a key cytokine to control key cellular mechanisms such as apoptosis, inflammation and cell proliferation (Ahmed and Othman 2013).

5.5 Honey and Its Anti-inflammatory and Immunomodulatory Activities

Chronic inflammation of the cell leads to cancer formation if left untreated. There is extensive damage of the tissue due to prolong and excessive inflammation of that tissue. Honey has the ability to show anti-inflammatory activity according to various studies (Vallianou et al. 2014). The research focused on the anti-Inflammatory activity of honey was carried on cell cultures, animal models as well as clinical trials. Various types of chemicals, biological agents and proinflammatory enzymes are responsible for the inflammatory process (Othman 2012). The enzyme cyclooxygenase-2 accelerates conversion of arachidonic acid to prostaglandin and this accelerated or abnormal metabolism of arachodonic acid is responsible for inflammation and ultimately carcinogenesis (Ricciotti and FitzGerald 2011). Cyclooxygenase-2 is also over expressed in pre and malignant conditions. Phenolic compounds such as caffeic acid, apigenin, quercitin and kaempferol present in honey have anti-inflammatory activity (Hossen et al. 2017). This activity of the phenolic compounds is due to suppression of the COX-2 pro-inflammatory activity as well as of nitric oxide synthase. Honey is also thought to have a role in proteins regulation like tyrosine kinase, ornithine decarboxylase iNOS, and COX-2 (Pipicelli and Tatti 2009).

Different types of honey are found to enhance the production of TNF-α IL-1β and IL-6 (Tonks et al. 2003). Hence this effect is related to anticancer activity of the honey (Ahmed and Othman 2013). Honey is also responsible for stimulation of antibodies, lymphocytes, monocytes, neutrophils, eosinophils and other types of killer cell which are produced during the immune response of tissues (Al-Waili and Haq 2004). Honey stimulates T and B cells, macrophages to produce the antitumor effect and also has a role in formation of short chain fatty acids which has immunomodulatory actions (Ahmed et al. 2018). Moreover, various components of honey act through different ways as immunomodulator like arabinogalactans acts as monocytes activator (Gannabathula et al. 2012), 261 molecular weight component as neutrophils activator (Fukuda et al. 2011), 5.8 kDa component as monocytes activator (Tonks et al. 2007) major royal jelly protein 1 as macrophages activator (Majtan et al. 2006) and as keratinocytes activator (Majtan et al. 2010) and apigenin and kaempferol as matrix metalloproteinase 9 inhibitor (Majtan et al. 2013).

5.6 Honey and Its Antimutagenic Activity

Mutagenicity is the ability to produce mutation in genes (Bose 2014) and this is linked with carcinogenicity of cell (Griffiths et al. 2000). Honey is thought to have excellent anti mutagenic property (Meo et al. 2017). This effect of honey was checked on *Escherichia coli* cells. Radiation (UV or γ) exposed *Escherichia coli* cells shows SOS response (SOS is an error prone repair pathway contributing to mutagenicity) (Saxena et al. 2012). In a study some important genes were knocked

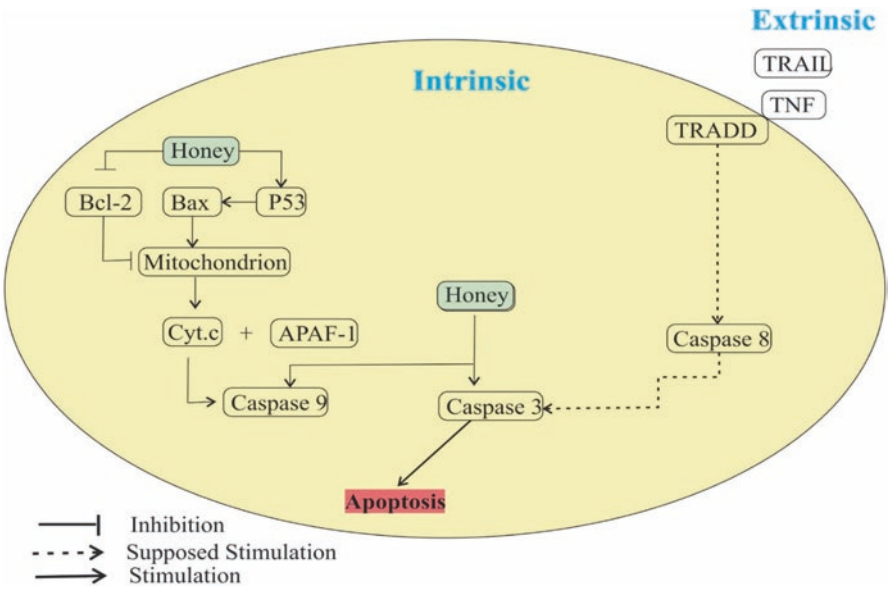

Fig. 15.4 Mechanisms of action of honey for prevention of cancer

out such as *umuC*, *recA,* and *umuD* which are responsible for SOS mediated mutagenesis. However, honey strongly inhibits these changes which confirm the strong antimutagenic effect of honey (Fig. 15.4). Honeys from various floral sources also show inhibition of Trp-p-1 mutagenicity (Wang et al. 2002).

6 Synergistic Effects of Honey

Different compounds show synergistic effect with honey. For instance, the synergistic effect of honey with different antibiotics was evaluated. These antibiotics were amikacin, gentamicin and ceftazidime. These antibiotics were tested against seven Pseudomonas and eight Klebsiella species. The minimum inhibitory concentration of honey with these antibiotics was checked. The synergetic effect was observed in case of Pseudomonas specie but not in case of Klebsiella specie (Ng et al. 2017).

Accelerating wound healing is nowadays considered important for clinical treatment. Honey and propolis are found to have a synergistic effect on wound healing of rat skin. The macroscopic as well as microscopic examination revealed that the percentage of wound healing on different days in combined application of propolis and honey experimental group was considerably different from that of control group. Therefore, the combined effect of both propolis and honey has synergistic effect on healing of the wound (Ng et al. 2017).

A study showed that honey also have the ability to enhance antibacterial activity (Mandal and Mandal 2011). The substance responsible in honey for this activity is amylase (Babacan and Rand 2007) and activity of amylase was shown both in presence and absence of starch on two different types of pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*). So, from this study it was concluded that amylase present in the honey have a synergistic effect on antibacterial activity. The proposed mechanism is that the amylase hydrolyzed the starch chains to produce dextrin along with maltose and also increase the osmotic effect of the media which is then responsible for increased antibacterial activity (Boukraâ et al. 2009).

Many studies have shown that honey has antibacterial activity which may be due to its low pH, high osmolarity, hydrogen peroxide (Molan 1995), presence of phenolic acids, lysozyme and flavonoids (Cliver and Snowdown 1996). Honey and thyme powder also found to have a synergistic effect against different types of bacteria like *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. This is due to thymol and carvacol, the main components with antibacterial properties (Ettayebi et al. 2000; Ultee et al. 2000). Starch is also having a synergistic effect on antifungal effect of honey (Erejuwa et al. 2012). It may be due to amylases present in honey which hydrolyze starch to dextrin and maltose leading to an increase in honey osmotic effect and subsequently increase the antibacterial activity (Boukraâ and Amara 2008).

6.1 Synergistic Effect with Substances That Produces Hypoglycemia

Honey contains different types of sugars like fructose. It is thought that fructose and glucose have the ability to increase the secretion of insulin from pancreatic cell which then in turn produce the hypoglycemic effect (Erejuwa et al. 2012).

6.2 Synergistic Effect with Substances That Produces Antioxidant Effect

The oxidative stress having free radicals is responsible for carcinogenic process according to many literatures. Reactive oxygen species as well as reactive nitrogen species like hydroxyl radical (OH), nitric oxide, hydrogen peroxide, superoxide, peroxynitrite as well as many others agents are considered as stress agents which is responsible for the destruction of lipids, proteins and DNA in the target cells. These cells having defense system against these oxidative destructions. This defense system includes antioxidative agents like catalases, peroxidases, ascorbic acid, superoxide dismutase, polyphenols and tocopherol. The job of these agent is to inhibit or scavenge these free radicals therefore inhibit the

process of cancer inside the body. The exact mechanism of working is unknown but it is thought that this antioxidant property is because of hydrogen donation, metallic ion chelation, free radical sequestration, flavonoids substrates for hydroxyl and superoxide radical actions. Honey having the antioxidant property is having role in the prevention of different types of acute and chronic disorders (Erejuwa et al. 2012).

6.3 Synergistic Effect with Substances That Produces Antihypertensive Effect

Antioxidants found in honey like Vitamin C, polyphenolics, and monophenolics are responsible for a lower risk of cardiovascular failures. These antioxidants reduce the risk of CHD mainly by three mechanisms: (a) increasing coronary vasodilatation, (b) decreasing the platelets ability in the blood to clot (c) preventing low-density lipoproteins from oxidizing (Samarghandian et al. 2017).

7 Clinical Trials

Cancer is mainly treated by chemotherapy as well as with radiotherapy but it is also harmful for normal cells of the body and there are many risks associated with this therapy. In this line, the recent research is mainly focus on the search of natural products with ability to prevent and treat tumorous cells. Honey is one of the most extensively researched products for its abilities to prevent and treat cancer. However how honey prevent cancer is still a mystery and there are different opinions. However, the honey mostly produces its effect through apoptosis, antitumor necrosis factor (anti-TNF), antiproliferative, antioxidant, anti-inflammatory and immunomodulatory activities (Ajibola et al. 2012).

Research conducted by (Samarghandian et al. 2017) also reveals that honey produces its anticancer effect through different mechanisms. Those researches showed that honey producing its anticancer effect by interfering with multiple cell signaling pathways including apoptosis, anti-proliferative, anti-mutagenic and anti-inflammatory pathways. By these mechanisms honey showed to produce its effect on skin cancer cells, cervical cancer cells, bladder cancer cell, renal cancer cells, prostate cancer cells and many more types of cells.

Studies shown by Premratanachai and Chanchao (2014) also suggest that there are different anticancer activities related to honey. honey, a rich source of antioxidants, also shown to have an active role in cancer prevention and treatment. different researchers have been experimenting honey against various types of cancers and getting highly valuable results regarding apoptosis produced by honey (Kumar Jaganathan et al. 2015).

8 Conclusion

Many studies have scientifically proved that honey is a potential anticancer agent through many mechanisms as shown in Fig. 15.3. These mechanisms are not yet fully understood but interference of honey with multiple pathways of cell-signaling, like apoptosis induction, anti-inflammatory, antiproliferative and antimutagenic pathways are involved. Also it modulate the immune system of body. Apart from the cancer activity honey also has wide range of nutraceutical applications and other therapeutic benefits. The botanical source of honey also plays a very important role on the bioavailability of honeys phytochemical compounds which ultimately influence the biological effects of honey. Although irrespective of botanical source, type of honey, variety of honey, all consists of antioxidants and having varying degree of biochemical properties representing value of honey as nutraceutical and therapeutic agent. The adverse effect of honey contamination identified should be minimized to promote the availability of honey as wholesome natural product for use on domestic level as well as in international market. This in addition will also help in preventing the problems associated with health due to honey toxicity especially lead poisoning. In nutshell the production and use of honey should be promoted in order to provide the best and easy source of nutrition and a very best alternative for different types of medicaments in different diseases.

Conflict of Interest The authors have no competing interests.

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Chapter 16
Soybeans and Phytoestrogen Rich Foods (Genistein, Daidzein) Against Cancer

Azadeh Manayi

Abstract The importance of diet and nutrition in cancer chemoprevention were indicated by a remarkable amount of evidence from experimental, clinical, and epidemiological studies. Phytoestrogens with remote structure similarities to estradiol enable to bind to receptors of estrogen. Isoflavones and lignans represent the main class of phytoestrogens, while other groups of phytoestrogens like coumestans and stilbenes are in a lower amount in food and less investigated. The most important representative of isoflavonoids are genistein, daidzein, and glycitein which accumulate to high levels in soy and legumes. Estrogen-like ability of isoflavones raise concerns in some individuals and more specifically in certain types of malignancies like breast or endometrial and prostate cancers. The available data regarding the important molecular processes of these compounds and clinical trials or observational studies were identified in chemoprevention and cancer to reveal whether there is association between soy food consumption and increase of the cancer risk. Molecular mechanisms of isoflavonoids can vary depending on their concentrations or physiological status of the cells or individuals (age, ethnicity, intestine microflora, and diet habits). Therefore, administration of these compounds has to be tailored according to the individual characteristic. Additionally, more clear recommendations can be provided by further high-quality, full powered, placebo-controlled studies considering individual characteristics as subgroups.

Keywords Daidzein · Equol · Genistein · Isoflavones · Phytoestrogen

1 Introduction

The health effects of soy and other phyto-estrogen rich foods have been investigated in recent years. The isoflavones have similarities in structure with 17β-estradiol and

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therefore are able to attach to receptors of estrogen and as such behave as natural selective estrogen modulators (Wu et al. 2008b).

According to the results of the researches, soy foods provide several health benefits especially in prevention of chronic diseases. However, *in vitro* and experimental studies doubtfully are relevant to the activity of these products in human. Since, *in vitro* tests do not represent complexity of living organisms and animal studies have limitations to predict effects in human because of difference in the anatomical characteristics and physiological. More debates present from confliction of *in vitro* and experimental studies, some studies showed that isoflavones can induce cell proliferation and opposite effects suggested by other studies (Chen et al. 2011; Imhof et al. 2008; Morito et al. 2001).

Considering observational studies, case controls showed stronger relation between soy and reduced risk of breast cancer, while less likely prospective studies do so (Fritz et al. 2013). Randomized clinical trials (RCTs) demonstrated a lack of pro-estrogenic properties by soy consumption, while isoflavones behave as natural selective estrogen receptor in cell lines (Fritz et al. 2013). Differential effects may observe in equol producers compared with non-producers depending on the intestinal microflora (Fritz et al. 2013; Yuan et al. 2007). Genetic variations present other factors that affect soy consumption outcomes in different ethnicities (Nechuta et al. 2012). The present chapter aims to discuss various aspects regarding the phytoestrogens, mostly isoflavonoids, of soy considering molecular targets of these compounds in cancer cells, observational and clinical studies in the breast and prostate cancers.

2 Phytoestrogens Rich Foods

The family of plants Leguminous such as soy, red clover, alfalfa, are almost exclusively rich in isoflavones. These naturally occurring compounds are reported in more than 300 specious of the plants particularly in seeds and roots (Ko 2014). Other reported sources of these phytochemicals are apple, blackcurrant, apricot, cherry, sweet potato, barley, cauliflower, lupin, plum, cabbage, date, wheat, onion, and melon pineapple (Durazzo et al. 2019).

Soy with scientific name of *Glycine max* includes a class of isoflavones known as phytoestrogens, more specifically daidzein, genistein, glycitein, formononetin, and biochanin A. Red clover also contains isoflavones with higher amount of formononetin and biochanin A, while dominant isoflavones in soy are genistein and daidzein (Fritz et al. 2013). The most significant food sources of isoflavones include soy flakes, soy flour, food containing isolated soy protein, fermented soybean products, tofu, soy milk, soybean paste, soy sauce and natto (Zaheer and Humayoun Akhtar 2017). The amounts of isoflavones in soy depend on varieties, climate conditions, and growing locations. The total isoflavones are lower in the soybeans which are cultivated in hot and dry weather. Generally, total isoflavones (around 90%) are

in sugar conjugates of daidzein, genistein, and glycitein with slight amounts of their aglycones. Isoflavones are also synthesized in legumes other than soy and red clover like kidney beans, navy beans, mung bean sprouts, and Japanese arrowroot (Zaheer and Humayoun Akhtar 2017).

Textured soy protein is commonly applied instead of meat and cheese and in sports drinks, cereals, infant formula, ice cream, doughnuts, and imitation of dairy products (Ko 2014). Asian populations intake more amount of isoflavones comparing to the western populations in their diet (Mortensen et al. 2009).

3 Chemical Properties of Isoflavones

Isoflavones with similar structure with estradiol biosynthesized through interaction of rhizobial bacteria and defense responses of leguminous plants. These are synthesized through phenylpropanoid pathway similar to the other flavonoids. Phenylalanine reacts with malonyl-CoA and converts to 4-hydroxycinnamoyl-CoA which is converted to 4,2′,4′,6′-tetrahydroxychalcon (naringenin chalcone) by chalcone synthase and reduced by chalcone reductase to 4,2′,4′-trihydroxychalcone (isoliquiritigenin). Ring closure catalyzed by chalcone isomerase to form 7,4′-dihydroxyflaone (liquirintigenin) and 5,7,4′-trihydroxyflavone (naringenin). Isoflavone synthase moves B-ring from 2-position to 3-position followed by dehydration to generate 2,3-double bond in heterocyclic ring by isoflavone dehydratase. Daidzein (7,4′-dihydroxyisoflavone) and genistein (5,7,4′-trihydroxyisoflavone) are generated by the mentioned reactions (Mortensen et al. 2009).

Isoflavones are physiologically active members of the large class of secondary metabolites, flavonoids. The main isoflavone aglycones of soy are chemically named 5,7,4′-trihydroxyisoflavone (genistein), 7,4′-dihydroxyisoflavone (daidzein), and 7,4′-dihydroxy-6-methoxyisoflavone (glycitein) (Zaheer and Humayoun Akhtar 2017). Free and conjugated forms of genistein and daidzein composed about 60% and 30% of total isoflavones in soy, respectively. The minor amount of soy isoflavones is glycitein, which is around 10% of total isoflavones. Overall, isoflavones are in four chemical forms including aglycones (genistein, daidzein, and glycitein), glucosides (genistin, daidzin, and glycitin), malonylglucosides (malonylgenistin, malonyldaidzin, and malonylglycitin) and acetylglucosides (acetylgenistin, acetyldaidzin, and acetylglycitin). Malonyl derivatives are predominant conjugates in soybeans (Barnes 2010). Glycosides to aglycones proportion varies among kind of food and depends on the processing of the foods. Glucosidic group is removed during fermentation by action of β-glucosidases from the fermentation microorganisms (Nakajima et al. 2005). In the small intestine, the bioactive contents of aglycones in fermented foods are rapidly absorbed to the blood (Patisaul and Jefferson 2010).

4 Metabolism of Soy-Isoflavonoids

Isoflavones are hydrolyzed after ingestion by intestinal microorganisms or enzymes to bioactive aglycones like genistein, daidzein, and glycitein (Cederroth and Nef 2009). The major isoflavones, daidzein and genistein, can be produced by intestinal glucosidases from formononetin and biochanin A. These compounds extensively metabolized in the liver and intestine. Formononetin is metabolized to daidzein that can metabolized to equol *in vivo* among equol producers (Jou et al. 2008; Setchell et al. 2005; Wong et al. 2012). Desmethylengolensin and equol are products of metabolism of daidzein, while p-ethyl-phenol is metabolite of genistein. Glycitin is resistant to enzymatic hydrolysis and undergo little metabolism by gut bacteria prior the excretion (Barnes 2010; Cederroth and Nef 2009). Conjugated isoflavones have enterohepatic circulation, they are deconjugated by intestinal microflora and then reabsorbed (Barnes 2010).

In the liver isoflavones are metabolized by isoenzymes of phase-I and II. Daidzein and genistein are hydroxylated by phase-I enzyme (cytochrome P450) and glycetin hydroxylated to mono- or dihydroxy glycetin. In phase-I, P450 1A1, 1A2, 1B1, 2E1, and 3A4 are involved in metabolism of daidzein and genistein (Mortensen et al. 2009). Phase-II enzymes like uridine 5′-diphospho-glucuronosyl transferase and sulfotransferase synthesize conjugate metabolites. In upper small intestine aglycones of isoflavone are actively absorbed. The glucuronide and sulfated derivatives of these compounds are excessively excreted into urine (5–35%) and few isoflavones excreted in feces (1–4%) (Ko 2014; Mortensen et al. 2009).

As intestinal bacteria play an important role in the isoflavones metabolism, bioavailability of soy isoflavones highly associated to the activity of the intestinal microflora and not all persons produce equol, which is the most active metabolite of isoflavones. However, underlying interaction is not well understood, each person who consume soy may produce different metabolites that can explain variation in health effect of soy. In fact, about 30–50% of individuals can produce this metabolite (Hedlund et al. 2003). Equol is highly efficiently absorbed via colon wall than daidzein and assessed in the plasma longer than daidzein and genistein. Subjects who ingested glycoside of daidzein showed equol in the plasma, while persons who consumed aglycone of daidzein did not show equol in the plasma. It is possibly because aglycone is absorbed passively, whereas glycosides cannot taken by enterocyte and delivered for further metabolism by bacteria in the distal small intestine and colon (Yuan et al. 2007). Longer time of stay in the intestine for glucoside forms may cause both bacterial and intestinal enzymes metabolism (Zubik and Meydani 2003). Beside, comparing the Asian populations with western populations revealed that Asians have higher equol producer prevalence (Decroos et al. 2005; Song et al. 2006). The variability of equol production is presumably attributed to the difference in capability and composition of the intestinal microflora. Good equol producers often consume carbohydrates more than non-equol producers (Yuan et al. 2007). After digestion of soy products, isoflavonoids were found in the in prostate fluid of men breast and tissue of premenopausal women. The concentration of equol in the

breast tissue was higher, while levels of daidzein and genistein were similar to those in plasma. Similarly, the content of isoflavonoids in prostate fluid is higher than plasma (Hedlund et al. 2005; Morton et al. 1997).

5 Molecular Targets of Isoflavonoids

Isoflavones with similar structure to 17β-estradiol have effects as estrogenic and anti-estrogenic agents. These compounds can bind to estrogen receptors ERα and ERβ and manipulate intracellular pathways which are important in cellular growth and protection. Isoflavones activity usually is lower than 17β-estradiol since they have weaker affinity to ERs compared to estradiol (Ko 2014). Aberrant proliferation, inflammation and the malignancy development are attributed to ERα, while ERβ oppose the activity of ERα on cell proliferation via modulation of expression of several genes, exhibiting anti-invasive and anti-migratory properties of cancer cells (Thomas and Gustafsson 2011). Soy isoflavones show selectivity toward ERβ over ERα *in vitro* that may suggest insight into activity of these compounds biologically. For instance, daidzein presents five-fold higher affinity for ERβ and genistein has 20- to 30-fold higher affinity toward ERβ than that for ERα (Kuiper et al. 1998). Comparing with natural ligand of ERs, 17β-estradiol, binding affinity of isoflavones is one to three order less (Sotoca et al. 2008). An active metabolite of daidzein, S-equol, provides binding preference comparable to genistein and greater than daidzein. In contrast, R-equol selectively binds to ERα (Muthyala et al. 2004). Balance of ERα/ERβ is changed in favor of ERα as a result of upregulation of ERα mRNA levels in the tumors. Therefore, response of cell by exposure of isoflavones depends on receptor affinity of the compounds as well as ERα/ERβ expression level (Leygue et al. 1998).

Isoflavones function is related to the kind of ER that they affect. In different cells and organs, the ERs receptors are expressed in different concentrations. In the breast, uterus, ovaries, testis, hypothalamus, pituitary glands, and liver that are the most target tissues of estrogen, ERα is mostly expressed. While, ERβ is mainly found in the lung, kidney, bladder, prostate, bones, heart, blood cells, intestine, and thymus and the receptor seems to relate to inflammation (Yakimchuk et al. 2013). Isoflavones bind to the ligand binding domain of the ERs and this receptor-ligand complex binds to estrogen response elements of DNA triggering transcription of target genes. Beside the classical genomic pathway, regulation of gene transcription by ERs can be perform by phosphatidylinositol 3-kinase/Akt (PI3K/Akt), Src/mitogen-activated protein (Src/MAP) kinase, and other direct DNA binding transcription agents such as specificity protein 1 (SP1), activation protein 1 (AP1), nuclear factor-κB (NF-κB), cAMP response element binding protein (CREB) or p53 (Castoria et al. 2001; Migliaccio et al. 1996; Thomas and Gustafsson 2011).

Levels of endogenous estradiol affect the biological function of isoflavones. In presence of high level of natural estrogens, isoflavones bind with ERα that inhibit effect of natural estrogens. Isoflavones bind with ERβ when the levels of natural

estrogens are low in women like in postmenopause and oophorectomy (Pilsakova et al. 2010). Depending on local concentrations of estrogen, isoflavones exert partial ER agonist and antagonist activity. In premenopausal women with high levels of estrogen, isoflavones have antagonist activity (Hwang et al. 2006; Ju et al. 2006; Qin et al. 2009; Velders et al. 2010).

Beside ERs, other main molecular targets of isoflavonoides include protein tyrosine kinase (PTK) and mammalian topoisomerase II. Kinases are one of the main targets of new specific anticancer medicines and genistein inhibits PTK in breast cancer cells (Davis et al. 2014; Ferrarelli 2013). In the ocean of the cellular kinome, 183 phopho-proteins are regulated by genistein. These data revealed that genistein regulated growth of cancer cells by inhibition of PTK, epidermal growth factor receptor (EGFR), platelet-derived growth factor receptors (PDGF-R), insulin receptor, Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene homolog (Fgr), proto-oncogene tyrosine-protein kinase Fyn (Fyn), and proto-oncogene tyrosine-protein kinase Src (Src) (Yan et al. 2010).

5α-Reductase is suppressed by isoflavones, the enzyme that converts testosterone to 5α-dihydrotestosterone. Aromatase which converts of testosterone to estradiol, is inhibited in low concentrations of isoflavones, while its activity improved when the isoflavones concentrations are high (Ko 2014). Sex hormone binding globulin (SHBG) is increased in the presence of isoflavones, since these compounds bind to SHGB and hence stimulate SHBG synthesis that alter concentrations of steroidal hormones. Total testosterone levels in men can be raised with high concentrations of isoflavones but levels of free testosterone are not changed due to increased SHBG uptake (Berrino et al. 2001; Celec et al. 2005). Follicle stimulating hormone (FSH) and luteinizing hormone (LH) can be increased with isoflavones rich diets in premenopausal women, while estradiol is increased in postmenopausal women (Hooper et al. 2009).

Thyroid hormone levels may be affected by high content of soy diet through stimulating of thyrotropin, however the levels of T3 (triiodothyronine) and T4 (thyroxine) do not change too much (Dillingham et al. 2007). Isoflavones competitively can bind to thyroid peroxidase that converts T3 to T4 and inhibit the conversion, however the effect may be small. Isoflavones themselves are changed to triiodoisoflavone (Doerge and Chang 2002).

Mitochondrial dependent pathways are main reporting mechanisms for pro-apoptotic effects of isoflavones. Status of caspase-3 beside ER status provides another pivotal determinant in different response of breast cancer cells in the presence of isoflavones. Caspase-3 knockdown in MDA-MB-231 causes the resistance to genistein, while transfection of caspase-3 induces apoptotic death in MCF-7 by genistein exposure (Yang et al. 2007). Several proposed molecular targets regarding isoflavonoids are indicated in Table 16.1.

Hypoxic microenvironment usually triggers angiogenesis by activation of various oxygen sensors, angiopoietins, growth factors, endothelial sensors, junctional molecules that finally will lead to enhanced vascularization and tumor growth (Hoff and Machado 2012). Cancer cells can spread and invade nearby tissues creating metastases (Berman et al. 2013). Medium and high concentrations of isoflavones

Table 16.1 Molecular targets and mechanisms of isoflavonoids effects against breast cancer cell lines

Cell line	Isoflavone	Mechanism	Reported effect	References
MCF-7	Equol with 4-hydroxy-tamoxifen (4-OHT)	Activate caspase-9 and caspase-7, and cytochrome-c release into cytosol	Potent apoptosis induction	Charalambous et al. (2013)
MCF-7	Daidzein	Mitochondrial caspase dependent pathway	Induces apoptosis	Jin et al. 2010)
MCF-7	Genistein	Activates calpain-caspase	Induces apoptosis	Shim et al. (2007)
MCF-7	Genistein	Induces DDIT3 and IRE1α associated withcell death	Induces apoptosis	Obiorah et al. (2014)
MDA-MB-231	Genistein	Inhibits the MEK5/ERK5/NF-κB pathway, down-regulation Bcl-2 and up-regulation of Bax	Inhibits cell growth and induces apoptosis	Li et al. (2008))
MCF-7	Genistein with 17β-estardiol	Induce phosphatidylserine externalization, LC3A/B immunopositivity modulates antioxidant enzyme, increase proapoptotic BAX/Bcl-2 ratio	Reduce cell proliferation independent of ER activation	Prietsch et al. (2014)
MDA-MB-231	Genistein and 17beta-Estradiol	Increase proapoptotic BAX/Bcl-2 ratio, concomitant decrease in ERK1/2 phosphorylation	Induce apoptosis and autophagy	Rajah et al. (2012)
MCF-7	Daidzein	Generates ROS, down-regulates of Bcl-2, and up-regulates of BAX, release of cytochrome C from the mitochondria into the cytosol activating caspase-9 and caspase-7	Induces apoptosis	Jin et al. (2010)
MCF-7	Daidzein, genistein, glycitein, coumestrol, resveratrol	Genistein, glycitein and resveratrol, all increase apoptosis and increase the Bax/Bcl-2 ratio	Reduce cell growth and induce apoptosis	Sakamoto et al. (2010)
MCF-7	Genistein (20 μM)	Inactivates the IGF1R-PI3K/Akt pathway and increases the Bax/Bcl-2 mRNA with protein expressions	Inhibits proliferation of cells and induces apoptosis	Chen et al. (Chen et al. 2015b, c)

(continued)

Table 16.1 (continued)

Cell line	Isoflavone	Mechanism	Reported effect	References
MCF-7	Genistein (1 μM)	Proliferative and stimulatory effects on IGF-1 receptor triggered by genistein by ER	Stimulation of growth of the ER positive human breast cancer (MCF-7) cells	Chen et al. (2007))
MCF-7	Genistein (10 nM) for 10–12 weeks	Down-regulates of the PI3-K/Akt signaling pathway	Inhibits estradiol-stimulated growth	Anastasius et al. (2009)
MCF-7	Calycosin and genistein	Reduction in activation of the PI3K/Akt, downregulated expression level of HOTAIR	Calycosin more potently inhibits cells proliferation than genistein in dose dependent manner, both induce apoptosis	Chen et al. (2015c)
MCF-7	Genistein and equol (10 μM)	Induce delayed and prolonged phosphorylation of ERK1/2 which is necessary for transactivation of ERα	Induce cell proliferation and S-phase entry	Liu et al. (2010)
ER-negative / erbB-2-overexpressing MCF-7 cells	Genistein (>10 μM)	Tyrosine kinase inhibitory activity	Inhibition of cells growth	Yang et al. (2010)
erbB-2-transfected ER+ MCF-7 cells	Genistein (2–10 μM)	p27/kip1 downregulation and increases ER-erbB-2 cross talk	Enhance cells proliferation	
MCF-7, MDA-MB-231, and BG-1 cells	Genistein (>10 μM)	Downregulation CXCR4 in MCF-7 (ER-positive), MDA-MB-231 (ER-negative), and BG-1 (ER-positive ovarian cancer cells),; genistein downregulates CXCL12 (unique ligand for CXCR4) in both MCF-7 and BG-1 cells	Inhibition of migration and invasion of the cells toward CXCL12	Hsu et al. (2009)
MCF-7 cells	Genistein (1–10 μM)	Upregulates CXCL12 mRNA levels	Stimulates invasion and metastasis of cells	

(continued)

Table 16.1 (continued)

Cell line	Isoflavone	Mechanism	Reported effect	References
HCC1395 cells	Genistein	Upregulation of TFPI-2, DNMT1, ATF3, and MTCBP-1, suppress invasion and metastasis, downregulation of MMP-2, MMP-7, and CXCL12 (they induce invasion and metastasis)	Decrease cell viability and inhibition of the invasion potential	Lee et al. (2007)
MDA-MB-231	Daidzein, R- and S-equol (50 μM)	Down-regulate of MMP-2 expression	Inhibit the invasion of cells	Magee et al. (2014)
MDA-MB-231	Treatment with genistein, genistin or daidzein (10–30 μM)	Unaffected MMP-3 activity	The compounds had minimal effects on cell invasion even at high concentrations	Phromnoi et al. (2009)
T47D	Genistein (1 μM)	Improves the antioxidant enzyme response, enhances mitochondrial biogenesis and functionality, a significant up-regulation of UCP2 and SIRT1	Reduction of oxidative stress	Nadal-Serrano et al. (2013)
MDA-MB-231 and MDA-MB-468	Genistein	Modulation of oxidant enzymes, generation of ROS and mobilization of endogenous copper ions, irreversible DNAdamage	Inhibits proliferation of the cells	Ullah et al. (2011)

BG-1 ovarian cancer cells, *MCF-7* MDA-MB-231, HCC1395, *T47D* breast cancer cells

(10–150 μM) provide anti-angiogenetic effects through inhibition of vascular endothelial growth factor/basic fibroblast growth factor (VEGF/bFGF) which is a key regulator of tumor angiogenesis. While low doses of genistein (0.1–10 μM) enhance secretion of VEGF in MCF-7 (ER positive) MELN (derived from MCF-7 cells) and MELP (derived from MDA-MB-231 cells and transfected with ERα), but not in MDA-MB-231 cells (ER negative), suggesting that for stimulation of VEGF presence of ERα is necessary (Buteau-Lozano et al. 2008; Fotsis et al. 1993; Kim 2003).

Regarding antioxidant activity of isoflavonoids, it was proposed that genistein has ability to chelate metals, scavenge radicals, inhibit production of hydrogen peroxide, and stimulate gene expression of catalase and superoxide dismutase genes (Mortensen et al. 2009). Equol is more active antioxidant than its precursor which is attributed to the absence of the 2,3-double bond in conjugation with a loss of the 4-oxo group. Genistein is more potent antioxidant than daidzein since it has three hydroxyl groups (Arora et al. 1998; Rüfer and Kulling 2006). Metabolites of

isoflavones including equol, 8-OH-daidzein, O-desmethylangolensin, and 1,3,5-trihydroxybenzene are also potent scavengers and chelate ferrous compound. Sulfate conjugation of isoflavones can reduce antioxidant property of the compounds. Reaction of isoflavonoids with lipid radicals can reduce oxidation of low-density lipoprotein (LDL) (Mortensen et al. 2009). Evidences suggest that soy proteins significantly decrease LDL-cholesterol (4–6%) and hypo-chlosterolemic effect of the soy protein is higher in hypercholestrolemic individuals than normal persons. In addition, soy protein also lower triglyceride levels (5%) and increase high-density lipoprotein (HDL) level (1–3%) (Anderson and Bush 2011; Harland and Haffner 2008; Messina 2016; Tokede et al. 2015; Zhan and Ho 2005).

6 *In Vitro* Studies

6.1 Breast Cancer

The leading cause of death among women is breast cancer and accounts for about 30% of diagnosed cancer in women (Siegel et al. 2016). Depends on the mutations that affect the cellular pathways considerable genetic heterogeneity is characterized in breast cancer which resulted in various prognosis and treatments (Davis et al. 2014). Over 60% of the anti-cancer medicines are derived of natural compounds or their derivatives. As a part of the daily diet, individuals consume many of these compounds creating the opportunity to use them as a preventing agent mostly in the early stage of the cancers (Uifălean et al. 2016). The potential of cytotoxic activity of soy isoflavones extensively studied in the last years, however there are much controversy regarding dose-dependent effects of these compounds (Russo et al. 2016; Uifălean et al. 2016). Low micromolar concentration of genistein (about 1 μM) inhibits expression of human epidermal growth factor receptor 2 (HER2), phosphorylation and promotor activity through ER-independent mechanism on BT-474 human breast cancer cells that expressed only ERβ (Sakla et al. 2007). HER2 positive breast cancer cells are fast growing and more aggressive with resistant to chemotherapy. Results of another study revealed that genistein and quercetin cause antiproliferation and apoptotic activities against MCF-7 cells which overexpress HER2 without inhibition of expression of HER2 or its phosphorylation activity. In MCF-7/HER2 cells, quercetin and genistein induce cell death through CD95/Fas/Apol receptor cell death. In addition, decrease in the level of phosphorylation of IκBα resulted in inhibition of phosphorylation of p65 and nuclear translocation in the nucleus (Seo et al. 2011a). Interference of methoxy form of genistein, biochanin, with upper kinases like IKKa/b may be the explanation of the event. Inhibition of NF-κB pathway can be another indirect target of genistein in MCF-7/HER2 cells (Manna 2012).

About 15% of breast cancers mostly in young and pre-menopause women is triple negative breast cancer (TNBC), that do not express ERα, HER2 and

progesterone receptor. This subtype is resistant to chemotherapy and cannot be approached by novel therapies like hormones. Genistein dramatically inhibits the growth of TNBC cells in dose and time dependently. In MDA-MB-231 cells, genistein induce apoptosis and G2/M cell cycle arrest through modification of expression of Bcl-xL and Bcl-2 as a NF-κB inhibition consequence (Pan et al. 2012).

In the absence of ERβ and constant ERα expression in T47D breast cancer cell line, genistein stimulate cell proliferation. While, growth of cancer cells which express ERβ fully is prohibited more effectively than that cells with no expression of ERβ (Pons et al. 2014; Sotoca et al. 2008). Therefore, isoflavones can influence proliferation of cancer cells, cell cycle arrest, and apoptosis depending on ERα/ERβ ratio (Pons et al. 2014). Similar to estrogen, isoflavones at high levels of ERα induce changes including promotion of DNA replication, upregulation of multiple factors in cell cycle, inhibition of apoptosis, and chromosome segregation in breast cancer cells. In the presence of inducible promotor to reconstitute the expression of ERβ, isoflavones attenuate cell growth factors and stimulate cell proliferation arrest agents (Dip et al. 2008). Although, genistein shows higher affinity for ERβ, in some cases that characterized by loss of ERβ expression and high level of ERα like high grade of lobular cancers and low to higher grade of ductal cancers, genistein can lead to detrimental effects (Huang et al. 2014). For these patients, consumption of soy and daily intake of phytoestrogens should be re-evaluated with special attention. Additionally, proliferation of ER positive breast cancer cells is induced by phytoestrogens at estrogen physiological concentrations, while growth inhibited and apoptosis promote in unexposed cells to estrogen *in vitro* (Chen et al. 2015a). As it mentioned, isoflavones exert both estrogenic and antiestrogenic effects based on the estrogen level, they act as antagonist in high concentrations of 17β-estradiol and as agonist in the low levels of estrogen (Hwang et al. 2006). Another factor associated with heterogeneity effects of isoflavones is their dose. At low concentrations of genistein, cell growth is promoted by its estrogenic effect, while higher concentrations of the compound inhibit cell growth with antiestrogenic effect (Allred et al. 2001; Choi et al. 2014; Hsieh et al. 1998; Maggiolini et al. 2001). This dual effect is not observed in ER negative cells and genistein exerts antiproliferative effects especially with high doses. Therefore, it seems that effect of genistein in low doses is mediated by ER and at high doses its activity is ER independent. Also, genistein in cells with high ERβ expression can exert additional antiproliferative effects (Uifalean et al. 2018). Possibly, genistein with low doses induces cell death via BAX/Bcl-2 pathway, while the compound with higher doses causes cytotoxicity through other mechanisms. Apoptosis is triggered by genistein in ER negative cell lines or knockdown of ERα suggesting that some apoptotic mechanisms may not require the expression of ER (Rajah et al. 2012; Sakamoto et al. 2010). Some studies suggest biphasic effect of genistein on growth of ER positive cells like MCF-7, in which low concentration of the compound (10^{-6} M) stimulates proliferation of the cancer cells and high concentration (>10^{-5} M) inhibits cancer cells growth (Chen et al. 2007; Chen and Wong 2004).

Genistein induces cell proliferation not exclusively via ERα interaction, but also through G protein coupled receptor 1 (GPER1), an alternative non-genomic

signaling pathway (Lucki and Sewer 2011). Production of cAMP, c-Src activation, and intracellular Ca^{2+} immobilization is stimulated by activation of GPER1. The transactivation growth factor receptor is activated followed by downstream pathways like mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) and PI3K/Akt. Even in the absence of ERs in MCF-7 cells, genistein induced c-fos expression and acid ceramidase gene (ASAH1) expression through GPER1-dependent pathway (Maggiolini et al. 2004).

Most of the studies have evaluated influence of genistein on the growth of breast cancer cells for short time of exposure, 48 and 72 h, which cannot reflect the long-time exposure to isoflavones by diet. The growth promoting effect of estrogen reduced by long term genistein treatment (10–12 weeks) in MCF-7 cancer cells. However, expression of ERα was not changed, the PI3-K/Akt decreased significantly (Anastasius et al. 2009). The PI3K/Akt/mammalian target of rapamycin (mTOR) and the Raf/mitogen-activated and extracellular signal-regulated kinase (MEK)/extracellular signal-regulated kinase (ERK) signaling pathways are interconnected and dysregulated in several human cancers. Targeting of these pathways can lead to superior activity toward multi-drug resistance in breast cancer (Ma et al. 2014; Saini et al. 2013). In both ER positive and negative breast cancer cells, genistein inhibits MAPK signaling pathway but via different mechanisms. High concentration of genistein (100 μM) causes release of Ca^{2+} from endoplasmic reticulum and activates p38 MAPK which trigger apoptosis in MCF-7 cells (Shim et al. 2007). While, genistein (5–20 μM) suppresses protein level of MEK5, total ERK5 with phospho-ERK5 dose dependently (Li et al. 2008).

Genistein and equol with low dose (10 μM) induce cell proliferation of breast cancer cell (MCF-7) through ERα and ERK signaling pathway. Co-treatment of cells with an ERK inhibitor abolishes the ERα transactivation suggesting that MAPK/ERK signaling pathway is essential for ER-mediated transcription (Liu et al. 2010). Low doses of genistein also stimulates growth in erbB-2-transfected ER positive MCF-7 cells through enhanced activation of ER, MAPK/ERK1/2 and PI3K/Akt signaling pathways sowing close cross-talk of ER-erbB-2 in breast cancer cells (Yang et al. 2010). The obtained results suggested that various concentrations of genistein cause cell responses via different signaling mechanisms (Table 16.1).

6.2 Prostate Cancer

The most protective roles of soy isoflavonoids in prostate cancers is growth inhibition of epithelial cells of prostate cancer. Genistein suppresses growth of LAPC-4 and PC-3 cells by anti-androgenic activity dose dependently (Mahmoud et al. 2013). Other contributed mechanisms include ability of isoflavones in modification of expression of involved central genes in cell cycle, cell survival, and apoptosis (Mahmoud et al. 2013; Moiseeva et al. 2007; Touny and Banerjee 2006). In several prostate cancer cell lines, soy isoflavonoids decrease androgen receptor, level of

PSA, inhibit activity of mTOR, and trigger growth arrest in several cancer cell lines of prostate (Mahmoud et al. 2014).

Mutational status of androgen receptors (AR) in prostate cancer cells beside dose of genistein affects biological outcomes of the isoflavonoids. In LAPC-4 cells with wild type of AR, genistein induces apoptosis and inhibits cell proliferation in a linear dose dependently. While, in LNCaP cells that express mutant AR endogenously, genistein showed a biphasic effect, in which genistein with physiological concentrations (less than 10 μM) stimulates cell proliferation and suppresses cell growth at doses of 25 μM and above. The stimulatory effect of genistein in LNCaP cells with low concentrations is abolished with Casodex, antagonist of androgen receptor, indicating that stimulatory activity of genistein in LNCaP cells is probably through T877A mutation in the androgen receptor of this cell line. Therefore, the results of the study suggested that bioactivity of genistein against prostate cancer cell lines depends on mutational status of androgen receptor. Genistein can activate mutant androgen receptor resulted in the stimulation of growth of prostate cancer cells, while in cancer prostate cells with wild type of androgen receptor shows inhibitory effect (Mahmoud et al. 2013).

Soy isoflavones directly up-regulated p21, a cyclin dependent kinase inhibitors (CDKIs), inducing apoptosis and cell cycle arrest in both LNCaP (androgen sensitive cell line) and PC-3 (androgen independent cell line) (Seo et al. 2011b). In another androgen independent prostate cancer cell line, DU145, genistein cause a significant induction of p27 and p21, reduction of cyclin dependent kinase 4 (CDK4) and moderate supression of CDK2, cyclin E and cyclin D1 (Agarwal 2000). Combination of daidzein and genistein increase expression of p53 and reduce cyclin B1 protein in LNCaP and PC-3 prostate cancer cell lines (Wang et al. 2009). High intake of soy decreased activity of hepatic aromatase and 5α-reductase, expression of AR, forkhead box A1 (FOXA1) proteins, urogenital tract weight, and tumor progression with upregulation of protective FOXO3 in TRAMP mouse model (Christensen et al. 2013).

Expression of several antioxidant enzymes is induced by isoflavones and these compounds preserve cells against DNA damage by free radical-induced. In LNCaP and PC-3 cells, high dose of genistein (100 μM) induces expression of GPx-1 gene leading to elevation of glutathione peroxidase enzyme activity (Suzuki et al. 2002). Role of GPx-1 protein is pivotal in protection of cells from oxidative damage by detoxifying H_2O_2 and reduction lipid hydroperoxides. Other essential antioxidant proteins like microsomal glutathione S-transferase 1, glutathione reductase, and metallothionein are also induced by genistein in LAPC-4 cells (Raschke et al. 2006). Genistein with dose of 10 μM induces expression of catalase and manganese-superoxide dismutase (Mn-SOD) in DU-145 prostate cancer cell lines, which is associated in reduction of ROS (Park et al. 2010). Genistein directly controls genes expression that their protein products are associated in DNA damage repair and programmed cell death and therefore offers preserve against carcinogenesis (Oki et al. 2004). Further molecular targets of isoflavonoids in prostate cancer cell lines are provided in Table 16.2.

Table 16.2 Molecular targets and mechanisms of isoflavonoids effects against prostate cancer cell lines

Cell line	Isoflavone	Mechanism	Reported effect	References
LNCaP	Genistein, daidzein, and equol	Inhibit MAPK related pathway genes, increase FOXO protein expression	Regulate cell cycle	Takahashi et al. (2006)
LNCaP, DU-145, PC-3	Genistein and daidzein	Modulation of DNA damage-signaling pathway and cyclin-dependent kinase-related pathway genes, down-regulation of EGF and IGF	Regulate cell cycle and angiogenesis	Rabiau et al. (2010)
PC-3	Genistein	Inhibits TCF/LEF-dependent transcriptional activity and inhibiting downstream of IGF-1R activation	Inhibit cell growth	Lee et al. (2012)
PC-3 and DU-145	Genistein	Abrogates TGF-β mediated enhancement in HSP27 and MMP-2 activity	Inhibits cell invasion and metastasis	Huang et al. (2005); Xu and Bergan (2006)
PC-3	Genistein	Reduces the mRNA expression of TGF-β and other genes wich regulated by TGF-β like MMPs	Downregulates invasion and angiogenesis	Li and Sarkar (2002a)
PC-3	Genistein	Increases E-cadherin protein leading to decrease in nuclear localization of β-catenin, Wnt/β-catenin inhibition resulting in an inhibition of TCF/LEF dependent transcriptional activity	Effective inhibition of cell growth	Rabiau et al. (2010); Takahashi et al. (2006)
LNCaP, DU-145, and PC-3	Genistein and daidzein	Interference with the growth stimulatory factor, IGF-1 and/or modulating its downstream signaling pathways via adjusting the IGF-1R/IRS-1 ratio	Inhibit cell proliferation	Lee et al. (2012)
PC-3	Genistein	Suppression of tyrosine kinase activity, reduction of the phosphorylation of Akt, GSK-3 and p70S6k and inhibition the Akt/FOXO3a/GSK-3/AR signaling network and Akt/mTOR signal transduction	Growth inhibition and apoptotic cell death	Oh et al. (2010)
LNCaP	Genistein and biochanin A	Downregulation of Bcl-2 and increase Bax mRNA and protein levels	Increase apoptosis	Kumar et al. (2011)
LNCaP and DU-145	Genistein	Enhances activity and expression of caspase-3	Induces apoptosis	Kumi-Diaka et al. (2000)

(continued)

Table 16.2 (continued)

Cell line	Isoflavone	Mechanism	Reported effect	References
LNCaP and PC-3	Soy extract, genistein or daidzein	Induce caspase activation, increase Bax expression in PC-3 cells	Induce cell cycle arrest and apoptosis, soy extract is more potent	Hsu et al. (2010)
PC-3	Genistein	Down-regulates the expressions of MMP-9, uPA, uPAR, protease M, PAR-2, VEGF, VEGFR, TGF-β, BPGF, LPA, and TSP, and up-regulated the expressions of connective tissue growth factor and connective tissue activation peptide	Inhibits angiogenesis, invasion and metastasis	Li and Sarkar (2002b)
LNCaP and PC-3	Genistein	Decreases COX-2 mRNA and protein expression, reduces the secretion of PGE2, EP4 and FP PG receptor mRNA	Exerts anti-proliferative effect	Swami et al. (2009)
PC-3	Genistein	Significant inhibition of both the basal and the hypoxia-stimulated VEGF expression partly by the ability of genistein to reduce nuclear accumulation and activity of HIF-1, downregulation of APE1/Ref-1, which is responsible for redox activation of HIF-1	Inhibits prostate tumor angiogenesis	Guo et al. (2007); Pines et al. (2005); Raffoul et al. (2007)
LNCaP, PC-3, and DU-145	Genistein and daidzein	Down regulate ECGF1, FGF1, IGF1, FGFR3, IL-1, IL-6, IL-8, CXCL10, platelet/endothelial cell adhesion molecule (CD31 antigen or PECAM1)	Inhibit angiogenesis	Handayani et al. (2006); Rabiau et al. (2010)

LNCaP, DU-145, PC-3: human prostate adenocarcinoma cells
ATF3 activating transcription factor 3, *APE1/Ref-1* apurinic apyrimidinic endonuclease redox effector factor-1, *Bcl-2* B-cell lymphoma 2, *BAX* Bcl-2-associated X protein, *BPFG* bone-derived growth factor, *COX-2* cyclooxygenase-2, *DDIT3* DNA damage-inducible transcript 3, *DNMT1* DNA methyltransferase 1, *ER* estrogen receptor, *EGF* epidermal growth factor, *ERK* extracellular signal-regulated kinase, *FGF1* fibroblast growth factor 1, *FOX* Forkhead box, *HSP* heat shock protein, *HOTAIR* homebox transcript antisense RNA, *IRE1α* inositol requiring protein 1 alpha, *IGF-1R* insulin-like growth factor 1 receptor, *LPA* lysophosphatidic acid, *MAPK* mitogen-activated protein kinase, *MMP* matrix metalloproteinase, *MTCBP1* membrane-type 1 matrix metalloproteinase cytoplasmic tail-binding protein-1, *MEK5-ERK5* mitogen-activated protein kinase kinase 5-extracellular signal-regulated kinase 5, *NF-κB* nuclear factor κ-B, *ECGF1* platelet-derived endothelial cell growth factor, *EP4* prostaglandin receptor subtype, *FP* prostaglandin receptor subtype FP, *PAR-2* protease activated receptors, *Akt* protein kinase B, *PI3K* phosphoinositide 3-kinases, *ROS* reactive oxygen species, *SIRTs* Sirtunins, *(TCF/LEF* T-cell factor/lymphoid enhancer factor, *TSP* thrombospondin, *TFPI-2* tissue factor pathway inhibitor-2, *HIF-1* transcription factor hypoxia-inducible factor-1, *TGF-β* transforming growth factor-β, *uPAR* urokinase plasminogen activator receptor, *UCPs* uncoupling proteins, *VEGF* vascular endothelial growth factor

7 Observational and Clinical Trials

7.1 Breast Cancer

Soy consumption among Asian and Western populations is greatly different, therefore, these two groups have to be evaluated separately. The first research of protective property of phytoestrogens intake against breast cancer was published in 1991 for Chinese women (Lee et al. 1991). A meta-analysis of observational studies which include eight case-control and a cohort studies assessed total soy intake in Asian-American or Asian populations (cut off point 5–20 mg/day). The analysis of the study revealed a statistically significant risk reduction of breast cancer by 29% (OR = 0.71; 95% CI = 0.6–0.85) in Asian women. Consumption of moderate amount of isoflavones (10 mg/day) caused a significant risk reduction of 12% (OR = 0.88; 95% CI = 0.78–0.98). The study suggested a significant inverse correlation between isoflavones consumption and risk of breast cancer in both pre- and postmenopausal women (Wu et al. 2008b). The findings of a large prospective cohort (include 35,303 Singapore Chines women) were also confirmed a significant risk reduction of breast cancer by consumption of soy (>10.6 mg isoflavone/1000 kcal) compared with lower daily intake (RR = 0.82; 95% CI = 0.70–0.97). In a subgroup analysis of postmenopausal women with body mass index (BMI) > 24 kg/m^2, even more significant risk reduction was found (RR = 0.67; 95% CI = 0.51–0.88) Wu et al. 2008a). Similarly, more protective effect of soy was found among women with higher waist-to-hip ratio or BMO or higher serum estradiol (>5.73 pg/mL) (Dai et al. 2003). Among cohort studies, some showed no association while no studies indicated higher risk of breast cancer in connection with soy isoflavones. An inverse association was reported between breast density as a marker of breast cancer by soy consumption or soy intake in equol producers only (Frankenfeld et al. 2004; Nagel et al. 2005).

Among 44 case control studies, 32 revealed that high ingestion of soy foods or isoflavones were related to lower risk of primary breast cancer. No study declared significant increase risk of breast cancer in relation with soy intake. Soy intake greater than 1 serving of soy food or 6.25 g soy protein or 12.5 mg isoflavones daily was related with higher protective effect compared to lower intake (Fritz et al. 2013). Although, these studies revealed overall protective effect of soy in breast cancer, no modification of this effect by receptor status was found. The results of a research suggested that intake of soy isoflavones was not related with risk of breast cancer in adulthood for any receptor type (ER+/progesterone receptor (PR+), ER−/PR−, or ER+/PR−). While, higher intake was associated with lower odds in the mixed receptor type (OR = 0.77; 95% CI = 0.60–0.99) during adolescence (Anderson et al. 2013). Soy provides protective effect among all ER+, ER−, PR+, and PR− tumor types, but the activity was highest toward ER+/PR+ and ER−/PR− tumors, as opposed to mixed types ER−/PR+ and ER+/PR− (Zhang et al. 2009). Significant protective effect of soy against ER+, PR+ and Her2− tumors was also confirmed by other studies (Suzuki et al. 2008; Touillaud et al. 2005). In addition, higher soy food consumption in childhood ranging from ≥1 to ≥4 serving per day has protective

effect against breast cancer in adulthood (Korde et al. 2009; Shu et al. 2001; Thanos et al. 2006; Wu et al. 2002, 2009).

The nested case control studies that embedded within larger cohorts' studies showed small significant association between serum levels of daidzein and serum and urine levels of equol and increase risk of breast cancer. No significant activity in either direction was revealed in five studies. (Goodman et al. 2009; Li et al. 2005; Maskarinec et al. 2006b; Shannon et al. 2005; Ward et al. 2008). Doubling of daidzein and equol levels increased 30–45% odds of breast cancer. Thirty-nine percent of the involved population were equol producers. There were no association found between other isoflavones like glycitein, genistein, or O-desmethylangolensin (O-MDA), another daidzein metabolite (Grace et al. 2004).

Among prospective studies on recurrence and survival of soy intake in breast cancer patients some reported decrease in breast cancer recurrence and mortality, while others found no significant association (Boyapati et al. 2005; Caan et al. 2011; Fink et al. 2007; Kang et al. 2012; Kang et al. 2010; Woo et al. 2012; Zhang et al. 2012). However, other studies showed protective effects or even significant decrease in longer disease-free survival or recurrence of breast cancer risk associated with higher soy intake (Caan et al. 2011; Guha et al. 2009; Kang et al. 2010; Shu et al. 2009; Woo et al. 2012). Another research reported lower risk of recurrence only among post-menopausal patients (HR = 0.67; 95% CI = 0.54–0.85) (Kang et al. 2010). Protective effects to reduce recurrence were indicated for >15.31 g soy protein or >62.68 mg soy isoflavones and >42.3 mg soy isoflavones in post-menopausal women (Kang et al. 2010; Shu et al. 2009). The influence of soy intake on breast cancer mortality were seen for >15.78 mg soy protein or >35.3 mg soy isoflavones (Kang et al. 2012); >28.83 mg soy isoflavones or >13.03 soy protein (Zhang et al. 2012); or >7.48 mg soy isoflavones (Fink et al. 2007). Risk of death was also reported to decrease in tamoxifen users by consumption of total isoflavones ≥6.3 mg/day (median 26.7 mg; HR = 0.26, 95% CI = 0.06–1.01) and in ER+ and PR+ status (HR = 0.31, 95% CI = 0.10–0.98) but was not significant (Caan et al. 2011).

The results of several uncontrolled trials provide evidences of no harm of soy consumption relevant to breast cancer risk (Hall et al. 2007; Palacios et al. 2010). In some randomized controlled trials, no significant impact on levels of circulating estrogens (E1, E2, and E3) from soy was found compared to control group (Brown et al. 2002; Khan et al. 2012; Kumar et al. 2002; Martini et al. 1999; Maskarinec et al. 2002, 2004; McLaughlin et al. 2011; Morimoto et al. 2011; Nagata et al. 1998; Nikander et al. 2003; Pop et al. 2008; Steinberg et al. 2011; Wu et al. 2005; Zittermann et al. 2004). However, a significant decrease in circulating estrogens was found in other studies compared to the control group (Duncan et al. 1999, 2000 Lu et al. 2001). One study revealed that taking soy protein caused reduction of estrone (E1), androstenedione, testosterone, dehydroepiandrosterone, and cortisol with increase in progesterone, estradiol ratio, follicle stimulating hormone (FSH) and sex hormone binding globulin (SHBG) only in equol producers compared to non-producers (Duncan et al. 2000). Same researchers reported no significant effect on levels of serum estradiol, FSH, or LH with significant reduction in estrone (E1) and

increase in SHBG (Duncan et al. 1999). No RCTs showed significant increase in circulating estrogens. Mammographic density was not significantly influenced by soy compared to the control group after adjustment of baseline density (Kataoka et al. 2008). Genetic markers of breast cancer risk including BRCA1 and 2 mRNA levels due to soy intake showed no changes (Marini et al. 2008). After 2 weeks delivering 60 g soy protein containing 45 mg isoflavones daily, *in vivo* markers of breast epithelial proliferation increased which showing significant increase in cells in S-phase and expression of progesterone receptor (McMichael-Phillips et al. 1998). Consumption of supplement containing genistein (150 mg), daidzein (74 mg), and glycitein (11 mg) had no effect on Ki-67labeling index, a cellular marker of proliferation, for 6 months (Khan et al. 2012).

7.2 Prostate Cancer

The most common malignancy in men is prostate cancer and despite of its high occurrence, little is known about the etiology. Although, incidence of prostate cancers is less in Asians, this malignancy has an increased incidence in Asian migrants (Jemal et al. 2011). Therefore, environmental factors like diet may have a pivotal role in prostate carcinogenesis. Asian individuals traditionally consume food containing soy which is rich in isoflavonoids, genistein and daidzein (Kimura 2012). In Japanese men, plasma concentrations of isoflavonoids are 10–100 times higher than European men (Adlercreutz et al. 1993). In addition, levels of daidzein and equol in prostate fluid in Asian are much higher than Western men. Epidemiologic studies have shown a lack of association between soy foods and isoflavones intake and prostate cancer (Mahmoud et al. 2014). The meta-analysis of epidemiologic studies showed that phytoestrogens including genistein (OR = 0.87; 95% CI: 0.78–0.98), daidzein (OR = 0.85; 95% CI: 0.75–0.96), and glycitein (OR = 0.89; 95% CI: 0.81–0.98) were associated with decrease in prostate cancer risk. While, there is no relation between total isoflavones (OR = 0.93; 95% CI: 0.84–1.04), total lignans (OR = 0.05; 95% CI: 0.54–2.04), equol (OR = 0.86; 95% CI: 0.66–1.14), secoisolariciresinol (OR = 1.02; 95% CI: 0.83–1.24), enterolactone (OR = 0.94; 95% CI: 0.73–1.20), matairesinol (OR = 0.91; 95% CI: 0.75–1.11), and coumestrol (OR = 0.89; 95% CI: 0.76–1.06). The findings of the study revealed that some phytoestrogens may play role in reduction prostate cancer risk (Zhang et al. 2017). Association between different types of soybean consumption and risk of prostate cancer were analyzed in another meta-analysis of observational studies. Total soy foods (OR = 0.69; 95% CI: 0.57–0.84) and nonfermented soy foods (OR = 0.75; 95% CI: 0.62–0.89) reduce risk of prostate cancer. While, digestion of soybean milk, natto, or miso did not significantly decrease risk of prostate cancer. Lower risk of prostate cancer was found in association with genistein and daidzein. The authors of the meta-analysis concluded that soy food ingestion may lower the risk of prostate cancer, however the findings have to be interpreted by caution since the results of meta-analysis can be affected by various biases (Hwang et al. 2009).

By consumption of lignans which have estrogenic effect, enterolactone is formed in the human gut that has association with risk of prostate cancer. A nested case-control study evaluated the association between enterolactone plasma levels and prostate cancer. Results showed that there is no significant association between plasma enterolactone levels and incidence of all prostate cancers (OR = 0.99; 95% CI: 0.77–1.280). While, in certain subgroup of men with abdominal obesity there is an association between high enterolactone levels and lower odds of high-risk prostate cancer (Wallström et al. 2018). Data from a population-based case-control study in Italy indicated that isoflavones (OR = 0.28) and specially genistein (OR = 0.40) were associated with reduced risk of prostate cancer (Russo et al. 2018). Subgroup analysis in a systematic review with meta-analysis showed association between phytoestrogen consumption and reduced risk of prostate cancer in Asians and Caucasians but not among Africans (M Zhang et al. 2016).

The findings of some clinical trials revealed that soy protein or isoflavonoids has no effects on serum levels of prostate-specific antigen (PSA) in healthy men, men with high risk of prostate cancer, men with localized prostate cancer, or men with failure of biochemical therapies (Adams et al. 2004; Bosland et al. 2013; deVere White et al. 2010; Hamilton-Reeves et al. 2007a; Jenkins et al. 2003; Kumar et al. 2004; Maskarinec et al. 2006a, b; Urban et al. 2001). While, other trials revealed that soy isoflavones can reduce levels of PSA in men after surgical intervention with biochemical failure, or in those with high PSA before therapy and localized prostate cancers (Dalais et al. 2004; Grainger et al. 2008; Hussain et al. 2003; Kwan et al. 2010; Lazarevic et al. 2011; Pendleton et al. 2008). Additionally, other endpoints rather than PSA were addressed in small number of clinical studies such as VEGF, prostaglandins, anti-apoptotic and proliferative markers, and expression of androgen receptor (Grainger et al. 2008; Hamilton-Reeves et al. 2007a, b; Swami et al. 2009). In a randomized, double-blind, placebo-controlled crossover study, administration of soy-based dietary supplement containing lycopene 15 mg, isoflavone 62.5 mg, silymarin 160 mg, Vit. E 75 mg, Vit. C 225 mg, pyridoxine 2.6 mg, riboflavin 2.5 mg, folic acid 400 g, cyanocobalamin 3 g, carotenoids 3 mg, CoQ10 4 mg, bioflavonoids 19 mg, zinc 18 mg, selenium 128 g, copper 2.7 mg, manganese 5 mg, and N-acetyl-L cysteine 500 mg delayed progression of PSA in a significant fashion after potentially curative treatment in men with prostate cancer (Schröder et al. 2005).

Another double-blind, placebo controlled, randomized trial was performed in 53 men with prostate cancer that provided supplement containing 300 mg daidzein and 450 mg genistein for 6 months. Serum concentrations of daidzein and genistein were higher significantly than baseline in subjects, while equol levels did not change. The findings revealed that dietary supplements did not decrease levels of PSA in men with low volume prostate cancer (deVere White et al. 2010). A systematic review of randomized trials analyzed findings of phytotherapeutic interventions in the management of recurrent prostate cancer. Soy isoflavones, lycopene, sulphorphane, pomegranate extract, and turmeric, pomegranate, broccoli sprout, and green tea extract are safe and well-tolerated in subjects. However, high-quality studies are lacking, limited evidence are available to support that they can affect PSA

dynamics. The review concluded that no recommendation can be provided until high-quality, full powered, placebo-controlled studies are conducted (van Die et al. 2016).

8 Conclusion

Isoflavones with similar structure and molecular weight with 17β-estradiol show estrogen like properties and more weakly binds to ERs compared to physiologic estrogen. In addition, *in vitro* studies suggested that ERα/ERβ ratio in cancer cells can determine whether the compounds cause apoptosis or cell proliferation (Pons et al. 2014; Sotoca et al. 2008). Isoflavonoids have higher affinity for ERβ and in the cases of loss of ERβ expression with high level of ERα like lobular cancers, genistein can cause detrimental effects and consumption of soy and phytoestrogen have to be consider by caution (Huang et al. 2014).

Concentrations of estrogens also affect whether isoflavones exert estrogenic or antiestrogenic properties, they act as antagonist in high concentrations of 17β-estradiol and as agonist in the low levels of estrogens (Hwang et al. 2006). Heterogeneity effects of isoflavones attributed to their doses as well. At low concentrations of genistein, cell growth is promoted by its estrogenic effect, while higher concentrations of the compound inhibit cell growth with antiestrogenic effect (Allred et al. 2001; Choi et al. 2014; Hsieh et al. 1998; Maggiolini et al. 2001).

Besides *in vitro* studies, observational reports suggested inverse correlation between isoflavones consumption and risk of prostate or breast cancers. While, some cohort studies showed no association, case control studies suggested higher association between serum levels of isoflavonoids and breast cancer (Dai et al. 2003; Frankenfeld et al. 2004; Hwang et al. 2009; Wu et al. 2008a; Zhang et al. 2017). Similarly, some prospective studies reported decrease in breast cancer recurrence and mortality, however some others found no significant association (Kang et al. 2012; Woo et al. 2012; Zhang et al. 2012). The results of these studies have to be interpreted by caution since their findings can be affected by various biases. In spite of observational studies, RCTs did not find significant changes in cancer markers in individuals. Overall, considering the obtained facts about molecular mechanisms of isoflavonoids activity which can vary depending on their concentrations or physiological status of the cells or individuals including the age, ethnicity, intestine microflora, and diet habits, it seems that administration of these compounds has to be tailored according to the individual characteristic of each person. Additionally, more clear recommendations can be provided by further high-quality, full powered, placebo-controlled studies considering individual characteristics as subgroups.

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Chapter 17
Tea (Catechins Including (−)-Epigallocatechin-3-gallate) and Cancer

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Abstract Catechins, a group of phenolic compounds (flavan-3-ols), are one of most widely studied plant secondary metabolites regarding their diverse pharmacological actions. Found in many foods and beverages including tea, catechins are reported to be useful for the prevention and treatment of cancer in *in vitro* and *in vivo* studies. Various signalling mechanisms have also been explored for the cancer chemopreventive activities of tea and tea catechins. However, the translational research on these compounds to clinical studies have not been performed in detail as compared to *in vitro* and *in vivo* studies. This chapter critically discusses the role of catechins in cancer prevention and treatment with special focus on their mechanism of action on signaling pathways.

Keywords Tea · Catechins · Cancer · Cancer signalling · Cancer chemoprevention

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1 Introduction

Natural polyphenols present in various fruits, vegetables, tea, coffee, legumes, among others, have played important role in maintaining of human health having nutraceutical, disease preventive and therapeutic effects (Pietta 2000; Petti and Scully 2009; Ganesan and Xu 2017). The young, tender leaves of tea plant (*Camellia sinensis* (L.) Kuntze, Syn.: *Thea sinensis* L., Theaceae) (Fig. 17.1) are used from the ancient time to prepare diverse tea formulations such as green tea, oolong tea, black tea, white tea and matcha powder among others (Kim et al. 2011; Carloni et al. 2013). Tea leaves are also used in traditional medicines in China, Korea and Japan. In Japan, crude drug obtained from tea leaves known as "Chyayou" is used in head and eye disorders such as headache and blindness. It is also included in official Kampo formulations such as "Senkyuchyatyousan" and "Shirenmeimeto". In

Fig. 17.1 Photographs of tea plants and black and green tea; (**a**) tea plantation, (**b**) tea flower, (**c**) young tea leaves, (**d**) green tea and (**e**) black tea

general practice, the tea is used as gargle to prevent from sore throat and cold. Strong tea is recommended in bacterial diarrhea (Watanabe et al. 2018). Tea is believed to be the second most consumed drink worldwide only after water (Kim et al. 2011). The global tea market in 2018 was valued at over 52 billion USD and it is expected to rise to more than 81 billion USD by 2026 (https://www.statista.com/statistics/326384/global-tea-beverage-market-size/). At current times, China is reported to be the leading producer of tea followed by India and Kenya. With the increasing scientific studies on tea and its constituents and their effects in human health, tea is becoming more and more popular as casual drink and also for its functional properties.

Various polyphenolic compounds are present in the tea leaves including flavan-3-ols, commonly known as tea catechins, and phenolic acids such as gallic acid (Carloni et al. 2013). Tea catechins are one of the most widely studied plant natural products for the chemical and pharmacological aspects such as antioxidant, cancer chemopreventive, anti-inflammatory, immunomodulatory activities (Wai et al. 2018; Khan et al. 2019). Among these catechins, (−)-epigallocatechin-3-gallate (EGCG) is the most studied for such activities. The main aim of this chapter is to critically analyse the role of catechins in cancer prevention and treatment with special focus on their mechanism of action on signalling pathways.

2 Chemical Aspects of Tea Catechins

The quantity and composition of catechins and other chemical constituents in tea formulations depend upon various factors related to tea leaves cultivation and collection. This includes variety of tea plant, environmental factors, conditions related to cultivation, collection time, processing of tea leaves after collection and further formulations such as extraction conditions (Zhao et al. 2011; Carloni et al. 2013). Flavan-3-ols, known as catechins (eg. (+)- catechin, (−)-epicatechin (EC), (−)-epicatechin-3-gallate (ECG), (−)-epigallocatechin (EGC) and (−)-epigallocatechin-3-gallate (EGCG)) (Fig. 17.2) are the most widely studied chemical constituents in both tea leaves and tea formulations. However, there are many other biologically important chemicals present such as flavonols (e.g. kaempferol and quercetin derivatives), phenolic acids (gallic acid, caffeic acid, gallic acid glucosides and their derivatives), proanthocyanidins, amino acids (e.g. L-theanine, gamma amino butyric acid (GABA)), methyl xanthines (caffeine, theophylline, theobromine) and volatile compounds (e.g. pentanal, heptanal, 2-butanone) (Kim et al. 2011; Zhao et al. 2011; Ananingsih et al. 2013; Carloni et al. 2013).

After the collection, young tea leaves are further processed and on the basis of processing they are classified into three main categories e.g. non-fermented (white tea, green tea), partially fermented (oolong tea) and fully fermented (black tea) (Zhao et al. 2011). However, there are many other varieties such as matcha tea powder in which tea plants are cultivated under shade (about 90%). Then the leaves are picked, dried then ground to make powder (Kurauchi et al. 2019). During the

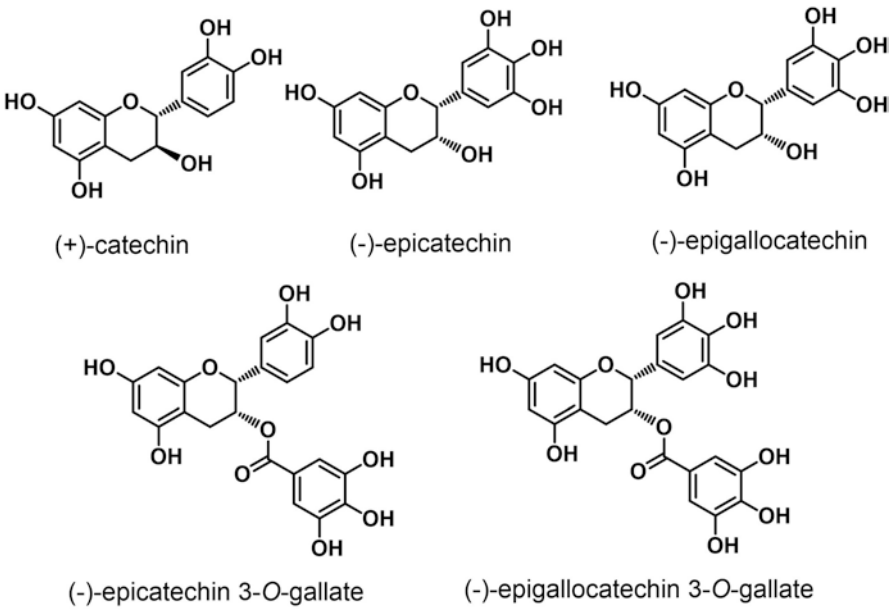

Fig. 17.2 Chemical structures of major tea catechins

fermentation process, the enzymatic oxidation of polyphenols by the enzyme polyphenol oxidase results in the generation of theaflavins and thearubigins, which are responsible for characteristic color and aroma (Zhao et al. 2011). Various research articles have reported the reduced antioxidant activity of the tea infusion/extracts obtained from fermented tea varieties as compared to green tea and its extracts. Studies have reported the anticancer and other health beneficial activities of the extracts obtained from these various varieties and pure isolated compounds such as EGCG (Carloni et al. 2013; Sonoda et al. 2014).

3 Catechins: Bioavailability and Metabolism

Though consumed widely around the globe and the majority of studies have shown the biological activities of tea and catechins in *in vitro* systems, one of the main obstacles in obtaining the similar data in *in vivo* systems is the poor bioavailability of tea catechins (Cai et al. 2018). Lin et al. (2007) investigated the pharmacokinetic profile of EGCG in freely moving rats and reported that the oral bioavailability was only about 5%. The plasma protein binding was about 92%. EGCG crossed blood-brain barrier at lower concentration. Further, the elimination half-life was reported to be 62 ± 11 and 48 ± 13 min for intravenous (10 mg/kg) and oral (100 mg/kg) administrations, respectively. In another study in rats, only about 14% of EGC, 31%

of EC and less than 1% of EGCG was reported to be measured in blood after oral administration (Chen et al. 1997).

Warden et al. (Warden et al. 2001) investigated the absorption of tea catechins in men and women after drinking the black tea containing 15.48, 36.54, 16.74, and 31.14 mg of EGC, EC, EGCG and ECG, respectively, at four time points (0, 2, 4 and 6 h). Only about 1.68% of administered catechins were reported to be present in plasma, urine and faeces after tea ingestion over 6 h and the bioavailability of the gallated forms of catechins was lower than that of the free forms. In another study, Yang et al. (1998) reported that the maximum plasma concentration for EC, EGC, and EGCG, were 0.6, 1.60 and 0.57μM, respectively in humans after administration of 3 g of decaffeinated green tea.

Absorbed catechins undergo phase II metabolism by enzymes such as uridine 5′-diphospho (UDP)-glucuronosyltransferases (UGTs), sulphotransferases (SULTs) and catechol-*O*-methyltransferase (COMT) in liver and are converted to their respective glucoronosyl, sulphate and methylated metabolites (Lambert et al. 2007; Cai et al. 2018). Catechins, their conjugated metabolites and other simple phenolic acid metabolites are then distributed to various organs and tissues.

4 Therapeutic Potential of Catechins

Tea catechins are well studied for their health beneficial activities related to not only cancer but many other pharmacological activities such as antioxidant, anti-obesity, anti-hyperlipidemic, aging, diabetes and many others (Zaveri 2006). A Scopus search (www.scopus.com) with the keyword “tea AND catechins” resulted total 6998 documents and the keyword “tea AND catechin AND activity” resulted total 3798 documents (accessed on May 29, 2020). Cancer chemopreventive activity is one of the widely studied and discussed activity of tea formulations, tea extracts and catechins which are discussed in detail in this chapter. Many review articles are also published in these aspects of tea catechins (Boehm et al. 2009; Khan and Mukhtar 2010; Yang and Wang 2016). Review articles have also extensively covered the other activities such as antioxidant activity (Higdon and Frei 2003; Gramza and Korczak 2005), obesity, diabetes and other metabolic diseases (Higdon and Frei 2003; Gramza and Korczak 2005; Kao et al. 2006; Zaveri 2006; Park et al. 2009; Masterjohn and Bruno 2012; Legeay et al. 2015), cardiovascular diseases (Hodgson and Croft 2010), cognitive functions (Weinreb et al. 2004; Da Silva Pinto 2013; Pervin et al. 2018), antimicrobial activities (Taylor et al. 2005; Reygaert 2014) among others. Not only as a drink or as a potential medicines, tea infusion and catechins are also widely used as food supplements and functional foods (Hara 2011; Namal Senanayake 2013; Sanna et al. 2015; Kurauchi et al. 2019).

5 Catechins and Cancer

Activities of tea extracts and isolated compounds including catechins have been extensively studied for the prevention and treatment of cancer through *in vitro* and *in vivo* systems. A Scopus search results with different key words (e.g. tea AND catechins AND breast cancer, tea AND catechins AND lung cancer) related to cancer is represented in Fig. 17.3. As per the results, activities related to breast cancer, lung cancer, skin cancer and colon cancer are widely reported. Some of the studies are reported below.

5.1 Lung Cancer

Lung cancer is the most common form of cancer worldwide with more than half of the patients in developing countries (Wong et al. 2017). Lung adenocarcinoma, squamous cell carcinoma, small cell carcinoma and and large cell carcinoma are the main types of lung cancers (Wong et al. 2017; Malyla et al. 2020). Various plants based single compounds has been explored for their promising activity against lung cancer. Deng and Lin (2011) reported the potent inhibitory activity of EGCG against matrix metalloproteinase-2 (MMP-2) which in turn inhibited the invasion of highly invasive CL1-5 lung cancer cells. Wnt signalling is an important pathway in non-small cell lung cancer (NSCLC) progression as overexpression of Wnt-1, -2, -3, -5a is common in resected NSCLC and associated with poor survival while inhibition of Wnt reduces NSCLC proliferation. Xie et al. (2017) reported that ECGC

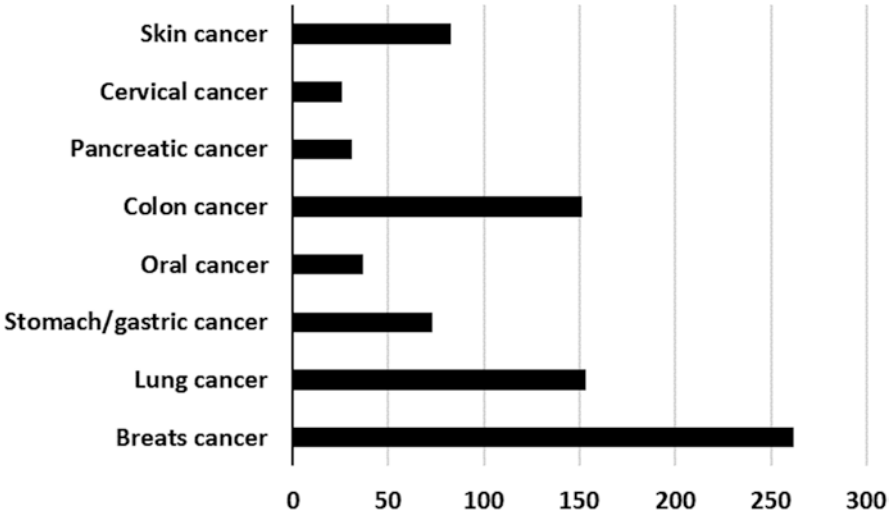

Fig. 17.3 No. of publication related to tea catechins and cancer of different organs

inhibited the lung cancer stem cells through Wnt/b-catenin pathway along with suppression of proliferation and induction of apoptosis. Similarly, Zhong et al. (2012) reported the *in vitro* anti-proliferative activity of green tea catechins against human lung cancer cells (NCI-H446 and MSTO-211H) by upregulation of let-7 (a microRNA function as tumor suppressor). Furthermore, another *in vitro* study on human lung adenocarcinoma cell line (A549) showed that green tea catechin, EGCG possess potent anti-cancer activity by attenuating the cell proliferation *via* Bcl-xL expression (Sonoda et al. 2014) .

5.2 Colorectal Cancer

Colorectal cancer also known as colorectal adenocarcinoma is reported to be the third leading cause of cancer mortality globally (Rawla et al. 2019). The application of EGCG to HT-29 colon cancer cell lines resulted into ER stress by upregulation of immunoglobulin-binding (BiP), PKR-like endoplasmic reticulum kinase (PERK), phosphorylation of eukaryotic initiation factor 2 alpha subunit (eIF2α), and activation of transcription 4 (ATF4), and inositol-requiring kinase 1 alpha (IRE1α) (Md Nesran et al. 2019). Haratifar et al. (2014) studied the effects of casein micelles of EGCG in HT-29 cells and reported that nanoencapsulation did not reduce the antiproliferative activity of EGCG and can be a good drug deliver carrier for EGCG.

5.3 Breast Cancer

Breast cancer is the most common cancer in women (Hu et al. 2019). Various in vitro, in vivo and human studies have been performed to evaluate the effectiveness of tea catechins in breast cancer. For example, Zhang et al. (Zhang et al. 2012) studied the effect of oral administration of 400 mg EGCG (three times/day) in breast cancer patients receiving radiotherapy. Compared to the group receiving only radiotherapy, patients receiving radiotherapy+EGCG expressed lower serum levels of vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), and reduced activation of metalloproteinase-9 and metalloproteinase-2 (MMP9/MMP2). Treating cultures of highly-metastatic human MDA-MB-231 breast cancer cells with the serum obtained from radiotherapy + EGCG treated patients resulted into supressed cell proliferation and invasion, arrest of cell cycles at the G0/G1 phase. Treated cells also showed reduced activation of MMP9/MMP2, expressions of Bcl-2/Bax, c-Met receptor, NF-κB, and the phosphorylation of Akt. Based on these data, authors suggested that EGCG may act as an effective adjuvant in radiotherapy for breast cancer patients.

5.4 Prostate Cancer

Prostate cancer, the second most commonly diagnosed cancer in men globally, is the six leading cause of death worldwide (Culp et al. 2020). Among different tea catechins, EGCG is reported as most effective agent in prostate cancer based on *in vitro* and animal studies (Davalli et al. 2012; Du et al. 2012; Miyata et al. 2019). However, the randomized, placebo-controlled clinical trial of polyphenon-E (a standard mixture of tea catechins including EGCG) in 97 men with high-grade prostatic intraepithelial neoplasia (HGPIN) and/or atypical small acinar proliferation (ASAP) showed that the administration of the catechin mixture for 1 year did not reduce the likelihood of prostate cancer with baseline HGPIN or ASAP (Kumar et al. 2015).

5.5 Gastric Cancer

Gastric cancer is one of the most common cancers worldwide as it is reported to be fifth most common cancer with third highest rate of mortality (Rawla and Barsouk 2019). Hibasami et al. (1998) reported that the treatment of human stomach cancer KATO III cells with green tea extract and EGCG resulted into growth inhibition and apoptosis. Similarly, Yang et al. reported that the EGCG inhibited the proliferation and induced apoptosis in SGC-7901 cells in vitro by canonical Wnt/β-catenin signalling pathway. EGCG also inhibited gastric tumour growth by inhibiting Wnt/β--catenin signalling in vivo (Yang et al. 2016).

6 Molecular Mechanisms of Anticancer Activity of Tea Catechins

Various molecular mechanisms have been purposed for the chemopreventive activities of tea catechins. Some of these mechanisms are discussed in brief in sections below. A graphical representation of these mechanisms is presented in Fig. 17.4.

6.1 Induction of Apoptosis

Lim et al. (2006) studied the effects of epicatechin gallate on cell growth and apoptosis in squamous carcinoma cell line, SCC7. Authors reported that epicatechin gallate suppressed the cyclin D1 expression in SCC7 cells by 90% in a dose- and time-dependent manner. It also inhibited the cell growth by 50% *via* G1 cell cycle arrest. Qin et al. (2007) reported that EGCG promotes apoptosis of human bladder cancer cells (T24) *in vitro* by inhibiting PI3K/Akt activation and modulation of

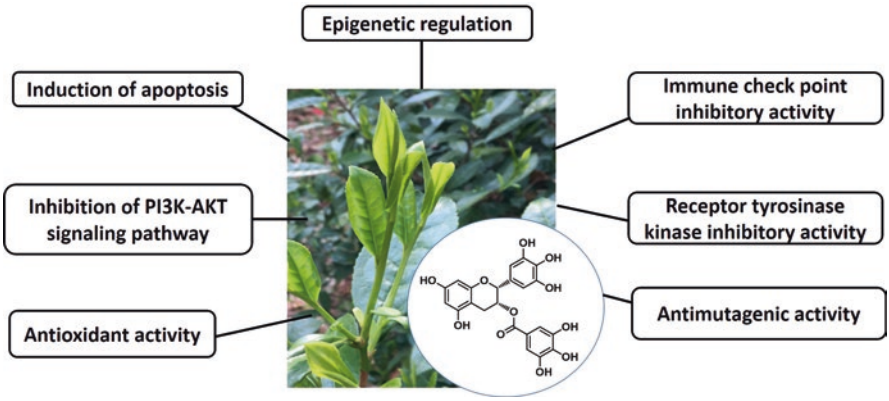

Fig. 17.4 Molecular mechanisms of actions of anticancer activity of tea extracts and catechins

Bcl-2 family proteins. Apoptosis of T24 cells were further supported by activation of caspase-3 and poly (ADP-ribose) polymerase protein expression. Similarly, to identify the molecular pathways involved in EGCG-induced apoptosis of human bladder cancer cells (TCCSUP), Philips et al. (2009) analyzed various gene expression following treatment of 40μg/mL EGCG for 24 h. The results showed down-regulation of key genes involved in cell survival (*Tmepai-* by fourfold, Wnt2 by 3.2-fold) and inflammation (*Ccl20* and *IL-8* by >10-fold).

6.2 Autophagy

Autophagy is an intracellular process that is involved in the degradation of cellular components via lysosomal pathway triggered by several stressful conditions such as organelle damage, the presence of misfolded proteins, and nutrient deprivation (Levy et al. 2017; Yun and Lee 2018). Autophagy is believed to play dual role in cancers through tumor suppression and promotion (Yun and Lee 2018). Effective targeting of autophagy stimulation is being considered as an therapeutic option in various cancers (Levy et al. 2017). Zhao et al. (2017) studied the molecular mechanisms of autophagy regulation by EGCC in human hepatocellular carcinoma HepG2 cells and found that EGCG reduced the α-fetal protein (AFP) secretion, which is involved in malignant differentiation, and induced the AFP aggregation which was further degraded by autophagic process.

6.3 PI3K-AKT Signaling Pathway

The phosphoinositidine-3-kinase/Akt/mammalian target of rapamycin (PI3K/Akt/m-TOR) signaling pathway is considered the most common pathway in human cancers (Van Aller et al. 2011). Various therapeutic targets are being studied for the treatment of cancer in PI3K-Akt pathway including dual PI3K–mTOR inhibitors, PI3K inhibitors, Akt inhibitors and mTOR complex catalytic site inhibitors (Engelman 2009). Van Aller et al. (2011) studied the inhibitory activity of tea catechins, catechin gallate, epicatechin gallate, gallocatechin gallate and EGCG on the PI3K-Akt pathway and reported that EGCG acted as dual inhibitor of PI3K/mTOR. Gu et al. (2018) investigated if EGCG induce apoptosis of human lung cancer cell (H1299) targeting PI3K/Akt signaling pathway. As compared to control (EGCG untreated), the expression of PI3K and Akt showed no significant differences, while expression levels of their phosphorylated form (p-PI3K and p-Akt) were significantly reduced.

6.4 Receptor Tyrosinase Kinase Inhibitory Activity

Receptor tyrosine kinases (RTKs) exert crucial function to control cellular processes and balance between cell proliferation and death. RKTs are promising therapeutic targets for the management of cancer. The tea catechins, including EGCG have ability to suppress RTK signaling thus exert protective effects against dysregulated RTKs in cancer cells (Larsen et al. 2010). EGCG suppress the activation of epidermal growth factor receptor family (ErbB1), HER2 (neu/erbB2) and HER3 (neu/erbB3) belonging to subclass I of the RTK superfamily, in different human cancer cells. The activation of insulin like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) receptors are also downregulated by EGCG (Shimizu et al. 2008). EGCG also inhibits the tumorigenicity of human lung cancer cell (H1299) stimulated by AXL RTK. In addition, oral administration of EGCG and green tea extract suppressed tumour growth in SCID/Beige mice and reduced p-AXL, ALDH1A1, and SLUG in tumours (Namiki et al. 2020).

6.5 Epigenetic Regulation

EGCG and other catechins are also widely studied for their epigenetic regulatory activity (Khan et al. 2020). EGCG was reported to reduce the cellular proliferation and induce apoptosis in MCF-7 breast cancer cell lines and HL60 promyelocytic leukemia cell lines through downregulation of human telomerase reverse transcriptase (hTERT) gene expression (Berletch et al. 2008). EGCG also reduced the level of B-cell-specific Moloney murine leukemia virus integration site 1 (Bmi-1) and

zeste homolog 2 (Ezh2) in SCC-13 cells, which was associated with the reduction in histone H3 lysine 27 trimethylation, a hallmark of PRC2 complex action (Balasubramanian et al. 2010). Similarly, in human prostate cancer LNCap cells, treatment of green tea polyphenols resulted into the re-expression of glutathione-S-transferase p1 (GSTP1) (Pandey et al. 2010). Similarly, in HCT 116 human colon cancer cells, EGCG treatment reduced the expression of histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) (Moseley et al. 2013).

6.6 Immune Check Point Inhibitory Activity

There is a growing interest in the research of immune check point inhibitors as therapeutic targets in cancer specially in immunotherapy. However, there is also growing attention towards the small molecule immune check point inhibitors (Sasikumar and Ramachandra 2018; Smith et al. 2019). Rawangkan et al. (2018) reported the inhibitory activity of EGCG against programmed cell death ligand 1 (PD-L1) expression in non-small-cell lung cancer cells which was initiated by interferon and epidermal growth factor (EGF).

6.7 Anti-mutagenic Activity

Various mutagenic processes are believed to play crucial role in carcinogenesis. Many studies have evaluated the antimutagenic activities of green tea extracts and catechins. Yamada and Tomita (1994) studied the antimutagenic activities of aqueous extracts of green tea, oolong tea and black tea using *Salmonella typhimurium* test strains, TA 98 and TA 100. These extracts reduced the reverse mutation induced by Trp-P-1, Glu-P-1, and B[a]P, and crude dimethyl sulfoxide (DMSO) extracts of grilled beef.

6.8 Antioxidant Activity

Reactive oxygen species and reactive nitrogen species play important role in human body physiology. However, the over production of these agents results in various disease conditions including cancer (Valko et al. 2006; Reuter et al. 2010; Sosa et al. 2013). Tea extracts and catechins are well studied agents for their antioxidant activities *in vitro* and *in vivo* and these activities are often reported to be related with the reduced incidence of cancer (Koo and Noh 2007; Almajano et al. 2008; Lambert and Elias 2010; Kim et al. 2014; Bernatoniene and Kopustinskiene 2018) in individuals taking green tea. However, many detailed clinical trials did not show such effects (Yuan et al. 2011).

7 Combination Therapy with Other Anticancer Drugs

Various studies have also been performed to investigate the combination of cancer chemopreventive drugs/compounds with other anticancer drugs (Suganuma et al. 2011; Fujiki et al. 2015). Przystupski et al. (2019) studied the effect of catechin pretreatment on the cytotoxic effects of cisplatin on human ovarian cancer cells' SKOV-3. Authors reported that the pretreatment of cells with catechin enhanced the cytotoxicity of cisplatin by promoting apoptosis and by changing the activity of membrane proteins involved in cisplatin uptake, metabolism, and efflux. Similarly, La et al. (2019) reported that the EGCG enhanced the colorectal cancer cells' sensitivity to 5-FU through GRP78/NF-κB/miR-155-5p/MDR1 pathway inhibition.

8 Conclusions

Different tea formulations are used worldwide as drink and for potential health beneficial activities. The isolated compounds such as catechins are widely used in food supplements and are also widely studied for their cancer chemopreventive and other pharmacological activities. Various *in vitro* and *in vivo* studies have also revealed the molecular mechanisms of these compounds as anticancer agents, but the clinical and epidemiological studies have provided mixed results (Yuan et al. 2011). The bioavailability and pharmacokinetic properties of these compounds are also of great concern. Future studies should explore more detailed evidence in clinical studies and evaluate the long-term safety and efficacy as therapeutic agents along with clear understanding of their pharmacokinetic properties.

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Chapter 18
Probiotics and Cancer

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Abstract Probiotics are living microorganisms which provide benefits to the host. They have been demonstrated to be effective and safe depending on the quantity, dose, route of administration and ingredients in a wide range of diseases such as intestinal inflammation or infection, ischemic heart diseases, urogenital infections, respiratory diseases and protective and treatment role against cancer. Most of the probiotic products currently available comprise lactic acid bacteria (LAB) that belong to the *Lactobacillus* and *Bifidobacterium.* There are different sources of probiotic like dairy products, fermented milk, kefir, kimchi, kombucha, kiom-ma, utonga kupsu, noni, soymilk and yogurt. The current paper investigated the major probiotic sources and their safety and efficacy and their mechanisms in the prevention and treatment of several types of cancer including breast, colon, gastric, liver, pancreatic, cervical, oral, lung, leukemia and melanoma cancer at three levels *in vitro*, animal and clinical studies. Several mechanisms are suggested for prophylactic and antitumor functions of probiotics including production of short chain fatty acids, alteration of colonic motility and transit time, alteration of differentiation process in tumor cells, anticarcinogenic effects, antimutagenic properties, modulation of inflammatory response, inhibition of the bacteria that convert pro-carcinogens to carcinogens, alteration of tumor gene expressions, decrease of intestinal pH to diminish microbial activity, antioxidant activity, modulation of gut microbiota, antiproliferative and apoptotic effects, antiangiogenesis, enhancement of barrier function, their interference in the enterohepatic cycle of estrogen. Several clinical trials revealed probiotics efficacy against prevent of post-operative complications and chemotherapy and radiation therapy related toxicity such as diarrhea and inflammation. However, numerous studies revealed that probiotics had either no or positive influences in associated with safety results like infection and mortality. Nevertheless,

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more clinical trial studies are essential to recognize the potential strains, dosages and administration regimes with highest efficacy and safety as an adjuvant therapy for cancer treatment for particular types and stages of cancer.

Keywords Probiotics · Herbal medicine · Molecular mechanism · Gut microbiota · Cancer

Abbreviations

APCs	Antigen presenting cells
Bad	Bcl-2 associated agonist of cell death
Bak	Bcl2-antagonist/killer
Bax	Bcl-2-associated X protein
Bcl-2	B-cell lymphoma 2
Bcl-xL	B-cell lymphoma-extra-large
CAT	Catalase
CCL	Chemokine (C-C motif) ligand
CD8	Cluster of differentiation
CLA	Conjugated linoleic acid
CLRs	C-type lectin receptors
COX-2	Cyclooxygenase-2
CRP	C-reactive protein
CXCL	Chemokine (C-X-C motif) ligand
DCs	Dendritic cells
ECM	Extracellular matrix
EGFR	Epidermal growth factor receptor
GABA	Gamma-amino butyric acid
G-CSF	Granulocyte-colony stimulating factor
GM-CSF	Granulocyte macrophage-colony stimulating factor
GPR	G-coupled protein receptors
GPx	Glutathione peroxidase
GSH	Glutathione
GSH-Px	Glutathione peroxidase
GSK3β	Glycogen synthase kinase 3β
HO-1	Heme oxygenase 1
ICAM	Intercellular adhesion molecule
IFN-γ	Interferon gamma
IL	Interleukin
iNOS	Nitric oxide synthase
JNK	c-Jun N-terminal kinases
MAPK	Mitogen activated protein kinase
M-CSF	Macrophage colony-stimulating factor
MDA	Malondialdehyde

MMPs	Matrix metalloproteinases
MnSOD	Manganese superoxide dismutase
NF-κB	Nuclear Factor kappa B
NK cells	Natural killer cells
NLRs	NOD-like receptors
NO	Nitric oxide
Nrf2	Nuclear factor erythroid 2-related factor 2
PRRs	Pattern recognition receptors
ROS	Reactive oxygen species
SCFAs	Short-chain fatty acids
SOD	Superoxide dismutase
TGF-β	Transforming growth factor beta
TIMP-1	Metallopeptidase inhibitor1
TLRs	Toll-like receptors
TNF-α	Tumor necrosis factor-α
T-SH	Total sulfhydryl
VEGF	Vascular endothelial growth factor

1 Introduction

Probiotics were defined by Fuller R in 1989 as live microorganisms of feed supplements that usefully impressed the host through developing of its intestinal microbial balance (Fuller 1989).

The word *probiotic* is extracted from two Greek words *pro*, meaning "promoting," and *biotic*, meaning "life" (Maia et al. 2019). Nowadays, lactic acid bacteria (LAB) belonging to the family *Lactobacillus* and *Bifidobacterium* are the most widely available bacteria in probiotic products (Javanmard et al. 2018).

Many studies highlighted probiotics consumption as safe to prevent or treat a wide range of diseases, like diabetes, obesity, liver disease, urogenital infections, respiratory diseases, intestinal inflammation or infection, Crohn disease, ulcerative colitis, pouchitis, arthritis, ischemic heart diseases, improved intrinsic and acquired immunity and prophylactic usage to prohibit the adverse events of cancer (Turner et al. 2017; Markowiak and Śliżewska 2017; Maia et al. 2019). Nevertheless, their health influence depends on the quantity, dose, way of prescribing and ingredients used to production of probiotic (Jonkers et al. 2012).

Exact mechanisms for the therapeutic advantage of probiotics are not completely understood due to the several microorganisms applied as probiotics (Wasilewski et al. 2015). However, gastrointestinal tract action of probiotics is explained by several mechanisms. Probiotics are genetically stable and survive when they pass from the gastrointestinal tract depending on a number of variables, such as the type of probiotic because of their resistance to bile, digestive enzymes and low pH (Jonkers et al. 2012). Their Gastrointestinal transfer and adhesion of probiotics to

the intestinal mucosa supply the basis for the colonization and secretion of metabolites (Maia et al. 2019). Then, interaction between probiotic microorganism and epithelial cells of small intestine, such as induction of proinflammatory markers and mucin production that prohibit apoptosis. Finally, intrinsic and acquired immune system modulation via interactions with macrophages, lymphoid tissue present in the intestinal mucosa, resident leukocytes in the B and T lymphocytes and dendritic cells (Rosenberg et al. 2016) that cause to reduction of inflammatory markers and the production of immunomodulatory indicators such as tumor necrosis factor-α (TNF-α), interleukin-2 (IL-2) and IL-10 (Gomes et al. 2017).

Microbiota in cancer progression and development is very important, so analysis of the microbiome over time can supply knowledge around microbial exposure, cancer risk, occurrence and development and treatment response (Abedin-Do et al. 2015).

Furthermore, the direct probiotics advantage on the host gut microbiota improvement, numerous studies have demonstrated potential probiotics effects in cancer prevention and treatment via decreased bacterial translocation, microbiota modulation, anti-inflammatory and antipathogenic activity, immune modulation, increased gut barrier function, reduction tumor formation and metastasis (Yu and Li 2016).

The current review investigated the major probiotic sources and safety and efficacy of probiotics and their mechanisms in the inhibition and treatment of several types of cancers including breast, colon, gastric, cervical, oral, melanoma cancer at three levels *in vitro*, animal and clinical studies.

2 Sources of Probiotics

Probiotics cultures are part of fermented dairy products, fermented foods, and some other products when ingested, these products can produce a good population of probiotics in the digestive system for health benefits. The sources of probiotics are listed below:

2.1 Chicken Cecum

The gastrointestinal (GI) tract of chickens has various strains of microbiota that play important role in digestion and absorption of useful nutrients and promotion of the immune system. The largest microbial community is in the chicken cecum that this microbiota recycles nitrogen by the breakdown of uric acid, prepares B vitamins and essential amino acids and digests non-starch polysaccharides. The cecum is a place for the *Clostridia* genus followed by genera *Lactobacillus* and *Ruminococcus* and also *Enterococcaceae*, *Enterobacteriaceae* and *Bacteroidaceae* families. Some of these probiotics were purified for various studies focused on the chicken cecum (Shang et al. 2018).

2.2 Dairy Products

Dairy products as a source for probiotics have been considered that consumers are familiar with. The most common probiotic strains used in dairy foods are *Lactobacillus* and *Bifidobacterium*. Consuming dairy products do to useful probiotics have beneficial impacts on human health including decrease of lactose intolerance disorders, decrease of blood pressure, cholesterol and/or triglycerides levels, anti-carcinogenic activity, immune system improvement, and they can produce low molecular weight ingredients including bacteriocins, gamma-amino butyric acid (GABA) and conjugated linoleic acid (CLA) (Ranadheera et al. 2017).

2.3 Fermented Milk

Fermented milk has an important role in the nutrition of people. It is prepared by the fermentation process with lactic acid bacteria (LAB), such as *Streptococcus thermophilus*, *Bifidobacterium*, and *Lactobacillus* (Aragon et al. 2014). Goat milk is widely used for fermentation. The fermentation process makes the fermented milk product easier to digest. Different types of fermented milk such as yogurt and kefir were prepared by specific culture organisms that used for fermentation. Fifty-four strains of lactic acid bacteria were obtained from fermented milk which therapeutic effects were investigated in some studies (Amraii et al. 2014). These studies suggested that the consumption of fermented milk is related with reduced risk of cancer; nevertheless, these findings depend on the production and the kind of cancer. Effects of probiotics isolated from fermented milk on colon and breast cancer has been discussed in many studies (Zhang et al. 2019). It is useful for people with a lactase deficiency because lactose is hydrolyzed to produce galactose and glucose by the fermentation process (Redondo-Useros et al. 2019).

2.4 Goat Milk

Caprine (goat) milk is a dairy product known as a source of probiotics and the usage of goat milk as a source of probiotics has rapidly enhanced in recent years. Goat milk also has a high nutritive value, easy digestibility and better digestibility, alkalinity and buffering capacity than cow or human milk (Salva et al. 2010).

2.5 Kefir

Kefir water is made from kefir grain by lactic acid bacteria including *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactococcus lactis*, yeast, and acetic acid

bacteria. These microorganisms embedded in a matrix of proteins, lipids, and polysaccharides of kefir grains as a symbiotic culture. Kefir's ancient origins are in the Caucasian mountains. For many years it is believed that kefir can promote good health and some studies were investigated that kefir could induce cytotoxicity and had a cancer-preventing effect and immunomodulatory capacity (Zamberi et al. 2016).

2.6 Kimchi

Kimchi is a traditional Korean food prepared by fermenting vegetables. *Baechu* cabbage and radish and many others like cucumber, green onion, leek used for the preparation of various types of kimchi. The fermentation process is done by lactic acid bacteria (LAB) to produce the most popular kimchi in Korea named *Baechu kimchi* (Park et al. 2014). Kimchi is rich in vitamins and minerals, such as iron, vitamin K and riboflavin (vitamin B2). Many previous studies have reported anticancer, antioxidative, anti-atherosclerotic, anti-diabetic, anti-obesity effects of kimchi (Lee et al. 2016).

2.7 KIOM-MA

KIOM-MA is a Korean Herbal Medicine used for the treatment of many inflammatory diseases combined of some plants such as *Cnidii rhizoma*, *Polygoni cuspidati radix*, *Arctii fructus*, *Sophorae radix*, and *Glycyrrhizae radix*. Some traditional oriental medicine in Asia is prepared from these herbs. KIOM-MA128 is a fermented version of KIOM-MA produced by probiotics (*Lactobacillus acidophilus*) to enhance its therapeutic features by increasing absorption and bioavailability of the active ingredients. In some studies, antiinflammatory and anti-allergic effects of KIOM-MA and anti-atopic and anticancer effects of KIOM-MA128 was investigated (Kim et al. 2017).

2.8 Kombucha

Kombucha is a fermented tea that has been consumed in central and eastern Asian countries more than Europe and the USA for many years. This beverage contains some probiotics such as yeasts like *Zygosaccharomyces rouxii*, *Saccharomyces ludwigii*, *Schizosaccharomyces pombe*, *Pichia membranaefaciens*, Candida spp., *Acetobacter* strains like *Acetobacter syzygii* and *Acetobacter aceti* and and fungi so it is rich in beneficial probiotics (Gaggia et al. 2018). According to previous studies, kombucha has favorable effects on human health due to probiotic strains and several

amounts of polyphenols, including flavonoids depending on the herbal tea. Results were obtained from studies that investigated the antioxidant, hepato-nephron protective, anti-cancer, antimicrobial, anti-stress, and hypercholesterolemia properties effects of kombucha (Greenwalt et al. 2000).

2.9 Kumys

Consumption of fermented milk products has increased due to beneficial effects. Kumys (koumiss or kumiss), an alcoholic drink, is a kind of fermented milk preparation like kefir, made from mare's milk, that is mostly consumed in Eastern Europe, Russia and Central Asia. Microbial activity by non-lactose-fermenting yeast (*Saccharomyces cartilaginosus*), non-carbohydrate-fermenting yeast (*Mycoderma* spp.), lactose-fermenting yeasts (*Kluyveromyces* marxianus var. *lactis*, *Saccharomyces lactis)* and lactobacilli (*L. delbrueckii* subsp. *bulgaricus* and *L. acidophilus*), causes to lactic acid and alcohol fermentations in the primitive culture. It is suggested that health-promoting effects of kumys include improvement of metabolism and promotion of immune system and antibacterial activities and protection of the nervous system (Nuraeni et al. 2014).

2.10 Noni

Noni (*Morinda citrifolia*) is a small evergreen tree that grows on the India, Australia, Southeast Asia, and Pacific islands. Different parts of the plant have some various biologically effective compounds to motivate the immune system, to prevent low-density-lipoprotein oxidation, to remove free radicals, to supply antiinflammatory advantages and to regulate cholesterol. Noni fruits traditionally are fermented for 4–8 weeks at a specific temperature in sealed containers to produce noni extract. Fermented noni extract was considered that has anti-adhesive and antiinflammatory properties during *Helicobacter pylori* infection and has antiinflammatory effects on colon (Wang et al. 2009).

2.11 Probiotics in Human Breast Milk

Probiotics in Human breast milk include several species, such as staphylococci, streptococci, micrococci, lactobacilli, enterococci, lactococci and bifidobacteria and play an important role due to transfer beneficial microflora to the gut of a newborn. The effects of each probiotic strain are different. Median numbers of 10^2–10^3 and a range of 10^1–10^7 colony forming units per ml of bacteria present in breast milk. In past studies have been demonstrated that *Lactobacillus* and *Bifidobacterium*

have effects on the prohibition and treatment of allergic disorders and childhood diarrheal and have immunomodulatory effects (Gotoh et al. 2018).

2.12 Soymilk

Soymilk has a large number of valuable proteins, unsaturated fatty acids, soluble and insoluble dietary fibers, and isoflavones. In some country's soymilk is fermented by lactic acid bacteria and this production can be applied by people who cannot digest milk for reasons like lactose intolerance, allergy to milk proteins, or vegetarians. Genera *Lactobacillus* and *Bifidobacterium* are the most probiotics applied for the fermentation process. Fermentation improves soymilk taste and nutritional characteristics (Haghighatdoost and Azadbakht 2015).

2.13 Utonga Kupsu

Utonga kupsu is a fermented fish prepared by Manipuri (meetei) people who live in northeast India. It is a mixture of various fish species. Fermentation is a useful technique to progress the quality of foods such as improving digestibility and enhancing some useful compounds like vitamins, unsaturated fats, amino acids, and protein. Also, fermented foods have some microflora that their medical roles were investigated in past studies that Utonga probiotics have anticancer activities (Singh et al. 2018).

2.14 Yogurt

Yogurt is prepared by the fermentation process of two microorganisms (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*) isolated from milk in which both bacterial species stay live in the final preparation. The beneficial effects of yogurt in many studies were investigated. Yogurt can act as health-promoting food because it is a source of live probiotics (Wang et al. 2013).

3 Mechanisms of Action of Probiotics in Cancer

3.1 Immunomodulatory Effects

The intestinal microbiota has an essential role in keeping immune homeostasis. Probiotics are applied to progress the intestinal microbiota balance (Han et al.

2015). Probiotics influence the immune system at several levels of humoral and innate immunity, including increasing levels of cytokines and immunoglobulins, increase proliferation of mononucleosis and activated macrophages against pathogenic bacteria and protozoa. The interaction of numerous elements of the immune system, including natural killer (NK) cells, antigen presenting cells (APCs), dendritic cells (DCs) and various subsets of B cells, T cells, is regularly activated via mutation, invasion or damage (Zhong et al. 2014).

Probiotics can induce DCs maturation, elevate NK cells cytotoxicity, and upregulate cytokine secretion, such as promoting Interferon gamma (IFN-γ) production (Hu et al. 2015a) Probiotics demonstrate immunomodulatory effects by binding to pattern recognition receptors (PRRs), that are existent on the surface of immune cells like intestinal epithelial cells, monocytes, macrophages and dendritic cells (Chong 2014). PPRs, including NOD-like receptors (NLRs), Toll-like receptors (TLRs) and C-type lectin receptors (CLRs) distinguish particular microorganism-related molecular patterns isolated from bacteria like probiotics. Probiotics express several ligands identified via NLRs, CLRs and TLRs (Bron et al. 2012).

3.2 Modulation of Inflammatory Response

Inflammation is a response of the body's defense against external factors. Existence of microorganisms in pathogenic sites is a significant beginning factor of the inflammatory process (Maia et al. 2019). The inflammation usually will remain till the pathogen is removed and may cause considerable damage. Inflammation is involved in carcinogenesis, so some strategies to prevent and treat several types of cancer centralized on the usage of nonsteroidal antiinflammatory drugs (Westbrook et al. 2010). Probiotic therapy is related to reduced systemic inflammation, principally at the inflammatory indicator C-reactive protein (CRP) and TNF-α. The CRP and TNF-α are strong proinflammatory indicator for most immune cells (Fischer and Maier 2015). Cytokine expressing inflammatory cells create great amounts of prostaglandin E2 (PGE2), nitric oxide (NO) and cytokines including IL-6, IL-1β, and TNF-α. PGE2 and NO are main pro-inflammatory mediators created via inducible cyclooxygenase-2 (COX-2) and NO synthase (Otte et al. 2008). Nuclear Factor kappa B (NF-κB) contains a family of transcription factors, that upregulate pro-inflammatory cytokines. These are identified as main targets in progressing therapeutic interventions for chemotherapy-induced mucositis, and also COX-2 suppression may be useful in decreasing the duration and severity (Mahendran et al. 2018).

Furthermore, there is a relation between gut microbiota health. Eubiosis is the normal condition of the intestinal microbiota. Every modification from eubiosis, such as enhancement of pathogenic bacteria or reduction of intestinal biodiversity, is called dysbiosis, which makes variation of the gut mucosa immunity and the increase of inflammation (Maia et al. 2019). Commensal bacteria indirectly influence on the inflammation occurs via preserving and reforming epithelial barriers,

that subsequently diminishes the effect of proinflammatory stimuli, including lipopolysaccharide (Mcloughlin et al. 2017). Also, particular bacterial species and their metabolic productions can modulate the NF-κB pathway, improves NK cell activity, induct T cell apoptosis and diminish the production of proinflammatory markers while rising the production of intestinal antiinflammatory cytokines including IL-10, and the synthesis of peptides with antimicrobial properties involved in inflammation eliminating pathways (Mcloughlin et al. 2017; Maia et al. 2019).

3.3 Antioxidant Activity

Oxidative stress is directly or indirectly responsible for causing numerous diseases. Free radicals and reactive oxygen species (ROS) made either exogenously or endogenously, may contribute to several pathological factors, such as cellular degeneration and DNA damage (Bai et al. 2016, 2). It is supposed that ROS play a main role in some cancers. Numerous studies demonstrated that some probiotics strains have antioxidant properties and deactivate ROS by enzymatic mechanisms including coupled NADH system and catalase (Zhong et al. 2014). Furthermore, they possibly dedicated to enzyme inhibition, ROS scavenging, and also decrease action or prohibition of ascorbate autoxidation in the intestine via neutralizing free radicals (Amaretti et al. 2013). Antioxidant activity of probiotics can be achieved through activity of free radical scavenging (H_2O_2, ABTS+, DPPH), capacity of tissues antioxidant enzyme [superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione (GSH), Total sulfhydryl (T-SH], capacity of mitochondria antioxidant enzyme [manganese superoxide dismutase (MnSOD), glutathione peroxidase (GPx)] and mRNA expression of antioxidant genes [GSH-Px, Nuclear factor erythroid 2-related factor 2 (Nrf2), SOD, Heme oxygenase 1 (HO-1), CAT], oxidative damage index [8-OHdG, ROS,Malondialdehyde (MDA), PC], and mitochondrial membrane potential (MMP) level (Bai et al. 2016).

3.4 Antiproliferative and Apoptotic Effect

Apoptosis is an innately programmed cell death that has significant roles in several and pathologic physiologic situations and regulation of cell numbers (Bonyadi et al. 2017). Reduction of ability to trigger apoptosis related to control procedures of cell proliferation modification is a chief pathogenetic occurrence in various types of cancers (Elmore 2007). The molecular regulation of cell survival and apoptotic death can have chemo protective and therapeutic effects. Several studies demonstrated which probiotics can have potentially critical roles in the prevention and treatment of cancers by regulation of cell apoptosis through intrinsic and extrinsic pathways (Fesik 2005).

The anti-proliferative influences demonstrated through probiotics are usually associated with modulation of apoptosis (Chondrou et al. 2018). These effects were related to the upregulating of the pro-apoptotic Bak and Bax, upregulating of the tumor necrosis factor-related apoptosis-inducing ligand TRAIL, downregulating of the production of the anti-apoptotic proteins Bcl-2 and Bcl-xL (Tiptiri-Kourpeti et al. 2016), activation of p38 Mitogen Activated Protein kinase (MAPK) signaling pathways and c-Jun N-terminal kinases (JNK), inhibition of NF-κB nuclear translocation and subsequent activation (Chondrou et al. 2018).

3.5 Antiangiogenesis

Angiogenesis is the formation of new blood vessels from existent vessels that is essential for growth, progression and metastasis of tumors (Petrella et al. 2012). Several studies demonstrated probiotics reduced the level of angiogenic factors of IL-1β which progress invasiveness of tumor (Zamberi et al. 2016).

Hematopoietic growth factors included, granulocyte macrophage-colony stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF), granulocyte-colony stimulating factor (G-CSF) cause tumors progression. VEGF which is secreted via activated macrophages and peritoneal neutrophils, is an essential mediator for angiogenesis in many types of cancer malignancies (De Luca et al. 2012). G-CSF is recognized as a macrophage regulator to progress survival, proliferation and differentiation and also along with GM-CSF can be as proto-oncogenes (Zamberi et al. 2016). The disruption and remodeling of extracellular matrix (ECM) via matrix metalloproteinases (MMPs) influence on numerous properties of the cancer cells, like metastasis, angiogenesis and invasiveness (Wang et al. 2010). MMP-9 is one of the most important of MMP family, that progress vascular occurrence in tumors and stimulate VEGF (Zamberi et al. 2016). Furthermore, MMP-9 is regulated via tissue inhibitor of metalloproteinases (TIMPs) in cancer cells, so the balance between the upregulating of TIMP and downregulating of MMP-9 is significant in decreasing angiogenesis in cancer (Kudo et al. 2012). Moreover, angiogenin, PDGF, and cytokines including IL-8, TNF-α, transforming growth factor beta (TGF-β), erythropoietin, HGF, macrophage migration inhibitory factor, and neutrophil-activating factor have the chief roles in angiogenesis (Voronov et al. 2003).

3.6 Anticarcinogenicity

Carcinogenesis, likewise called tumorigenesis or oncogenesis, is the formation of a cancer, whereby normal cells are converted to cancer cells. The process is considered via transformations at the genetic, epigenetic, and cellular levels and abnormal cell division. The balance between proliferation and apoptosis, preserves the

integrity of organs and tissues (Gao et al. 2017). According to the somatic mutation theory that is predominant accepted theory of carcinogenesis, mutations in DNA and epimutations disturbed the normal balance between proliferation and cell death and uncontrolled cell division (Walia et al. 2018). Several studies show that probiotics have prophylactic potentials on chemically induced experimental carcinogenesis through prevention of DNA damage *in vitro* and *in vivo*, controlling the expression of glycogen synthase kinase 3β (GSK3β), MMPs, and nitric oxide synthase (iNOS) activation while inducing apoptosis in cancer cells, modulation of carcinogen formation and activation by gut microflora, suppression of pre-neoplastic changes, downregulation of procarcinogenic markers (β-catenin, NF-κB, COX-2) expression resulting into decrease of both Aberrant Crypt Foci (ACF) counts and proinflammatory cytokines (IFN-γ, IL-10, TNF-α) (Sharaf et al. 2018).

3.7 Production of Short-Chain Fatty Acids

Short-chain fatty acids (SCFAs), principally propionate, butyrate and acetate, produced by microbial fermentation influence colonic health (Van Der Beek et al. 2017). Probiotics are capable to produce SCFAs, which reduce the colonic pH and the growth of pathogenic organisms (Michail 2005). SCFAs have demonstrated direct immunomodulatory, antiinflammatory and anticarcinogenic effects via G-coupled protein receptors (GPR) activation and histone deacetylases (HDAC) (Correa-Oliveira et al. 2016). Inhibition of HDACs by SCFAs is related to cell cycle arrest, causing proapoptotic and antiproliferative effects (Van Der Beek et al. 2017). Furthermore, SCFAs can modulate TLR-4 signaling, enhance the production of the antiinflammatory cytokine IL-10, repress the production of proinflammatory cytokines IL-6, IL-12, and TNF-α and decrease the infiltration of colonic mucosa by leukocytes, so suppress the immune response (Tedelind et al. 2007).

3.8 Enhancement of Barrier Function

Probiotics through the improvement of intestinal barrier function by tight junctional protein phosphorylation and modulation of cytoskeletal can impress cellular stability and mucosal cell–cell interactions (Ng et al. 2009). Several studies have shown probiotic bacteria can improve barrier function by mechanisms including increase tight junction proteins activation and suppress the progress of a leaky intestine (Resta-Lenert and Barrett 2003), also some of them can decreased mucosal permeability and inhibit inflammation and programmed cell death of the intestinal epithelial cells (Michail 2005).

3.9 Inhibition of the Enzymatic Activity of Pathogenic Bacteria

Glucuronide conjugation process that is done in the liver is important for the deletion of toxins and carcinogenic agents and hormones' metabolism. Bacterial enzymes do deconjugation stage in the intestine. The intestinal microbiota imbalance leads to the release of plentiful quantities of azoreductase, nitroreductase, β-glucuronidase and β-glucosidase enzymes and the generation of carcinogenic agents (Vernazza et al. 2006). Probiotics consumption can decrease the activity of these enzymes via several mechanisms, for instance, *Lactobacillus* prohibits bacterial enzyme to decrease the dehydroxylation of primary bile acid and *L. rhamnosus* GG via decreasing the β-glucuronidase activity, *L. acidophilus* and *Bifidobacterium bifidum* by reduction of nitroreductase enzyme (Eslami et al. 2019).

4 Effects of Probiotics in Cancer Prophylaxis and Treatment

Tables 18.1, 18.2, and 18.3 show details of *in vitro* and *in vivo* and clinical studies investigating the effects of probiotics on prevention and treatment cancer.

4.1 Breast Cancer

Breast cancer is most second common kind of cancer worldwide and the first cause of cancer-related death among women (Ranjbar et al. 2019). Gastrointestinal bacteria play a significant role in the reabsorption, enterohepatic circulation, and modulation of systemic estrogens (Mendoza 2019). The gastrointestinal bacteria associate with estrogen level by secretion of β-glucuronidase enzyme that help to free deconjugates estrogen. Enhancement free estrogen amount for reabsorption increases the risk of progression of hormone-driven malignancies such as breast cancer (Kwa et al. 2016). Cholesterol metabolism, diet and alcohol that are some risk factors related with breast cancer, have a direct effect on the gastrointestinal bacteria composition and function (Muegge et al. 2011).

4.1.1 *In Vitro* Studies

Several *in vitro* and animal investigations have been carried out to evaluate the influence of probiotics against breast cancer. Table 18.1 demonstrates examples of these *in vitro* studies.

Lactobacillus plantarum is considered as potential chemotherapeutic agent because of decreasing proliferation, downregulation of NF-κB pathway and apoptotic activity against estrogen receptor negative (Nami et al. 2014; Kadirareddy

Table 18.1 *In vitro* studies on use of probiotics for prevention and treatment of cancer

Type of cancer	Probiotic strains	Cell line	Results	Reference
Breast cancer	Kefir Water	4T1	↓Lipid peroxidation of MDA and NO, inhibited cell mitosis and inhibited tumor proliferation via ↑tumor apoptosis ↑Immune response in spleen (T-helper CD4+/ CD3+ and cytotoxic T cells (CD8+/CD3+) ↓Expression of inflammation and metastatic-related genes and proteins (downregulation ICAM, iNOS, MMP-9, IL-1β, NF-κB, G-CSF, GM-CSF, IL-4, TNF-α genes)	Zamberi et al. (2016)
Breast cancer	Heat-killed cells of *Entrococcus feacalis* and *Staphilococcus hominis*	MCF-7	↓Cell line proliferation, cancer cell apoptosis	Hassan et al. (2016)
Breast cancer	*Bifidobacterium* sp. and *Lactobacillus acidophilus*	SKBR-3	Cytotoxicity against cell line, probiotics converted lapachol, an antitumor naphthoquinone, into a highly cytotoxic metabolite	Silva et al. (2014)
Breast cancer	Soy milk fermented with *Lactobacillus paracasei*, *Lactobacillus bulgaricus*, and *Saccharomyces cerevisiae*	MCF-7, MDA-MB-231	↓Proliferation, production of (S)-latifolicinin A(4b) (with antiproliferative effect)	Ke et al. (2015)
Breast cancer	Soy milk fermented with *Lactobacillus paracasei subsp. paracasei* NTU 101 and *Lactobacillus plantarum* NTU 102	MDA-MB-231	↓Cell line proliferation, apoptosis induction, ↑SOD activity and PGE2 synthesis	Liu et al. (2009)

Type of cancer	Probiotic strains	Cell line	Results	Reference
Breast cancer	*Saccharomyces cerevisiae*	MDA-MB-231, MBC	Apoptosis induction, ↓expression of Bcl-2, ↑Bax, ↑Bax: Bcl-2 ratio	Ghoneum and Felo (2015)
Breast cancer	Conjugated linoleic acid from *Lactobacillus plantarum*	MDA-MB-231	↓Cell line proliferation, apoptosis induction by downregulation of NF-ĸB pathway	Kadirareddy et al. (2016)
Breast cancer	*Lactobacillus plantarum*	MCF-7	↓Cell line proliferation, apoptosis induction	Nami et al. (2014)
Breast cancer	*Lactobacillus plantarum*	MCF-7	↑Cytotoxicity in a time and dose dependent manner, human health supplement and as anticancer preventive agent	Tan et al. (2015)
Breast, colorectal, cancer	*Lactobacillus plantarum*	MCF-7, HT-29	Cytotoxicity effects via antiproliferative effect and induction of apoptosis	Chuah et al. (2019)
Breast, cervical, colon adenocarcinoma, gastric cancer	*Enterococcus* strains isolated from fermented dairy products (*E. mundtii* 50H, E. durans 39C, *E. faecalis* 13C)	MCF-7, HeLa, HT29, AGS	Antiproliferative effects, ↑Apoptosis, anticancer similar to Taxol	Haghshenas et al. (2014)
Breast and lung cancer	*Lactococcus lactis* NK34	MCF-7, MRC-5, SK-MES-1, HT-29 cells	Inhibition of proliferation, >77% of cytotoxic activity, ↓NO, TNF-α, IL-18, and COX-2	Han et al. (2015)
Breast, colon, lung, gastric	*Lactococcus lactis* KC24 isolated from kimchi	MCF-7, HT-29 and LoVo, SK-MES-1, AGS	↓Cell line proliferation, ↑ apoptosis, antiinflammatory effects through NO production, antioxidant activity, strong cytotoxic effect	Lee et al. (2015)
Cervical cancer	Exopolysaccharides (EPSs) of *Lactobacillus gasseri*	HeLa	Inhibiting cell proliferation, apoptosis induction by upregulation of Bax and caspase3, antiinflammatory by ↓TNF-α and ↑IL-10 production	Sungur et al. (2017)
Cervical cancer	*Lactobacillus crispatus*	HeLa	Cytotoxic, anti-metastatic effect, downregulation of MMP2 and MMP9 genes expression	Adnan et al. (2018)

(continued)

Table 18.1 (continued)

Type of cancer	Probiotic strains	Cell line	Results	Reference
Cervical, colon adenocarcinoma cancer	*Lactobacillus crispatus* and *Lactobacillus rhamnosus*	HeLa, HT-29	Antimetastatic, cytotoxic, antiproliferative activity, ↓Expression of MMP2, MMP9, ↑expression of their inhibitors	Nouri et al. (2016)
Cervical cancer	*Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri*	Caskie cells	Regulation of the cell cycle, expression of HPV E6 and E7 oncogenes, antiproliferative, cytotoxic	Wang et al. (2018)
Cervical cancer	*Lactobacillus casei*, *Lactobacillus paracasei*	HeLa	Antioxidant activity, upregulating the expression of apoptotic genes Bax, Bad, caspase3, caspase8, caspase9, by downregulating the expression of the Bcl-2 gene	Riaz Rajoka et al. (2018)
Colorectal cancer	*Bacillus coagulans* Unique IS2	COLO 205	Antiproliferative activity and apoptotic effects via ↑Bax, ↓Bcl2, ↓mitochondrial membrane potential, ↑cytochrome c release, ↑caspase 3 activity	Madempudi and Kalle (2017)
Colorectal cancer	*Leuconostoc mesenteroides* isolated from traditional dairy products	HT-29 cells	Modulating NF-kappaB/AKT/PTEN/MAPK pathways, apoptosis induction by upregulation of MAPK, Bax, caspase 3, and downregulation of AKT, NF-ĸB, Bcl-XL expressions	Zununi Vahed et al. (2017)
Colorectal cancer	six microbial strains (*Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *L. casei*, *Bifidobacterium bifidum* and *Bifidobacterium longum*)	HT-29 and RKO	Downregulation of most cytokines like IL-1β, IFN-γ, IL-10 and IL-1 ↑production of TNF-α and IL-1β, modify the intestinal environment, ↓colon cancer development	Djaldetti and Bessler (2017)

Type of cancer	Probiotic strains	Cell line	Results	Reference
Colorectal cancer	peptide extract of probiotic yoghurt supplemented + pineapple peel powder	HT-29 cells	Antiproliferative activities via inducing apoptosis and cell cycle arrest in G2/M-phase, high scavenging activity against 2,2′-azino-bis (antioxidant)	Sah et al. (2016)
Colorectal cancer	*Lactobacillus acidophilus*	CTM, NRFC-028	↓*Aeromonas hydrophila* induced cytotoxicity in catla thymus macrophages by modulating oxidative stress and inflammation, ↑Expression of TNF-α and IL-10, ↓iNOS, COX-2, apoptosis induction, ↓ROS, RNS and DNA damage	Patel et al. (2016)
Colorectal carcinoma cancer	*Lactobacillus casei*	HT29	↓Tumor growth and volume, antiproliferative and proapoptotic effects, upregulation of TRAIL, downregulation of Survivin	Tiptiri-Kourpeti et al. (2016)
Colorectal cancer	*Lactobacillus casei*, *Lactobacillus acidophilus*	HT-29, WiDr, DLD-1 and CX-1 cells	↓Proliferation, ↑apoptosis induction, ↑antioxidative activity, ↑scavenging activity of the available DPPH free radicals	Choi et al. (2006)
Colorectal cancer	*Lactobacillus acidophilus* + *Lactobacillus casei*	LS513	↑Apoptosis-induction capacity of 5-fluorouracil	Baldwin et al. (2010)
Colorectal cancer	*Lactobacillus acidophilus*, *Lactobacillus casei*	CaCo-2	↓Cell proliferation, migration and invasion, ↑cell apoptosis	Soltan Dallal et al. (2015)
Colorectal cancer	*Lactobacillus paracasei* K5	Caco-2	Antiproliferative activity and apoptotic effects via ↓Antiapoptotic Bcl-2 and ↑Pro-apoptotic Bak and Bax	Chondrou et al. (2018)
Colorectal cancer	*Lactobacillus brevis*, *Lactobacillus paracasei*	HT-29	Inhibit the growth, apoptosis induction by regulation of Bax/Bcl2 and caspases pathway, ↑Bax, caspase-3, caspase-9, ↓Bcl2	Karimi Ardestani et al. (2019)

(continued)

Table 18.1 (continued)

Type of cancer	Probiotic strains	Cell line	Results	Reference
Colorectal cancer	*Lactobacillus brevis* SBL8803	SW620	Apoptosis induction through activation of the ERK pathway	Sakatani et al. (2016)
Colorectal cancer	*Lactobacillus paracasei* subsp. *paracasei* M5L	HT-29	Apoptosis induction through ↑ROS, ↓SOD and CAT	Hu et al. (2015b)
Colorectal cancer	*Lactobacillus delbrueckii*	SW620	Inhibit growth, apoptosis induction through intrinsic caspase 3-dependent pathway, ↓expression of Bcl-2, ↓ MMP9	Wan et al. (2014)
Colorectal cancer	*Lactobacillus plantarum*	Caco-2	↓IL-23 secretion, ↑HBD-2 expression	Paolillo et al. (2009)
Colorectal cancer	*Lactobacillus* spp. from traditional dairy products	HT-29, Caco-2	Prophylactic effects, ↓the viability of cancer cells, downregulation of ErbB-2 and ErbB-3 gene expression	Faghfoori et al. (2017)
Colorectal cancer	*Lactobacillus casei and Lactobacillus rhamnosus*	HCT-116	↓Cell invasion, ↓MMP-9	Escamilla et al. (2012)
Colorectal cancer	*Bifidobacterium* strain, *Lactococcus* strain	HT-29	Antiinflammatory and ↓Proliferation, induction of a differentiated phenotype, upregulation of intestinal alkaline phosphatase, ↓IL-8, ↑NF-κB activation	Grimoud et al. (2010)
Colorectal adenocarcinoma	*Lactobacillus rhamnosus* GG	Caco-2 cells	↓TNF-α-induced IL-8 production by affecting the NF-ĸĸB/IĸB pathway	Zhang et al. (2005)
Colorectal adenocarcinoma	*Lactobacillus rhamnosus* GG	Caco-2 cells	Upregulate gene expression of pro-inflammatory cytokines like TNF-α, MCP-1 and IL-12	Fang et al. (2014)
Colorectal adenocarcinoma	*Lactobacillus casei*, *Lactobacillus rhamnosus* GG	Caco-2 cells	Inhibite IL-1β-induced IL-8 production, inhibition of the NF-ĸB signaling pathway	Hwan Choi et al. (2008)
Colorectal cancer	*Saccharomyces cerevisiae*	SW480	Apoptosis induction by modulate Akt/NF-ĸB signaling pathway, ↓p-Akt1, Rel A, Bcl-xL, pro-caspase 3, and pro-caspase 9 expressions, ↑Bax, cleaved caspase-3, cleaved caspase-9	Shamekhi et al. (2019)

Type of cancer	Probiotic strains	Cell line	Results	Reference
Colorectal cancer	*Saccharomyces cerevisiae*	HT-29	Inhibits growth and metastasis, stimulates apoptosis, ↑expression of PTEN and caspas3 genes, ↓Bcl-xl, RelA	Sambrani et al. (2019)
Colorectal cancer	Nisin (polycyclic peptide produced by *Lactococcus lactis*)	SW480	antiproliferative and augmentation apoptotic index (Bax/Bcl-2 ratio)	Ahmadi et al. (2017)
Colorectal cancer	*Pichia kudriavzevii* AS-12	HT-29, Caco-2	Inhibit cell proliferation and induce intrinsic and extrinsic apoptosis, the expression level of proapoptotic genes (BAD, caspase-3, caspase-8, caspase-9, and Fas-R), downregulation of antiapoptotic gene (Bcl-2)	Saber et al. (2017)
Colorectal cancer	*Propionibacterium freudenreichii*	CRC	Inhibit proliferation and ↑cell death via production of short chain fatty acids, protective effects, prevention at early stages of the carcinogenesis process	Casanova et al. (2018)
Colorectal cancer	*Bifidobacterium adolescentis*	Colo 320	Anti-mutagenic activity against mutagens, regulate the expression of COX-2	Otte et al. (2008)
Colorectal adenocarcinoma	*Bifidobacterium Lactis* sp. 420	Caco-2 cells	Antiinflammatory and anticarcinogenic, upregulate COX-1 and downregulate COX-2 gene expression	Nurmi et al. (2005)
Colorectal, gastric cancer	*Lactobacillus paracasei* IMPC2.1, *Lactobacillus rhamnosus* GG	HGC-27, DLD-1	Antiproliferative and apoptosis induction, growth inhibition	Orlando et al. (2012)
Colorectal cancer, leukemia cells	*Lactobacillus* spp. from Philippine dairy products	HT-29, HCT116, THP-1	↓IL-1β and TNF-α, prevention of inflammation, ↑apoptosis	Shyu et al. (2014)
Gastric cancer	*Lactobacillus reuteri*	AGS	Inhibits cell proliferation, suppression cell invasion by downregulation of pathways such as uPA and uPAR	Rasouli et al. (2017)
Gastric cancer	*Lactobacillus rhamnosus* GG	HGC-27	↓Ornithine decarboxylase mRNA and activity as well as polyamine content and neoplastic proliferation	Linsalata et al. (2010)
Gastric cancer	*Lactobacillus acidophilus*	CRL 5822	↑COX-1, cytoprotective	Mahkonen et al. (2008)

(continued)

Table 18.1 (continued)

Type of cancer	Probiotic strains	Cell line	Results	Reference
Gastric cancer	*Lactobacillus* kefir	AGS	Apoptosis induction, ↓polarization of mitochondrial membrane potential (MMP), ↓Bcl2 expression	Ghoneum and Felo (2015)
Gastric cancer	Milk Fermented by *Propionibacterium freudenreichii*	HGT-1	↑Apoptosis, ↑chromatin condensation, ↑formation of apoptotic bodies, ↑DNA laddering, ↑cell cycle arrest, ↑ROS accumulation, ↑ mitochondrial transmembrane potential disruption, ↑caspase activation, ↑cytochrome c release	Cousin et al. (2012)
Hepatic cancer	*Lactobacillus rhamnosus*	HepG2	Cytotoxic, ↑apoptotic index (Bax/Bcl2 expression ratio)	Behzadi et al. (2017)
Myeloid leukemia	*Lactobacillus reuteri* ATCC PTA 6475	Human myeloid leukemia-derived cells	↑Apoptosis, modulation of NF-κB, ↑c-Jun N-terminal kinase, ↑p38 MAPK, ↓extracellular signal regulated kinases 1/2, cell proliferation regulation via ↓ IkBa ubiquitination and ↑pro-apoptotic MAPK signaling	Iyer et al. (2008)
Chronic myeloid leukemia	*Saccharomyces cerevisiae*	K562	Time-dependent antitumor activity, inhibit cell growth apoptosis and necrosis induction	Bonyadi et al. (2017)
Acute erythroleukemia	Kefir	KG-1	Inhibition of proliferation, apoptosis and necrosis induction	Jalali et al. (2016)
Lymphoblastic leukemia	Kefir	HTLV-1	Cell-cycle arrest and apoptosis induction, ↓ proliferation, downregulating TGF-α, upregulating TGF-β1 mRNA expression	Maalouf et al. (2011)
Oral cancer	*Lactobacillus plantarum*	KB cells	Apoptosis induction through upregulation of PTEN and downregulation of MAPK signaling pathways	Asoudeh-Fard et al. (2017)
Oral cancer (squamous cell carcinoma)	*Acetobacter syzygii*	KB cells	Prophylactic effect, cytotoxicity, ↑apoptosis	Aghazadeh et al. (2017)

A549 lung carcinoma; *AGS* human gastric adenocarcinoma epithelial cell line cells, *Bad* Bcl-2 associated agonist of cell death, *Bcl-2* B-cell lymphoma 2, *Bcl-xL* B-cell lymphoma-extra-large, *Bak* Bcl2-antagonist/killer, *Bax* Bcl-2-associated X protein, *CD8* cluster of differentiation, *COLO 205* human colon cancer, *COX* cyclooxygenase, *CRL 5822* human gastric adenocarcinoma cell line, *CTM* Catla thymus macrophage cell line, *DLD-1* colon cell lines, *ERK2* extracellular signal regulated kinases 1/2, *G-CSF* granulocyte-colony stimulating factor, *GM-CSF* granulocyte macrophage-colony stimulating factor, *HBD-2* human β-defensin 2, *HCT-15* colonic epithelial cell line, *HeLa* cervical cell line, *HGC-27* gastric cancer cell line, *HGT-1* human gastric cancer cells, *HT29* colon carcinoma cell lines, *HTLV-1* lymphotropic virus type I-negative malignant T-lymphocytes, *ICAM* intercellular adhesion molecule, *IFN-γ* interferon gamma, *IgA* immunoglobulin A, *IL-1β* interleukin, *iNOS* inducible nitric oxide synthase, *JNK* c-Jun N-terminal kinase, *K562* chronic myeloid leukemia, *KB* human oral cancer cell line, *KG-1* acute erythroleukemia cell line, *LS513* colorectal cancer cells, *MAPK* mitogen-activated protein kinase, *MBC* human metastatic breast cancer cells, *MCF-7* breast cancer cell, *MCP-1* macrophage chemoattractant protein-1, *MMP-9* matrix metallopeptidase, *NF-κB* nuclear factor kappa B, *TNF-α* tumor necrosis factor, *PGE2* prostaglandin E2, *PTEN* phosphatase and tensin, *Rel A* a subunit of NF-kB transcription factor, *RNS* reactive nitrogen species, *ROS* reactive oxygen species, *SOD* superoxide dismutase, *Th* T-helper, *THP-1* leukemia cell line, *TRAIL* TNF-related apoptosis-inducing ligand, *uPA* urokinase plasminogen activator, *uPAR* uPA receptor

Table 18.2 Animal studies on use of probiotics for prevention and treatment of cancer

Type of cancer	Probiotic strains	Animal	Results	Reference
Breast cancer	Kefir water, 28 days, 150 mg/kg body weight per day	BALB/c mice	↓Tumor size and weight, ↓metastasis to lung and bone marrow, ↓proinflammatory and procarcinogenic markers, inhibited cell mitosis and inhibited tumor proliferation via ↑tumor apoptosis, ↓IL-10, ↓IL-1β, ↑IFN-γ ↑IL-2), ↑T helper CD4+/CD3+, ↑cytotoxic T cells (CD8+/CD3+), downregulation ICAM, iNOS, MMP-9, IL-1β, NF-κB, G-CSF, GM-CSF, IL-4, TNF-α genes	Zamberi et al. (2016)
Breast cancer	*Lactobacillus acidophilus*	BALB/C mice	↓Tumor growth rate, ↑ IL-12	Yazdi et al. (2010)
Breast cancer	*Lactobacillus acidophilus*	BALB/c mice inbred female mice	↓Tumor growth rate, ↑lymphocyte proliferation, ↑Th1, ↓TGF-β	Maroof et al. (2012)
Breast cancer	*Lactobacillus acidophilus (ATCC4356)*	BALB/c mice	↑ Immune response, ↑Th1, ↑IFN- γ, ↓IL-4 and IL-10	Imani Fooladi et al. (2015)
Breast cancer	Soymilk with *Lactobacillus casei* Shirota	Female Sprague–Dawley rat	Cytotoxicity, ↓tumor growth, ↓angiogenesis, activation of immune cells like neutrophils and monocytes	Kaga et al. (2013)
Breast cancer	Milk fermented with *Lactobacillus casei* CRL431	BALB/c mice	↓Tumor growth rate, ↓tumor vascularity, ↓lung metastasis, ↓macrophage infiltration in tumor and lung, ↓ angiogenesis, ↑CD8+ and CD4+ cells, ↓IL-6	Aragón et al. (2014)
Breast cancer	Milk fermented by *Lactobacillus casei* CRL431	Mice	Modifies cytokine profiles	Utz et al. (2019)
Breast cancer	Milk fermented by *Lactobacillus casei* CRL431	Murine model, Female BALB/c mice	↑Survival rate, ↓lung metastasis, modulation of immune cells, ↓F4/80+, ↓IL-10/F4/80+ cells, ↓IL-6, ↑TNF-α, ↑IFN-γ	Mendez Utz et al. (2019)
Breast cancer	Milk fermented with *Lactobacillus casei* CRL 431	Mice	Inhibition of growth and lung metastasis, ↓tumor vascularity, ↓extravasation of tumor cells, ↓infiltration of macrophages, ↑CD4+ lymphocytes, immunomodulatory	Aragon et al. (2015)
Breast cancer	*Lactobacillus casei* CRL 431	BALB/c mice, murine breast cancer model	immunomodulatory effect	Aragon et al. (2014)

Type of cancer	Probiotic strains	Animal	Results	Reference
Breast cancer	*Lactobacillus casei*	BALB/c mice	↓Tumor growth rate, ↑survival, ↑ IL-12, ↑IFN- γ, ↑NK cells cytotoxicity	Dallal et al. (2012)
Breast cancer	Milk fermented with *Lactobacillus helveticus* R389 or L89	BALB/c mice	Delay in breast tumor growth, induction of cell apoptosis through ↓IL-6 and IL-10, ↑CD4+, ↑IgA	De Leblanc et al. (2005)
Breast cancer	*Lactobacillus helveticus* R389	BALB/c mice	↓Tumor growth, ↑apoptosis, antiproliferative activity, ↓IL-6 and IL-10, ↑CD4+	Rachid et al. (2006)
Breast cancer	*Lactobacillus reuteri ATCC*-PTA-6475	Swiss mice exposed to westernized diet	Inhibit neoplasa, microbially-triggered CD4+ CD25+ lymphocytes	Lakritz et al. (2014)
Breast cancer	*Lactobacillus plantarum* (with selenium nanoparticles)	BALB/C mice	↑Mouse lifespan, ↑IFN- γ, ↑TNF-α, ↑ IL-2, ↑NK cells activity	Yazdi et al. (2012)
Breast cancer	*Lactobacillus plantarum* LS/07	Female rats of Sprague-Dawley strain	Suppress tumor frequency, ↑CD4+ T-cells	Kassayova et al. (2016)
Breast cancer	*Bacillus subtilis* strain fmbj	Arbor Acres broiler chickens	↑Antioxidant activity, improvement tissues antioxidant enzyme capacity (SOD, CAT, GSH-Px, GSH, T-SH), mitochondria antioxidant enzyme capacity (MnSOD, GPx, GSH), mRNA expression of antioxidant genes (Nrf2, HO-1, SOD, CAT, GSH-Px) and mitochondrial function genes (avUCP, NRF1, NRF2, TFAM, PGC-1α), oxidative damage index (MDA, ROS, PC, 8-OHdG)	Bai et al. (2016)
Colorectal cancer	*Lactobacillus salivarius* ssp. *Salivarius* UCC118	IL-10 knockout mice	↓Faecal coliform and enterococci levels, ↓*Clostridium perfringens*, ↓prevalence of colon cancer and mucosal inflammatory act	O'Mahony et al. (2001)
Colorectal cancer	Lactic acid bacteria	Females BALB/c mice	Expressing antioxidant enzymes, ↑SOD activity, ↑catalase activity, ↑IL-10	Del Carmen et al. (2017)

(continued)

Table 18.2 (continued)

Type of cancer	Probiotic strains	Animal	Results	Reference
Colorectal cancer	*Lactobacillus pentosus* B281 and *Lactobacillus plantarum* B282	BALB/c mice, Caco-2	Antiproliferative and immunostimulatory properties, induced the expression of G-CSF, IL-1α, IL-1β, IL-6, CXCL-1, CCL-3, CCL-4, CXCL-2, ↓expression levels of sICAM, M-CSF, TIMP-1	Saxami et al. (2017)
Colorectal cancer	*Lactobacillus rhamnosus*	Rats	Prevents CRC via suppression of expressions of inflammatory and angiogenesis genes, and upregulation of apoptotic gene expression, upregulation of protein expressions of iNOS, TNF-α, NF-kB, COX-2, Bcl-2, VEGF-α, β-catenin and Bax genes	Huang et al. (2019)
Colorectal cancer	*Lactobacillus acidophilus*	BALB/c mice	↓CA19-9 tumor markers, ↑the serum levels of IFN-γ, IL-10, the number of CD4+ and CD8+ cells	Agah et al. (2019)
Colorectal cancer	*Lactobacillus rhamnosus*	Sprague Dawley rats	Prevents colon cancer development, ↓the expression of β-catenin and the inflammatory proteins NFκB-p65, COX-2 and TNF-α, the anti-apoptotic protein Bcl-2, ↑expression of the pro-apoptotic proteins Bax, casp3 and p53, protection effect against colon carcinogenesis inducing apoptosis and ameliorating inflammation	Gamallat et al. (2016)
Colorectal cancer	*Lactobacillus casei* BL23	C57BL6 mice	Immunomodulatory effect, through the downregulation of the IL-22 cytokine, antiproliferative effect, through the upregulation of caspase-7, caspase-9, and Bik	Jacouton et al. (2017)
Colorectal cancer	*Lactobacillus casei* BL23, *Lactobacillus lactis* MG1363	C57BL/6 mice	expression of regulatory cytokines (IL-6, IL-17, IL-10 and TGF-β) production, ↑the percentage of Th17 cells modulates host immune responses and protects mice against induced colorectal cancer	Lenoir et al. (2016)
Colorectal cancer	*Lactobacillus plantarum*, *Lactobacillus rhamnosus*	BALB/c mice + CT26 cells	↑the effector functions of CD8+ and NK cell infiltration into tumor tissue, upregulates IFN-γ production, promotes Th1-type CD4+ T differentiation, inhibited CT26 cells, prolonged the survival time of CRC-bearing mice by producing protective immunity	Hu et al. (2015a)

Type of cancer	Probiotic strains	Animal	Results	Reference
Colorectal cancer	*Lactobacillus acidophilus*, *Lactobacillus fermentum*	CRC cell + Apc (Min/+) CRC mouse model	↓Proliferation, ↑apoptosis, ↓intestinal tumor multiplicity, protection against intestinal tumorigenesis, biotherapeutic for the prevention of CRC	Kahouli et al. (2017)
Colorectal cancer	*Saccharomyces boulardii*	Apc (Min) mice	Inactivation of EGFR-Erk and EGFR-Akt pathways, inactivation of HER-2, HER-3, insulin-like growth factor-1 receptor, prevented EGF-induced proliferation, ↓cell colony formation, promoted apoptosis	Chen et al. (2009)
Colorectal Cancer	*Bifidobacterium longum*	CRC-mice	Suppresses murine colorectal cancer through the modulation of oncomiRs and tumor suppressor miRNAs, ↑necrosis and fibrosis of cells, inhibition of the proliferation, invasion, apoptosis	Fahmy et al. (2019)
Colitis-associated colorectal tumorigenesis	*Lactobacillus helveticus* NS8 separated from fermented koumiss	Male C57BL/6 mice	↓Tumor number and the degree of hyperplasia, suppressed proliferation, ↓apoptosis, ↓NF-κB, upregulated the antiinflammatory cytokine IL-10. downregulation of IL-17-producing T cells, modulating inflammatory development and microbial homeostasis	Rong et al. (2019)
Colon carcinogenesis	*Lactobacillus casei* shirota	C3H/HeN Mice	Chemopreventive mechanisms, cytotoxicity of NK cells by delays tumor onset	Takagi et al. (2001)
Colon carcinogenesis	*Bacillus polyfermenticus*	F344 rats	↓DNA damage and anti-oxidative ability, ↓DMH induced precancerous lesions	Park et al. (2007)
Colon carcinogenesis	*Lactobacillus plantarum*, *Lactobacillus rhamnosus*	Sprague–Dawley rats	Protection against oxidative stress, ↓the activity of GSH, GPx, GST, SOD and catalase, ↑the activity enzymes involved in the p53-mediated apoptotic pathway such as p53, p21, Bcl-2, Bax, caspase-9 and caspase-3	Walia et al. (2018)
Colon carcinogenesis	*Lactobacillus rhamnosus* GG, *Lactobacillus acidophilus*	Sprague-Dawley rats	Prophylactic intervention, modulate Bax-mediated apoptosis, downregulated the expression of anti-apoptotic Bcl-2, proto-oncogene K-ras, upregulated pro-apoptotic Bax, tumor suppressor p53	Sharaf et al. (2018)
Colon carcinogenesis	*Lactobacillus casei*	Mice	Delay the onset of cancer, protection against colon carcinogenesis, its antimutagenic activity, polyamine metabolism	Irecta-Najera et al. (2017)

(continued)

Table 18.2 (continued)

Type of cancer	Probiotic strains	Animal	Results	Reference
Colon carcinogenesis	*Lactobacillus reuteri*	BALB/c mice	↓Proinflammatory cancer-associated cytokines (IL-22, IL-6), ↓relative numbers of splenic CD11bþGr-1þ immature myeloid cells, suppress carcinogenesis, suppression of chronic intestinal inflammation and colorectal tumorigenesis	Gao et al. (2017)
Colon cancer and carcinogenesis	*Bifidobacterium longum*	F344 rats	Inhibits colon cancer and modulates the intermediate biomarkers of colon carcinogenesis	Singh et al. (1997)
Hepatocellular carcinoma	Prohep (*Prevotella* and *Oscillibacter*)	Mice	↓Tumor growth, ↓tumor size and weight, antiangiogenesis, antiinflammatory, by inducing Tregs in gut, downregulation of IL-17, suppressing Th17 cells differentiation, modulates the microbiota	Li et al. (2016)
Hepatocellular carcinoma	*Lactobacillus species*, *Bifidobacterium species*, *Enterococcus species*	Pathogen-free male Sprague–Dawley rats	Suppression of intestinal inflammation, ↓liver tumor growth and multiplicity, ↓tumorigenic inflammation in the liver, ↓IL-6, ↑IL-10, ↓C-reactive protein, higher levels of serum IgG and sIgA	Zhang et al. (2012)
Hepatocellular carcinoma	*Salmonella choleraesuis*	BALB/c mice	Prolonged the animal survival, ↓the tumor size, upregulated IFN-γ and induced IFN-inducible IP-10 productions, ↑infiltration of neutrophils, CD4+ and CD8+ T cells, induced cell death, tumoricidal and antiangiogenic activities	Lee et al. (2008)
Human acute monocytic leukemia	*Lactobacillus* isolates (*L. plantarum and L. pentosus*)	Swiss Albino Mice + THP-1 celle	Induced expression of TNF-α, IL-6, MCP-1, VCAM-1, ICAM-1	Aparna Sudhakaran et al. (2013)
Lung cancer	*Bifidobacterium infantis*-mediated sFlt-1 gene transferring system (recombinant)	LLC C57BL/6 mice	↓Tumor growth, ↑survival time	Zhu et al. (2011)
Lung cancer	*Bifidobacterium infantis*-mediated soluble kinase insert domain receptor (sKDR) (recombinant)	LLC C57BL/6 mice	↓Tumor growth, by increasing the necrosis rate of the tumor and ↑survival time	Li et al. (2012)

Type of cancer	Probiotic strains	Animal	Results	Reference
Lung cancer	*Lactobacillus acidophilus*	C57BL J mice	↑Anti-tumor effect of cisplatin, ↑survival rates	Gui et al. (2015)
Lung and ovarian cancer	*Enterococcus hirae*, *Barnesiella intestinihominis*	C57BL J mice	Increased cyclophosphamide-anticancer effects	Daillere et al. (2016)
Melanoma	Bifidobacterium cocktail (*B. bifidum*, *B. longum*, *B. lactis*, *B. breve*)	C57BL mice	Modulates the CD activity, improve the tumor-specific CD8+ T cell function, promotes antitumor immunity and facilitates anti-PD-L1 efficacy	Sivan et al. (2015)
Metastatic melanoma (MM) or non–small cell lung carcinoma (NSCLC)	*Bacteroides* (*B. fragilis* or *B. thetaiotaomicron*)	Germ Free mice	Immunostimulatory effects of CTLA-4 blockade	Vetizou et al. (2015)
Pancreatic cancer	*Lactobacillus delbruckei* sp. *bulgaricus* DWT1 and *Streptococcus thermophilus* DWT4	C57BL/6 mice + RAW 264.7 cells	Inhibits tumor growth by activating pro-inflammatory responses in macrophages, ↓cells cytotoxicity, upregulated IL1 β, IL6, IL12, TNF-α	Guha et al. (2019)

avUCP avian uncoupling protein, *Bax* Bcl-2-associated X protein, *Bcl-2* B-cell lymphoma 2, *CAT* catalase, *CCL* chemokine (C-C motif) ligand, *CD8* cluster of differentiation, *EGF* epidermal growth factor, *Erk* extracellular signal-regulated kinase, *G-CSF* granulocyte-colony stimulating factor, *CXCL* chemokine (C-X-C motif) ligand, *G-CSF* granulocyte colony-stimulating factor, *GM-CSF* granulocyte macrophage-colony stimulating factor, *GSH* glutathione, *GSH-Px* glutathione peroxidase, *HO-1* heme oxygenase 1, *ICAM* intercellular adhesion molecule, *IgA* immunoglobulin A, *IL-1β* interleukin, *iNOS* inducible nitric oxide synthase, *MCP-1* monocyte chemotactic protein-1, *M-CSF* macrophage colony-stimulating factor, *MDA* malondialdehyde, *MIP-1β* macrophage inflammatory protein 1 beta, MMP-9 matrix metallopeptidase, *MnSOD* manganese superoxide dismutase, *NF-κB* nuclear factor kappa B, *NK cells* natural killer cells, *NRF1* nuclear respiratory factor 1, *Nrf2* nuclear factor erythroid 2-related factor 2, *PGC-1α* peroxisome proliferator-activated receptor gamma coactivator 1α, *PGE2* prostaglandin E2, *sICAM* soluble intercellular adhesion molecule, *SOD* superoxide dismutase, *STAT3* signal transducers and activators of transcription 3, *TFAM* mitochondrial transcription factor A, *Th* T-helper, *TIMP-1* metallopeptidase inhibitor 1, *TNF-α* tumor necrosis factor, *T-SH* total mercapto, *VCAM-1* vascular cell adhesion molecule-1, *VEGF-α* vascular endothelial growth factor α, *XCL1* lymphotactin, *IP-10* chemokines CXCL10

Table 18.3 Human studies of probiotics interventions for prevention, post-operative complications and treatment of cancer

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Breast cancer	Prevention	Case-Control	Beverages containing *Lactobacillus casei* Shirota (BLS) and soy isoflavone	360 Breast cancer patients, 662 controls	Four times a week	↓Breast cancer risk	Toi et al. (2013)
Cervical, sigmoid or rectal, cancer	Chemotherapy and radiation therapy related toxicity	Double-blind, parallel-group, and placebo-controlled trial	VSL#3 (a mixture of eight probiotics *Lactobacilli* and *bifidobacteria*)	490 Cancer patients	From the first day of radiation therapy	↓Diarrhea, ↓daily bowel movements	Delia et al. (2007)
Cervical cancer	Prevention of radiation-induced diarrhea	Randomized double-blind placebo-controlled trial	*Lactobacillus acidophilus* LA-5 + *Bifidobacteriumanimalis* subsp. *lactis* BB-12	54 Patients (n = 26), Placebo (n = 28)	3 weeks after radiotherapy	↓Mild-to-moderate and severe diarrhea, ↓loperamide consumption, ↓abdominal pain in the probiotic group	Linn et al. (2019)
Cervical cancer	Prevention of radiation-induced diarrhea	Prospective, randomized, double-blind, placebo-controlled trial	*Lactobacillus acidophilus* + *Bifidobacterium bifidum*, one capsule (250 mg), two times a day before meals	63 Patients undergoing pelvic radiotherapy concurrent with weekly cisplatin (n = 32) (infloran), placebo (n = 31)	Beginning 7 days before starting radiotherapy and continuing every day during radiotherapy	↓ Diarrhea, ↓anti-diarrhea drug used, improved stool consistency	Chitapanarux et al. (2010)

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Cervical cancer	Prevention of radiation-induced diarrhea	Randomized double-blind, placebo-controlled clinical trial in two parallel groups	Probiotics (*Lactobacillus casei* DN-114001, *Streptococcus thermophilus*, *Lactobacillus delbrueckii*, subsp. Bulgaricus), a dose, five times weekly	85 Patients undergoing pelvic radiotherapy	5–6 weeks	↓ Diarrhea, improved stool consistency	Giralt et al. (2008)
Colorectal cancer	Prevention	Randomized, double-blind, placebo-controlled, two-period crossover trial	*Lactobacillus rhamnosus* LC705, *Propionibacterium freudenreichii* ssp., *shermanii* JS, two capsules daily	38 Healthy patients	4 weeks	↓β-glycosidase activity, ↓urease activity, ↑fecal counts of lactobacilli and propionibacteria	Hatakka et al. (2008)
Colorectal cancer	Prevention	Randomized, double-blind, placebo-controlled, crossover trial	*Bifidobacterium lactis* one sachet and one capsule daily	17 Healthy patients	4 weeks	Induced unique changes in fecal microflora, not significantly alter any other fecal, serum or epithelial variables	Worthley et al. (2009)
Colorectal cancer	Prevention	ND	*Lactobacillus gasseri* OLL2716: LG21 once daily	10 CRC patients and 20 healthy patients	12 weeks	Intestinal environment improvement in colorectal cancer patients, ↑short-chain fatty acid isobutyric acid, ↑IL-1β, ↑NK cells activity	Ohara et al. (2010)

(continued)

Table 18.3 (continued)

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Colorectal cancer	Prevention of post-operative complication	Randomized, double-blind, placebo-controlled trial	*Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium lactis*, *Saccharomyces boulardii*, one capsule b.i.d	124 CRC patients undergoing surgery (placebo group/ probiotics group n = 80/84)	15 days	↓All postoperative major complication, gene expression of TNF-α and circulating concentrations of IL-6 were under the control of SOCS3 in the probiotics group	Kotzampassi et al. (2015)
Colorectal cancer	Prevention of post-operative complication	ND	*Enterococcus faecalis*, *Clostridium butyricum*, *Bacillus mesentericus* six tablets daily	156 CRC patients undergoing surgery (placebo group/ probiotics group n = 81/75)	15 days	↓Superficial incisional surgical site infections (SSIs) in patients undergoing CRC surgery	Aisu et al. (2015)
Colorectal cancer	Prevention of post-operative complication	Randomized controlled trial	*Bifidobacterium longum*, *Lactobacillus acidophilus*, *Enterococcus faecalis*, gastric gavage	60 CRC patients undergoing surgery (placebo group/ probiotics group n = 30/30)	12 days	Faster recovery of bowel function, lower incidences of diarrhea, and slightly lower rate of bacteremia in probiotic group	Yang et al. (2016)

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Colorectal, liver metastasis	Prevention of post-operative complication	Double-center and randomized, double-blind clinical trial	*Bifidobacterium longum*, *Lactobacillus acidophilus*	150 patients control group/ probiotics group (n = 68/66)	6 days preoperatively and 10 days postoperatively	↓Concentration of serum zonulin and plasma endotoxin, ↓postoperative septicemia, ↓diarrhea, inhibit the p38 MAPK signaling pathway	Liu et al. (2015)
Colorectal cancer	Prevention of post-operative complication	Randomized, double-blind clinical trial	*Bifidobacterium longum* (BB536) + *Lactobacillus johnsonii*, Orally 2 doses daily	31 Patients	3 day preoperatively and postoperatively from day 2–4 days	↓Concentration of pathogens, modulates local immunity	Gianotti et al. (2010)
Colorectal cancer	Prevention of post-operative complication (effect on inflammatory markers)	ND	Glucose solution combined with postoperative probiotics (*Bifidobacteria* and *Lactobacillus*)	60 Patients after colorectal cancer surgery (placebo group/ probiotics group n = 30/30)	7 days	↓Plasma endotoxin, ↓IGF-I concentration, ↓D-lactic acid and urinary L/M levels, ↓duration of postoperative fever, ↓average heart rate WBC and CRP clinical indexes, improve the imbalance of intestinal flora, recovery of early inflammatory response after surgery	Xu et al. (2019)

(continued)

Table 18.3 (continued)

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Colorectal cancer	Prevention of post-operative infectious complications	Randomized, double-blind, placebo-controlled, prospective study	*Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Bifidobacterium longum*, orally, 2 g/daily	100 Patients with CRC scheduled for radical colorectomy, control (n = 50) and probiotics groups (n = 50)	16 days (6 days preoperatively and 10 days postoperatively)	Improved the integrity of the gut mucosal barrier and balance of the gut microbiota, ↓rate of post-surgical infection and diarrhea	Liu et al. (2011)
Colorectal cancer	Prevention of post-operative complication (effect on inflammatory markers)	Randomized double-blind placebo controlled trial	*Lactobacillus paracasei* subsp. paracasei, *Lactobacillus plantarum*, *Pediacoccus pentosaceus*, *Leuconostoc mesenteroides*, b.i.d	68 Patients undergoing colorectal surgery	3 days before the operation and 9–11 days after the operation	↓Postoperative inflammatory response (IL-6)	Horvat et al. (2010)
Colorectal cancer	Prevention of post-operative complication (effect on inflammatory markers)	Randomized clinical trials	*Leuconostoc mesenteroides*, *Lactobacillus plantarum*, *Pediacoccus pentosaceus*, *L. paracasei*, b.i.d	73 Patients with preceding colorectal operations	3 days before the operation and 10–11 days after the operation	↓Systemic inflammatory response	Krebs et al. (2013)
Colorectal cancer	Prevention of post-operative complication	Randomized double-blind placebo controlled trial	Probiotics *(Lacidofil)*, b.i.d	28 Participants in the probiotics group and 32 participants in the placebo group	12 weeks	↓The proportion of patients suffering from irritable bowel symptoms, improved quality of life	Lee et al. (2014)

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Colorectal cancer	Chemotherapy and radiation therapy related toxicity	Open-label, prospective, randomized, trial	*Lactobacillus rhamnosus* GG, gelatine capsules b.i.d	150 CRC patients undertreated	24 weeks	↓Frequency of severe diarrhea, ↓abdominal discomfort, less hospital care, and had fewer chemo dose reductions due to bowel toxicity	Österlund et al. (2007)
Colorectal cancer	Prevention of post-operative complication (effect on inflammatory markers)	Randomized double-blind placebo controlled trial	*Lactobacillus acidophilus*, *Lactobacillus lactis, Lactobacillus casei*, *Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium infantis*), orally b.i.d	52 Patients with CRC	From 4 weeks after surgery, for 6 months	↓Level of pro-inflammatory cytokine (TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C and IL-22)	Zaharuddin et al. (2019)
Colorectal cancer	Prevention of post-operative complication (effect on inflammatory markers)	Randomized double-blind trial	*Lactobacillus acidophilus*, *Lactobacillus lactis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Bifidobacterium infantis*, two sachets daily	140 Patients with CRC	4 weeks	↓Inflammatory biomarkers (IL-6, TNF-α, CRP), ↓side effects of chemotherapy, improve quality of life	Golkhalkhali et al. (2018)
Gastric cancer	Prevention of post-operative complication	Prospective randomized controlled trial	Probiotics (*Bifidobacterium* and *Lactobacillus*) combined with enteral nutrition	140 Postoperative patients with gastric cancer (underwent distal gastrectomy) (n = 70/70)	8 days	↑Immune function factors such as IgG, IgA and IgM, ↓inflammatory cytokines including IL-6, IL-8, TNF-α, ↓time to relief of abdominal distention and time to first flatus, ↓Diarrhea	Xie et al. (2018)

(continued)

Table 18.3 (continued)

Type of cancer	Type of intervention	Design	Probiotic strain	Participants	Length of treatment	Outcome	Reference
Gastric cancer	Prevention of post-operative complication	Randomized trial	Probiotics (*Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Enterococcus faecalis* and *Bacillus cereus*), three capsules, t.i.d	37 Patients	7 days after the partial gastrectomy	↑Immune response, ↓inflammation through modification of gut microbiota, ↓severity of physiological and microbial disorders induced by partial gastrectomy	Zheng et al. (2019)
Hepatic cancer	Prevention	Randomized, double-blind, placebo-controlled trial	*Lactobacillus rhamnosus* LC705, *Propionibacterium freudenreichii* subsp. *Shermanii*. One capsule b.i.d.	90 Patients (n = 45/45)	5 weeks	↓Urinary excretion of aflatoxin B1-N^7-guanine, ↓the biologically effective dose of aflatoxin exposure, ↓risk of liver cancer	El-Nezami et al. (2006)
Lung cancer	Chemotherapy and radiation therapy related toxicity	Prospective, randomized, double blind and placebo comparative	*Clostridium butyricum*, 420 mg/tablet, t.i.d	41 Patients with lung cancer	3 weeks	↓Diarrhea, ↓systemic inflammatory response system, ↑levels of CD3 + CD8+ and CD16 + CD56+	Tian et al. (2019)

b.i.d twice a day, *CRC* colorectal cancer, *CRP* C-reactive protein, *IGF-I* insulin-like growth factor-I, *IL-1β* Interleukin, *L/M* lactulose/mannitol, *NF-κB* nuclear factor kappa B, *t.i.d* means three times a day, *TNF-α* tumor necrosis factor, *WBC* white blood cell count

et al. 2016). Also, in other study postbiotic metabolites produced via *Lactobacillus plantarum* induced several levels of MCF-7 cancer cell death and demonstrated that can have potential as anticancer preventive agent and as human health supplement.

Saccharomyces cerevisiae via enhancement of Bax and reduction of Bcl-2 expression and resulting in alteration in the Bax: Bcl-2 ratio induced apoptosis in human metastatic breast cancer cells (Ghoneum and Felo 2015). *Lactococcus lactis* KC24 was derived from Kimchi demonstrated anticancer effect against gastric carcinoma (AGS), breast carcinoma (MCF-7) colon carcinoma (HT-29 and LoVo) and lung carcinoma (SK-MES-1) through decrease cell line proliferation, apoptosis induction, antioxidant activity and antiinflammatory effects by NO production (Lee et al. 2015). In another study, the antiinflammatory activity of *Lactococcus lactis* NK34 was also revealed in lipopolysaccharide-induced RAW 264.7 cells, where the production of NO and proinflammatory cytokines (COX-2, IL-18, and TNF-α) was decreased (Han et al. 2015).

In a study by Silva et al., an anticancer drug, Laphacol, was transformed to a more cytotoxic ingredient by *Lactobacillus acidophilus* and *Bifidobacterium* sp. (Silva et al. 2014). Anti-breast cancer influence of alive, heat-killed cells of *Staphylococcus hominis* and *Enterococcus feacalis* extracted from human breast milk have also been assessed. Bacteria demonstrates cytotoxic effect against MCF-7 cell line in a concentration and time-dependent manner (Hassan et al. 2016).

(S)-latifolicinin A(4b), a product of soy milk fermentation with *Saccharomyces cerevisiae*, *Lactobacillus bulgaricus* and *Lactobacillus paracase*was moderately inhibited the proliferation of MCF-7 and MDA-MB-231 (Ke et al. 2015).

Administration of the fermented soy-skim milk with *Lactobacillus* strains (*Lactobacillus paracasei* ssp. paracasei NTU 101 and *Lactobacillus plantarum* NTU 102) revealed antioxidant and anti-proliferative features by enhancement SOD activity and PGE2 synthesis (Liu et al. 2009).

Anti-proliferative effects of *Enterococcus* (*E. faecalis* 13C, *E. durans* 39C, *E. mundtii* 50H) strains isolated from fermented dairy products were evaluated on different cancer cell lines. The secreted metabolites of *E. durans* demonstrated anticancer properties against cancer cell lines, similar to Taxol without toxicity in the normal cell line (Haghshenas et al. 2014).

4.1.2 Animal Studies

Several studies in animals have revealed the advantage of probiotics against breast cancer.

Orally administration of *Lactobacillus casei* to mice using a standard gastric feeding tube for 2 weeks, significantly increased the production of IFN-γ, IL-12 and NK cytotoxicity in spleen cells culture and decreased the growth rate of tumor (Dallal et al. 2012). Milk fermented with *Lactobacillus casei* demonstrated inhibition of growth and metastasis of breast cancer in mice through increasing CD8+,

CD4+ cells lymphocytes (Aragon et al. 2015), activation of immune cells such as neutrophils and monocytes (Kaga et al. 2013), decreasing infiltration of macrophages, angiogenesis (Mendez Utz et al. 2019) and modifying cytokine profiles like IFN-γ, TNF-α, IL-6 (Aragón et al. 2014; Utz et al. 2019).

Oral administration of *Lactobacillus acidophilus* revealed a significant enhancement in the survival time. Also, it revealed that can reduce tumor growth rate progress the immune responses by enhancement of lymphocyte proliferation (Yazdi et al. 2010), suppression splenocyte production of TGF-β, IL-4, IL-10 and IFN-γ (Maroof et al. 2012) and stimulation of the production of pro-inflammatory cytokines such as IL-12, IFN-γ (Imani Fooladi et al. 2015). An enhancement of IFN-γ, TNF-α IL-2 levels and NK cells activity were reported in mice treated with selenium nanoparticles enriched with *Lactobacillus plantarum* (Yazdi et al. 2012). In addition, another study reported immunomodulatory effects of *Lactobacillus plantarum* via increasing CD4+ T-cells (Kassayova et al. 2016).

In another study, kefir water suppressed tumor proliferation *in vivo* and *in vitro* chiefly by cancer cell apoptosis, immunomodulation through stimulating cytotoxic T cells and T helper cells, and antiinflammatory, antimetastatic, and antiangiogenesis effects. Moreover, proangiogenic and proinflammatory markers were significantly decreased in this group (Zamberi et al. 2016).

Oral administration of fermented milk containing *Lactobacillus helveticus* revealed delay in breast tumor growth, an immunoregulatory response induction of cell apoptosis through reduction IL-6, IL-10 and increase CD4+ and IgA (De Leblanc et al. 2005; Rachid et al. 2006).

Dietary probiotic *Bacillus subtilis* strain fmbj enhanced oxidative stability and antioxidant capacity of chicken breast meat during storage (Bai et al. 2016).

4.1.3 Human Studies

In a Japanese population-based case-control study, 306 cases with breast cancer and 662 controls were assessed about their diet, lifestyle, and other risk factors of breast cancer using an interview and self-administered questionnaire. It was concluded that regular usage of *Lactobacillus casei* Shirota and soy isoflavones since adolescence was significantly related to reduce breast cancer risk in Japanese women (Toi et al. 2013).

4.2 Cervical Cancer

Cervical cancer, a human papillomavirus (HPV)-related sickness, is the fourth highest mortality rate among women of the world (Boccardo et al. 2010). Some factors such as the cervical ecosystem and its microbiome are involved in the progress of cervical intraepithelial neoplasia following HPV infection. Lactobacilli are dominant in the cervical and vaginal microbiota of a healthy woman (Wang et al. 2018).

Lactobacilli modulate the vaginal microbiota by numerous mechanisms, such as aggregation, coaggregation with pathogenic microorganisms, adherence to epithelial cells, and production of several antimicrobial substances, like hydrogen peroxide, bacteriocins, lactic acid and biosurfactants (Nami et al. 2014).

4.2.1 *In Vitro* Studies

In a study by Sungur et al., both live and exopolysaccharides of *Lactobacillus gasseri* strains were capable to suppress the cell proliferation, induce apoptosis in HeLa cells in a dependent manner through upregulation of Bax and caspase 3. Also, it showed an antiinflammatory influence by increasing the IL-10 and reducing the production of TNF-α production (Sungur et al. 2017).

The effects *Lactobacillus crispatus* probiotics on metastasis and proliferation of cervical cancer cell line were evaluated by Adnan et al. Results of this study indicated that *Lactobacillus crispatus* supernatant could be a probable healing agent for the treatment of progressive human cervical cancer via downregulation of MMP2 and MMP9 genes expression (Adnan et al. 2018).

Supernatants of these two lactobacilli (*Lactobacillus crispatus* and *Lactobacillus rhamnosus*) revealed cytotoxic and anti-metastatic effect on HeLa cells with reduction expression of MMP9, MMP2 and enhancement expression of their inhibitors (Nouri et al. 2016).

Inhibitory effects supernatants of *Lactobacillus crispatus*, *Lactobacillus jensenii*, and *Lactobacillus gasseri* on the viability of cervical cancer cells demonstrated through adjustment of cell cycle-related genes and HPV oncogenes (Wang et al. 2018). *Lactobacillus paracasei* SR4, *Lactobacillus casei* SR2, and *Lactobacillus casei* SR1, isolated from human breast milk demonstrated a potential therapeutic index and acceptable anticancer effects on HeLa cells via downregulating the expression of the Bcl −2 gene and via upregulating the expression of apoptotic genes, caspase3, caspase8, caspase9, Bax and BAD (Riaz Rajoka et al. 2018).

4.2.2 Human Studies

All four papers, which will be discussed below, investigated whether probiotics are effective at decreasing diarrhea caused by radiation therapy in patients with cervical cancer. Sixty-three advanced cervical cancer patients in a randomized, double blind, placebo-controlled trial have been examined (Chitapanarux et al. 2010). Results of this study demonstrated that a probiotic containing live *Bifidobacterium bifidum* plus *Lactobacillus acidophilus* had a significant advantage on stool consistency and decreased the occurrence of radiation-induced diarrhea and the need for antidiarrheal remedy in drug group (Chitapanarux et al. 2010).In another double-blind, placebo-controlled study, 450 patients who undergo adjuvant radiation therapy after surgery for cervical, rectal, and sigmoid cancer were evaluated with probiotic product VSL#3 or placebo beginning from the first day of radiation therapy.

Most patients who received placebo endured grade 3 or 4 diarrhea, and also had radiation-induced diarrhea compared with VSL # 3 recipients. Daily bowel movements and diarrhea were significantly decrease in VSL#3 group. So, probiotic VSL#3 is a safe, feasible and easy approach to preserve cancer patients against the risk of radiation-induced diarrhea (Delia et al. 2007). Also decrease diarrhea and improvement stool consistency in 41 patients which involved drinking a probiotic drink five times daily (Giralt et al. 2008).

A randomized double-blind placebo-controlled study was carried out by Linn et al., in 2019, on the effect of probiotics *Lactobacillus acidophilus* LA-5 and *Bifidobacterium animalis* subsp. *lactis* BB-12 on acute radiation-induced diarrhea among 54 cervical cancer patients. The incidence of diarrhea, daily episodes of abdominal pain and usage of loperamide as an anti-diarrhea drug were significantly decreased in the probiotic group (Linn et al. 2019).

4.3 Colorectal Cancer

Colorectal cancer is the third most prevalent cancer around the world with over a billion cases a year that shows a high mortality and increasing incidence (Javanmard et al. 2018). Evidence has demonstrated that using probiotics is a protecting approach for appropriate keeping of healthy gut microbiota and additionally decreasing the risk of colon cancer. Furthermore, many *in vitro* and *in vivo* studies in cancer cell lines of human, animal models and randomized placebo-controlled trials have investigated the impact of probiotics on prohibition of intestinal carcinogenesis and also prevention and treatment of colorectal cancer.

4.3.1 *In Vitro* Studies

Administration of live *Lactobacillus casei* demonstrated significance antiproliferative and proapoptotic effects upregulation of the TNF related apoptosis-inducing ligand (TRAIL) and downregulation of survivin (Tiptiri-Kourpeti et al. 2016). *Lactobacillus acidophilus* via modulating oxidative stress and inflammation, increasing expression of TNF-α and IL-10, reduction of iNOS, ROS, RNS and DNA damage, COX-2 induced macrophage mediated inflammatory response and apoptosis against *Aeromonas hydrophila* in CTM cells (Patel et al. 2016). In addition, treatment with *Lactobacillus casei* and *Lactobacillus acidophilus* decreased cell proliferation, increased cell apoptosis and scavenging activity in several studies and they enhanced immune system and efficiently repress the malignant phenotypes of colorectal cancer cells (Choi et al. 2006; Baldwin et al. 2010; Soltan Dallal et al. 2015).

Live and heat-killed *Lactobacillus rhamnosus* GG effectively ameliorate inflammation via upregulating gene expression of pro-inflammatory cytokines like TNF-α, MCP-1 and IL-12 by affecting the NF-κB/IκB pathway in Caco-2 Cells (Fang

et al. 2014; Zhang et al. 2005). Another study has shown that Cell-Free Supernatants from *Lactobacillus casei* and *Lactobacillus rhamnosus* reduced colon cancer cell invasion and MMP-9 and enhanced ZO-1 protein levels (Escamilla et al. 2012), also they inhibited IL-1 β -induced IL-8 production in Caco-2 and this impact happened at the transcriptional level, by inhibition of the NF-κB signaling pathway (Hwan Choi et al. 2008).

Supernatant of *Lactobacillus rhamnosus* and *Lactobacillus crispatus* have shown to possess antimetastatic, cytotoxic, antiproliferative activity by reduction of expression of MMP2, MMP9 and increasing expression of their inhibitors (Nouri et al. 2016). *Streptococcus thermophilus*, *Lactobacillus rhamnosus*, *Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum* and *Bifidobacterium longum* decline colon cancer development by downregulation of most cytokines like IL-1β, IFN-γ, IL-10 and IL-1, increasing production of TNF-α and IL-1β and modifying the intestinal environment (Djaldetti and Bessler 2017).

A recent study has shown *Lactobacillus paracasei* induces apoptosis cell cycle arrest and calreticulin translocation through the ROS generation followed by CRT accompanied endoplasmic reticulum (ER) stress and S phase arrest in HT-29 cells (Hu et al. 2015b), besides, anti-proliferative effects via induction of through apoptosis modulation of expression of specific Bcl-2 family proteins, revealed in another study (Chondrou et al. 2018). The growth-inhibitory properties of probiotic heat-killed *Lactobacillus brevis* and *Lactobacillus paracasei* on the HT-29 cell line revealed by inhibiting the growth, inducing apoptosis by regulation of Bax/Bcl2 and caspases pathway for the treatment and prevention of cancer (Karimi Ardestani et al. 2019).

As revealed by a study, polyphosphate derived from *Lactobacillus brevis* prevents developement of colon cancer induce apoptosis through through activation of the ERK pathway in SW620 cells (Sakatani et al. 2016). Immunomodulatory effects of *Lactobacillus plantarum* on Caco-2 cells proved by increasing in (Human β-defensin 2) HBD-2 expression, inhibiting TLR-2 and production of IL-23 (Paolillo et al. 2009). Fermentation supernatants of *Lactobacillus delbrueckii* has been shown to prevent growth of human colon cancer cells via induction of cell apoptosis through caspase 3-dependent pathway and can be as novel therapies for the treatment of colon cancer (Wan et al. 2014). Nisin that is a polycyclic peptide with 34 amino acids produced by *Lactococcus lactis* during fermentation could induce apoptosis by intrinsic pathways and lead to cancerous cell death (Ahmadi et al. 2017).

Bifidobacterium lactis sp. 420, which preparate acetate and lactate but no butyrate or propionate, exerted potential antiinflammatory and anticarcinogenic properties by upregulating COX-1 and downregulating COX-2 gene expression in a Caco-2 cell culture model (Nurmi et al. 2005). Furthermore, *Bifidobacterium adolescentis* regulates the expression of COX-2 in intestinal epithelial cells (Otte et al. 2008). Antiinflammatory and anti-proliferative effects *Bifidobacterium strain* and *Lactococcus strain* were revealed via upregulation of intestinal alkaline phosphatase, activation of NF-κB and reduction IL-8 (Grimoud et al. 2010).

Apoptotic effect of *Saccharomyces cerevisiae* by regulation of Akt/NF-κB signaling pathway by reduction of p-Akt1, Rel A, Bcl-XL, pro-caspase 3, and

pro-caspase 9 expressions was demonstrated on human colon cancer SW480 cells (Shamekhi et al. 2019; Sambrani et al. 2019). *Pichia kudriavzevii* AS-12 revealed significant cytotoxic effects, induction of apoptosis via increasing expression level of pro-apoptotic genes (BAD, caspase-3, -8, -9 and Fas-R) downregulating anti-apoptotic gene (Bcl-2) in treated HT-29 and Caco-2 cells (Saber et al. 2017).

Therapeutic and prohibition at early stages of the carcinogenesis process of *Propionibacterium freudenreichii* in colorectal cancer demonstrated by inhibiting proliferation and cell death induction through the production of SCFAs mainly acetate and propionate (Casanova et al. 2018). Heat-killed culture supernatant of *Bacillus coagulans* Unique IS2 revealed that can be considered for adjuvant therapy in the treatment of colon carcinoma by inducing apoptosis through enhancement caspase 3 activity, Bax, and reduce Bcl2, mitochondrial membrane potential (Madempudi and Kalle 2017).

4.3.2 Animal Studies

Anti-proliferative and immunostimulatory properties of probiotics of *Lactobacillus pentosus* B281 and *Lactobacillus plantarum* B282 isolated from fermented olives were demonstrated by inducing cytokine/chemokine production, increasing expression of granulocyte-colony stimulating factor (G-CSF), IL-1α, IL-1β, IL-6, chemokine (C-X-C motif) ligand (CXCL)-1, CXCL-2, chemokine (C-C motif) ligand (CCL)-3, CCL-4 and neutrophil and diminishing expression levels of soluble intercellular adhesion molecule (sICAM), macrophage colony-stimulating factor (M-CSF) and metallopeptidase inhibitor1 (TIMP-1) in mice (Saxami et al. 2017).

Tow animal study have shown that treatment with *Lactobacillus rhamnosus* prevents colorectal cancer and carcinogenesis development inducing apoptosis and ameliorating inflammation and angiogenesis by downregulation of β-catenin and the inflammatory and anti-apoptotic proteins, COX-2 and TNF-α, iNOS, NF-kB, Bcl-2, VEGF-α, NFκB-p65, Bcl-2 and increasing expression of the pro-apoptotic proteins Bax, casp3 and p53 (Huang et al. 2019; Gamallat et al. 2016).

A study suggests that *Lactobacillus plantarum* and *Lactobacillus rhamnosus* can supply protection against oxidative stress and apoptotic-related protein dysregulation via decreasing the activity of GSH, GPx, GST, SOD and catalase, and enhancement of the activity enzymes involved in the p53-mediated apoptotic pathway including p53, p21, Bcl-2, Bax, caspase-9 and caspase-3 and prohibit colon carcinogenesis induction in rats (Walia et al. 2018). Another study indicates enhancement the anti-tumor immune response and delay tumor formation by increasing the effector functions of CD8+ and NK cell infiltration into tumor tissue, upregulation of IFN-γ production, and promotion of Th1-type CD4+ T differentiation in BALB/c mice (Hu et al. 2015a).

Protective effect of *Lactobacillus casei* on 1,2-dimethylhydrazine dihydrochloride (DMH)-induced colon carcinogenesis and delayed the onset of cancer in mice demonstrated in two studies through cytotoxicity of NK cells (Takagi et al. 2001; Irecta-Najera et al. 2017). In addition, this probiotic significantly protected mice

against colorectal cancer development through reduction histological scores, immunomodulatory effect via the downregulating of the IL-22, and antiproliferative effect with the upregulating of caspase-7, caspase-9, and Bik (Jacouton et al. 2017), also, by modulating T_{reg} and Th17 T-cell of host immune responses by the expression of regulatory cytokines (IL-6, IL-17, IL-10 and TGF-β) (Lenoir et al. 2016).

Probiotics *(Lactobacillus acidophilus*, *Lactobacillus rhamnosus* GG) and celecoxib decreased tumor multiplicity, tumor burden, upregulated pro-apoptotic Bax as well as tumor suppressor p53, downregulated the expression of anti-apoptotic Bcl-2, proto-oncogene K-ras and demonstrated prophylactic intervention against colon carcinogenesis (Sharaf et al. 2018). Administration of *Lactobacillus helveticus* NS8 separated from fermented koumiss repressed colitis-associated colorectal tumorigenesis and demonstrated protective effects against colorectal cancer by modulating inflammatory development by inhibiting NF-κB activation and IL-17-producing T cells, upregulating IL-10 and microbial homeostasis (Rong et al. 2019).

Saccharomyces boulardii inhibited epidermal growth factor receptor (EGFR), EGFR-Erk, EGFR-Akt pathways and growth of intestinal tumor in mice, and it decreased formation of cell colony, and advanced apoptosis, inactivated HER-2, HER-3, and insulin-like growth factor-1 receptor, thereby it has therapeutic or prophylactic role in intestinal neoplasia (Chen et al. 2009). *Bifidobacterium longum* represses murine colorectal cancer via the modulation of oncomiRs and tumor suppressor miRNAs. The modulatory effect of *Bifidobacterium longum* on microRNAs may supply a significant therapeutic impact in colorectal cancer via inhibition of the apoptosis, invasion, proliferation, and cell cycle of tumor cells (Fahmy et al. 2019; Singh et al. 1997). A probiotic strain of *Bacillus polyfermenticus* indicated a protective influence on the antioxidant system and the colon carcinogenesis process, so suppressed the development of preneoplastic lesions in rats (Park et al. 2007).

4.3.3 Human Studies

The results of several clinical trial studies demonstrated the impact of probiotics on the alteration of the structure of gut microbiota, so certainly influence the host by inhibiting pathogens growth, decreasing metabolism of pro-carcinogenic substances, progressing intestinal barrier integrity, increasing production of short-chain fatty acids. Thus, probiotics are effective in avoiding and prohibiting the intestinal cancer growth (Hatakka et al. 2008; Worthley et al. 2009; Ohara et al. 2010).

In a randomized, double-blind, placebo controlled two-period crossover study, consumption of *Lactobacillus rhamnosus* LC705 together with *Propionibacterium freudenreichii* ssp. *shermanii* JS daily in thirty-eight healthy men indicated decrease in the activity of β-glucosidase and increase in the fecal counts of *lactobacilli* and *propionibacteria* (Hatakka et al. 2008). Furthermore, numerous randomized clinical trial studies reveal that the consumption of probiotics is a promising approach to the improvement of the integrity of gut mucosal barrier and prohibition of post-operative superficial incisional surgical site infections (SSIs) in patients undergoing abdominal surgery (Kotzampassi et al. 2015; Aisu et al. 2015; Yang et al. 2016). Additionally,

quality of life of the patients, the period needed for prescription of antibiotics and shortening the duration of post-operative hospital stay was also improved (Lee et al. 2014).

Radiotherapy and chemotherapy can modify the combination of the gut microbiota. These disruptions can impress on the development of mucositis, principally bacteremia and diarrhea (Touchefeu et al. 2014). Gastrointestinal mucositis is ulcers or inflammation of gastrointestinal tract. Symptoms of mucositis can include abdominal pain, fatigue, diarrhea, malnutrition, electrolyte imbalance bleeding dehydration and infections, with fatal complications (Javanmard et al. 2018). Two trial on CRC patients who were undergoing radiotherapy and chemotherapy demonstrated a significantly reduction incidence of diarrhea through administration of *L. rhamnosus* GG and VSL#3 (a mixture of eight probiotics) (Delia et al. 2007; Österlund et al. 2007). Also, *Lactobacillus plantarum*, *Lactobacillus acidophilus* and *Bifidobacterium longum* via promoting the faecal microbiota, and reducing infectious complications progress the integrity of gut mucosal barrier in CRC patients undergoing colorectomy (Liu et al. 2011).

Probiotics are capable to enhance immune status and modulate the inflammatory factors so specific probiotic genera such as the lactic acid probiotics have called "immunobiotics". Several clinical trial studies have revealed that probiotics comprisingmicroorganisms of *Lactobacillus* and *Bifidobacteria* strains are effective in patients after colorectal cancer surgery and have decreased pro-inflammatory markers such as TNF-α, IL-6, IL-10, IL-12, IL-17A, IL-17C and IL-22 (Horvat et al. 2010; Krebs et al. 2013; Zaharuddin et al. 2019; Golkhalkhali et al. 2018).

4.4 Gastric Cancer

Gastric cancer as fourth most common type of cancer in the world is developed from the lining of the stomach (Xie et al. 2018). This cancer can happen as a result of numerous factors, for example diet, smoking, infections and genetics. Symptoms of diarrhea or constipation, weakness of the stomach, weight loss, bloating, abdominal pain, and bleeding are associated with gastric cancer (Zheng et al. 2019). Studies on gastric cancer and probiotics are chiefly focused on removing *Helicobacter pylori* (*H. pylori*) infection as the main risk factors of gastric cancer (Bhandari and Crowe 2012). According to recently meta-analysis, consuming probiotics as a supplementation with antibiotic is beneficial to the *H. pylori* eradication (Losurdo et al. 2018). One of the suggested mechanisms for probiotic therapy is increasing the immune response and decreasing the effect of inflammation caused by *H. pylori* on the host gastric mucosa (Du et al. 2012).

4.4.1 *In Vitro* Studies

Lactobacillus reuteri activity against gastric cancer progression have been demonstrated by inhibiting cell proliferation and suppression cell invasion via downregulation of urokinase plasminogen activator/urokinase plasminogen activator receptor gene expression (Rasouli et al. 2017). Administration of *Lactobacillus rhamnosus* GG revealed an alternative approach to prevention of gastric cancer via decreasing ODC mRNA and activity as well as polyamine content and cell proliferation (Linsalata et al. 2010).

Cyclooxygenase (COX) profile forecasts gastric cancer prognosis. COX-1 repression is destructive to the intestinal mucosa. In contrast, COX-2 repression is protective against gastric cancer. *In vitro* study on CRL 5822 gastric cancer cell line showed *Lactobacillus acidophilus* can induce COX-1 expression (Mahkonen et al. 2008). Evaluation of the effect of *Lactobacillus kefir* demonstrated that it is safe for white blood cells and selectively induces apoptotic effects in gastric cancer cells by reducing Bcl2 expression and diminishing polarization of MMP (Ghoneum and Felo 2015). Milk fermented by *Propionibacterium freudenreichii* induced apoptosis of HGT-1 human gastric cancer cells and demonstrated that it can be useful as a food supplement to potentiate cancer therapeutic treatments and as part of a protective diet designed to avoid gastric cancer (Cousin et al. 2012).

4.4.2 Human Study

Probiotics (*Bifidobacterium* and *Lactobacillus*) combined with enteral nutrition in postoperative patients with gastric cancer, could decrease the inflammatory response and the incidence of diarrhea and progress the immune function (Xie et al. 2018). Also, another study demonstrated that administration of probiotic combination containing *Bifidobacterium infantis*, *Lactobacillus acidophilus*, *Enterococcus faecalis* and *Bacillus cereus* reduced the severity of inflammation and increased the immune response of patients by modification of gut microbiota (Zheng et al. 2019).

4.5 Leukemia

Leukemia is a cancer of the blood-forming tissues of body, such as the lymphatic system and the bone marrow that usually involves the white blood cells. Many types of leukemia exist such as acute lymphocytic leukemia, chronic lymphocytic leukemia, acute myeloid leukemia, and chronic myeloid leukemia. Some forms of leukemia are more common in children and other forms of occur mostly in adults (Bonyadi et al. 2017). Patients with leukemia demonstrate signs of anemia, fatigue, weakness, weight loss, joint pain and excessive bleeding due to malfunctioning of their platelets, leukocytes and erythrocytes (Maalouf et al. 2011). Erythroleukemia (acute myeloid leukemia, according to categorization of French, American, British:

FAB) is defined as neoplasia of erythroid and myeloid precursors in bone marrow (Jalali et al. 2016). Adult lymphoblastic leukemia is prevalent both in adults and children and is categorized into chronic or acute subtypes dependent on the abnormal T-cells number in peripheral blood (Maalouf et al. 2011).

4.5.1 *In Vitro* Studies

Probiotic *Lactobacillus reuteri* progressed TNF-induced apoptosis by modulation of NF-kB and MAPK signaling in human myeloid leukemia-derived cells (Iyer et al. 2008). Furthermore, in another study the cytoplasmic extract of *Saccharomyces cerevisiae* examined inhibited cell growth and induce necrosis and apoptosis in chronic myeloid leukemia cells (K562) (Bonyadi et al. 2017). Kefir induced apoptosis and repressed cell proliferation in human acute erythroleukemia (Jalali et al. 2016). Also, kefir induced apoptosis and cell-cycle arrest in human T-lymphotropic virus type I -negative malignant T-lymphocytes by decrease proliferation, downregulation TGF-α, upregulation TGF-β1 mRNA expression (Maalouf et al. 2011).

4.5.2 Animal Studies

Antiinflammatory response in human acute monocytic leukemia (THP-1) cells and mouse model at gene expression level with indigenous probiotic *Lactobacillus plantarum* and *Lactobacillus pentosus* was evaluated by Aparna Sudhakaran et al. MCP-1, IL-6, ICAM-1, VCAM-1, TNF-α and E-selectin expressions were significantly downregulated in this study (Aparna Sudhakaran et al. 2013).

4.6 Liver Cancer

Liver cancer is the ninth most common cancer in women and the fifth in men worldwide (Richman et al. 2017). WHO assesses that 788,000 death occure from primary liver cancer every year (WHO 2018). The gut microbiome has been associated with the progress of liver disorders including non-alcoholic fatty liver disease, liver fibrosis and more recently liver cancer (De Minicis et al. 2014).

4.6.1 *In Vitro* Studies

The inhibitory influences of *Lactobacillus rhamnosus* GG-derived extracellular vesicles on the growth of HepG2 cell line was evaluated by Behzadi et al., in 2017. Results demonstrated significant cytotoxic effect and increase of the apoptotic index (Bax/Bcl2 expression ratio) (Behzadi et al. 2017).

4.6.2 Animal Studies

Probiotics (*Prevotella* and *Oscillibacter*) are known producers of antiinflammatory metabolites modulated gut microbiota and represses hepatocellular carcinoma growth in mice by downregulation of IL-17 and suppressing Th17 cells differentiation (Li et al. 2016). Impression of gut homeostasis on chemically-induced protumorigenic inflammation in a diethyl nitrosamine model of rat hepatocarcinogenesis was investigated by Zhang et al. Modulation of the gut microbiota, suppression of intestinal inflammation by probiotics (*Lactobacillus* species, *Bifidobacterium* species, *Enterococcus* species) represented a new path for healing intervention to treat or prevent hepatocellular carcinoma development(Zhang et al. 2012). *Salmonella choleraesuis* demonstrated tumoricidal and antiangiogenic activities in mice through significantly protracting the animal survival, decrease the tumor size, upregulating IFN-γ and induction IFN-inducible chemokines CXCL10 (IP-10) productions, enhancement infiltration of CD41, CD81 T cells and neutrophils and induction cell death (Lee et al. 2008).

4.6.3 Human Study

Decrease the risk of liver cancer by the use of a probiotic was evaluated in a randomized double-blind placebo-controlled trial. In the probiotic group, patients received one capsule preparation of probiotic including mixture of *Lactobacillus rhamnosus* LC705, *Propionibacterium freudenreichii* subsp. *Shermanii.* two times daily, and another group received placebo. Results revealed that a probiotic supplement decreased the biologically impressive dose of aflatoxin exposure and suggested an impressive dietary approach to reduction the liver cancer risk (El-Nezami et al. 2006).

4.7 Lung Cancer

According to several studies on respiratory diseases, alcohol intake, smoking, toxic heavy metal consumption, exposure to radon gas (in mines or homes), genetic factors and exposure to, silica dust, asbestos are causes of the incidence of lung cancer (Sharma et al. 2018). Based on morphological cancer cells properties, there are two main types of lung cancer including **small cell lung cancer**, which grows faster and metastasize to other organs of the body, and **non-small cell lung cancer**, which progresses and spreads slowly and is further categorized into three types including large cell lung cancer, squamous cell carcinoma and adenocarcinoma (Sharma et al. 2018).

4.7.1 *In Vitro* Studies

Anticancer effect of probiotic *Lactococcus lactis* KC24 isolated from kimchi was investigated against lung carcinoma (SK-MES-1), colon carcinoma (HT-29 and LoVo), breast carcinoma (MCF-7) and gastric carcinoma (AGS). The results indicated that all the cancer cells resulted in antiinflammatory effects through NO production and strong inhibition of proliferation (Lee et al. 2015). In another study, antiinflammatory and anticancer activity of probiotic *Lactococcus lactis* NK34 was investigated on MCF-7, MRC-5 (human lung cell line), SK-MES-1 cells. These results suggested that probiotic showed cytotoxic activity, decreased NO, proinflammatory cytokines (TNF-α, IL-18, COX-2) and inhibited cell proliferation (Han et al. 2015).

4.7.2 Animal Studies

Antitumor effect of *Bifidobacterium infantis*-mediated sFlt-1 gene transferring system was investigated on Lewis lung cancer in mice (Zhu et al. 2011). This system prolonged survival time of LLC C57BL/6 mice safely and inhibited the tumor growth (Zhu et al. 2011). In another study, the *Bifidobacterium infantis* mediated sKDR prokaryotic expression system increased significantly the efficacy of tumor growth repression and prolongation of survival, the necrosis rate of tumor, and could obviously reduce microvessel density and the signals of blood flow in tumors (Li et al. 2012). Lewis lung cancer was investigated in mice models by Gui et al. (2015). They were divided into the following groups: cisplatin group, cisplatin/ABX (an antibiotic cocktail) group, and cisplatin/*Lactobacillus acidophilus* group. Results demonstrated positive effects on survival rates and reduction tumor size. The group cisplatin/*L. acidophilus* demonstrated longer survival rates in mice as compared to cisplatin and cisplatin/ABX groups. Furthermore, this study revealed an enhancement anti-tumor response in *Lactobacillus*-co-treated mice with upregulation IFN-γ, Gzmb, and Prf1 mRNA expression (Gui et al. 2015). Daillere et al., in 2016, investigated the role of two bacterial species, *Barnesiella intestinihominis* and *Enterococcus hirae,* in the progress of the efficacy of the most prevalent alkylating immunomodulatory compound cyclophosphamide. It was revealed, the two strains were represented as valuable "oncomicrobiotics" and specific-memory Th1 cell immune responses selectively predicted longer progression free survival in advanced ovarian and lung cancer patients treated with chemo-immunotherapy (Daillere et al. 2016).

4.7.3 Human Study

One clinical trial was carried out with 41 lung cancer patients to check stool flora, 16S ribosomal RNA sequencing (Tian et al. 2019). Patients were divided into the *Clostridium butyricum* and placebo group. The lymphocyte/monocyte ratio was

higher in the probiotic group compared with the placebo group. This study demonstrated that *Clostridium butyricum* reduced chemotherapy-induced diarrhea in lung cancer patients, encouraged homeostatic maintenance and decreased the systemic inflammatory response system (Tian et al. 2019).

4.8 Oral Cancer

The oral tumor is the second leading cause of cancer-related mortality in the world and the sixth most common type of cancer. Squamous cell carcinoma is a common oral cavity carcinoma (Asoudeh-Fard et al. 2017). Nowadays it is clear that oral cavity cancer is a multifactorial disease as well as other cancers, that progresses via several factors including habits, behaviors and lifestyle. Recently, dietary factors, including infectious agents such as human papillomavirus and iron deficiency have been revealed as effective factors in the progress of oral diseases (Meurman and Uittamo 2008). Although the oral flora carcinogenesis has not been confirmed, but cytokines and mediators secreted by bacteria can modify cellular metabolic pathways and cause to malignant transformation (Aghazadeh et al. 2017).

4.8.1 *In Vitro* Studies

Lactobacillus plantarum is one of the most chief bacteria that live in the oral system of human. This probiotic via downregulating of MAPK signaling pathways and upregulation of phosphatase and tensin homolog (PTEN) induced apoptosis in oral cancer KB cells (Asoudeh-Fard et al. 2017). Moreover, *Acetobacter syzygii* probiotic demonstrated prophylactic effect by induction of apoptosis in KB cancer cells (Aghazadeh et al. 2017).

4.9 Pancreatic Cancer

Pancreatic cancer is the 12th most common cancer worldwide and seventh most frequent cause of death worldwide, but the etiology is still unknown (Javanmard et al. 2018). Some studies demonstrated a multifaceted role of probiotics in prohibiting pancreatic cancer via modulating inflammation, pancreatitis, pancreatic necrosis, diabetes and obesity (Besselink et al. 2008).

4.9.1 Animal Studies

An established probiotic cocktail of *Lactobacillus delbruckei* sp. *bulgaricus* DWT1 and *Streptococcus thermophilus* DWT4 inhibited tumor growth. The results of this study demonstrated that the transition of M2 to M1 like state of macrophage cells of mouse origin *in vivo*, *ex vivo* and *in vitro* following treatment with the established probiotic cocktail, for the first time. Also, activation of pro-inflammatory responses in macrophages, decrease cells cytotoxicity, upregulating IL1 β, IL6, IL12, TNF-α was reported (Guha et al. 2019).

4.10 Melanoma

Melanoma, known as melanocytes, is a serious form of skin cancer that starts in cells. Although it is less common than squamous cell carcinoma and basal cell carcinoma, but melanoma is more dangerous because its ability to spread rapidly to other organs.

4.10.1 Animal Studies

In melanoma mouse model, *Bifidobacterium* cocktail (*B. bifidum*, *B. longum*, *B. lactis*, *B. breve*) promoted antitumor immunity like CD8+ T cell activation, cytokine-cytokine receptor interaction, augmented dendritic cell function and the chemokine-mediated recruitment of immune cells to the tumor microenvironment (Sivan et al. 2015).Another research demonstrated that the efficacy of CTLA-4 blockade depends on distinct *Bacteroides* (*B. fragilis* or *B. thetaiotaomicron*) species and described the main role of bacteroides in the immunostimulatory impacts of CTLA-4 blockade. An augmented immune response was observed when *Bacteroides fragilis* was orally administered to germ Free (antibiotic treated) mice and tumors did not react to CTLA-4 blockade (Vetizou et al. 2015).

5 Safety of Probiotics in Cancer Treatment

The long history of usage of probiotic bacteria, particularly *bifidobacteria* and *lactobacilli* in foods (for example fermented dairy products) and their presence in the human gastrointestinal tract, supplies rational evidence about the safety of dietary LAB (Snydman 2008). However, according to the mechanisms of probiotics activities as mentioned above, there are possible theoretical risks of probiotics usage. These risks include infection (bacteremia and endocarditis), metabolic actions, transmigration of pathogenic bacteria, adverse extreme immune stimulation and transfer of antibiotic-resistance from probiotics to pathogenic bacteria (like

vancomycin resistance in several *lactobacilli* strains, tetracycline resistance in *Lactobacillus plantarum*) (Boyle et al. 2006). LAB has been related to cases of infection, like bacteremia and infective endocarditis so, it is one of the main safety worries about LAB (Chong 2014). Nevertheless, two studies revealed that there was no significant enhancement in lactobacilli or probiotics associated with infection, though there had been a significant enhancement in *Lactobacillus* GG use over that decade between 1990 and 2000 (Salminen et al. 2002). The LAB, *lactobacilli* and *bifidobacteria* that are the most frequently consumed probiotics have been considered the 'generally recognized as safe' (GRAS) status (FDA 2019). In associated with the safety of probiotics consumption in patients receiving nutritional supplement, a systematic review of randomized controlled trials, nonrandomized trials and case reports, was conducted by Whelan and Myers in 2010, which involved 53 trials and 4131 patients received probiotics. This widespread review revealed that probiotics had either no or positive influences in associated with safety results (like infection, mortality) (Whelan and Myers 2010). Furthermore, other reviews with available clinical information reported the safety of LAB probiotics consumption (Chong 2014). Also, based on the systematic review and meta-analysis conducted by Qiu et al. (2019) there is no statistically significant related probiotics to any adverse effects in cervical cancer patients receiving radiation (Qiu et al. 2019).

However, it is notable that all probiotics don't have GRAS status, for example several members of bacilli, streptococci and enterococci probiotic, are not commonly considered as safe due to they include opportunistic pathogens, particularly enterococci (Snydman 2008). Because of the risk of infection including sepsis, pneumoniae and endocarditis, it is necessary that patients with immune deficiency consume probiotics with precaution (Floch 2013). Moreover, microorganisms with identified virulence genes should not be used for probiotics usage (Vankerckhoven et al. 2008).

6 Conclusion

Due to their beneficial properties on the host health, probiotics have become very important in medicine. Most of the currently available probiotic products comprise lactic acid bacteria (LAB) that belong to the *Lactobacillus* and *Bifidobacterium.* There are different sources of probiotics like dairy products, fermented milk, kefir, kimchi, kombucha, kiom-ma, utonga kupsu, noni, soy milk and yogurt. They seem to have capacities in prevention, treatment, and management of several cancers such as colon, gastric, liver, pancreatic, cervical, oral, lung, leukemia, melanoma and breast cancer. Numerous *in vitro* and animal studies demonstrate positive effects of probiotics on several cancers by numerous mechanisms, such as, production of short chain fatty acids, alteration of colonic motility and transit time, alteration of differentiation process in tumor cells, anticarcinogenic effects, antimutagenic properties, modulation of inflammatory response, inhibition of the bacteria that convert pro-carcinogens to carcinogens, alteration of tumor gene expressions, decrease of

intestinal pH to diminish microbial activity, antioxidant activity, modulation of gut microbiota, antiproliferative and apoptotic effects, antiangiogenesis, enhancement of barrier function, their interposition in the enterohepatic cycle of estrogen (in breast cancer).

Several human studies demonstrated that probiotics supplement could prevent of post-operative complication and chemotherapy and radiation therapy related toxicity such as diarrhea, particularly the incidence of Common Toxicity Criteria Grade ≥ 2 and Grade ≥ 3 diarrhea induced by radiotherapy. Moreover, probiotics reduced the use of anti-diarrheal medication such as loperamide for radiotherapy-induced diarrhea patients (Qiu et al. 2019).

Human intestinal microbiota that can be modulated by probiotic usage can metabolize chemotherapeutics drugs, therefore, may change their bioavailability under or over treatment (Ranjbar et al. 2019). Thus, to propose a regimen containing probiotics, all aspects of probiotic safety and efficacy must be considered. However, human clinical trials of the probiotic's usage as bio therapeutics against cancer with enough follow-up outcomes are still lacking. Therefore, more clinical trial studies are essential to recognize the potential strains, dosages and administration regimes with highest efficacy and safety as an adjuvant therapy for cancer treatment for particular types and stages of cancer.

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Part III
The Role of Nutrients in the Prevention of Cancer

Chapter 19
Vitamins (C, D and E) Against Cancer

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Abstract Cancer is the leading cause of death worldwide. This disease is described as accelerated and uncontrolled cell multiplication. In recent decades, chemoprevention has been advocated to reduce the risk of cancer or prevent its recurrence. An example of chemopreventives is antioxidant vitamins, which inhibit angiogenesis and cancer cell metastasis. In addition, vitamin C, vitamin D and vitamin E decrease the side effects caused by chemotherapy during cancer treatment. This review provides a complete update on the therapeutic potential of vitamins C, D and E against cancer. Unfortunately, most studies suggest the need to conducting rigorous clinical trials to confirm the benefits of these vitamins. In addition, chemopreventive regimens should be adjusted for a greater range of cancer types.

Keywords Cancer · Signaling · Vitamins · Chemoprevention

1 Introduction

Cancer refers to several diseases that are identified by abnormal cell proliferation. Cancer cells divide uncontrollably and can invade organs and tissues as well as spread through blood and lymphatic tissue (Alonso Castellanos et al. 2014).

With technological advances in medicine and the identification of novel drugs, cancer patients have new therapies to choose from. Therefore, currently, many patients do not depend only on chemotherapy and radiotherapy and are now choosing alternative treatments or complementary therapies.

Chemoprevention has been advocated to reduce the morbidity and mortality from diseases such as cancer. Specifically, this chemoprophylaxis involves the use of drugs or bioactive compounds to prevent or suppress carcinogenesis in patients at high risk of cancer (Adaniel et al. 2017). The targets of primary chemoprevention are healthy individuals predisposed to cancer.

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Vitamins and their derivatives are molecules provided by the diet that are required to maintain optimal body function and body growth. Some act as antioxidants, gene expression regulators or coenzymes. Therefore, vitamins are essential nutrients that have been associated with the etiology of cancer and the protective effect of chemoprevention (Jain et al. 2017).

Cancer patients are interested in novel treatments that are simultaneously nontoxic, economical and with effective clinical benefits (Drisko et al. 2018). In neuropathic and integrative oncology, vitamin C is applied in high doses (Raymond et al. 2016). In addition, vitamin C therapies are safe, without the side effects caused by chemotherapy and radiotherapy. Oncological therapies with intravenous vitamin C have shown selectivity for abnormal cells, but unfortunately, these results are strongly refuted by the scientific community. To date, this therapy is supported by laboratory and animal tests; therefore, more rigorous clinical trials are required.

Vitamin D can be obtained from food or supplements, or it can be synthesized by exposing the skin to sunlight (Mondul et al. 2017). In addition to the effects of vitamin D on phosphorus and calcium metabolism, other effects have been reported, such as preventing the formation of tumours that may become cancerous (Dou et al. 2016). In addition, in its active form, vitamin D inhibits the proliferation and replication of cancer cells, and reports indicate that it has therapeutic activity in prostate cancer (Jain et al. 2017).

The best known form of tocopherols is α-tocopherol, but clinical trials have not reported any role of α-tocopherol in cancer prevention. Antioxidant and antiinflammatory activities have been reported for γ-tocopherol, δ-tocopherol, γ-tocotrienol and δ-tocotrienol, which participate in cancer prevention (Jiang 2019). However, more studies are needed to strengthen this evidence.

It has been reported that the etiology of cancer is multifactorial; therefore, it is necessary to study the preventive impact or increased risk of vitamins during nutritional therapies (de Baptista and Melo 2014). There is evidence that antioxidant vitamins participate in carcinogenesis (Guo et al. 2015). Guo et al. found an inverse association between the dietary intake of vitamins E and C and the risk of cervical cancer. Therefore, to prevent cancer, fruits and vegetables consumption should be increased. Additionally, FAO /WHO experts recommend the intake of 400 g of fruits and vegetables per day or about 150 kg per person per year, all this to prevent heart disease, diabetes, obesity and cancer, particularly cancer of the gastrointestinal tract (The World Health Organization [WHO] 2003). Additionally, when evaluating the efficacy of vitamins and other micronutrients as chemoprotectors, it is important to consider the effect of food processing on the stability of these bioactive components (Mokbel and Mokbel 2019).

Older people around the world are the major consumers of dietary supplements for health promotion. However, supplement intake should be limited to individuals with a proven deficiency, or supplement availability should be limited to those with confirmed protective action.

In this review, we document the findings of the last decade that relate vitamin C, vitamin D and vitamin E to cancer prevention and treatment and those complemented by clinical trials that confirm or contradict the possible chemopreventive actions of these vitamins.

2 Structural and Biological Characteristics of Vitamins

Vitamins are essential organic nutrients, in small amounts, for humans to maintain health. Vitamins are essential because body tissues cannot produce them in sufficient quantities to satisfy the requirements of an individual under normal conditions (de Baptista and Melo 2014). Therefore, vitamins must be supplied through the diet, although some are synthesized endogenously in very small amounts. Vitamins K2, B1 and B2 are synthesized by intestinal bacteria, niacin (vitamin B3) is synthesized from tryptophan, and vitamin D is formed in the skin by sun exposure.

Vitamins are classified based on solubility as water soluble and fat soluble. Solubility determines their mode of action, body storage and toxicity. Vitamin C and B vitamins are not stored in the body, except B12; they are distributed in intra- and extracellular fluids and are excreted through the urine. Fat-soluble vitamins are stored in the body and are not quickly absorbed or eliminated. Vitamins A and D are stored in body fat and are toxic (Albahrani and Greaves 2016). Water-soluble vitamins are harmless, but their prolonged administration can alter cellular metabolism.

There are two chemical forms of vitamin C. Ascorbic acid is the reduced form, and dehydroascorbic acid is the oxidized form. In the human body, the reduced form is the most abundant and is recognized as an essential nutrient (Mamede et al. 2011). During vitamin C deficiency, prostaglandin metabolism, cellular immunity, and catecholamine formation are altered (Klimant et al. 2018). Vitamin C requirements in men and women depend on body size and lean body mass. Vitamin C requirements in healthy adults are higher for men (90 mg/day) than women (75 mg/day), and the requirements are the same for young and old adults (Institute of Medicine [IOM] 2006).

The dietary forms of vitamin D are ergocalciferol (D2) and cholecalciferol (D3). The biologically active hormonal form of vitamin D is 1,25-dihydroxyvitamin (D3) (Albahrani and Greaves 2016). Vitamin D deficiency causes rickets in children and osteoporosis in adults. This vitamin is synthesized by exposure to ultraviolet rays from sunlight. However, with age, the ability to synthesize vitamin D in the skin decreases.

There are two natural families of vitamin E: four tocopherols (α, β, γ and δ) and four tocotrienols (α, β, γ and δ) (Debier and Larondelle 2005). The four isomers differ in the position and number of methyl groups attached to the chromanol ring

(Albahrani and Greaves 2016). α-Tocopherol is the most abundant form in human tissues and in nature (Mamede et al. 2011). Fat malabsorption and protein-energy malnutrition are causes of vitamin E deficiency. α-Tocopherol protects polyunsaturated fatty acids in membranes from oxidation (Koudelka et al. 2015).

In epidemiological studies, vitamins C, D and E have been linked to diseases such as cancer. Specifically, vitamins C and E have protective effects in cardiovascular diseases and cancer due to their functions as antioxidants. Vitamin D deficiency has been associated with an increased risk of colon, breast and prostate cancer. To back these findings, more studies involving these three vitamins are needed to support their relationship with different types of cancer.

Table 19.1 shows the biological functions attributed to vitamins C, D and E and the foods through which they are supplied (Institute of Medicine [IOM] 2006). However, attention must be paid to food processing and culinary preparation methods that reduce vitamin losses. Vitamin C is affected by oxygen, pH, light, enzymes and metal catalysts. Vitamin D is sensitive to oxygen and storage conditions, but its stability is not important because the requirements of adults are ensured. All technological processes involving the separation of the lipid fraction affect the stability of vitamin E (Rodríguez et al. 1998). The consumption of nutritional supplements has become a lifestyle choice for some individuals to compensate for vitamin losses that occur during the processing of fresh foods. However, increased consumption of supplements should be aimed at the prevention of deficiencies or diseases.

Recently, Hamishehkar et al. (2016) documented the harmful or noxious properties of vitamins. To do so, they reviewed controlled clinical trials with water-soluble and fat-soluble vitamins. They found that vitamins A, E, D, and C and folic acid supplements in large amounts are harmful to health. Additionally, they explain that the reduced and oxidized forms of antioxidant vitamins in foods are stable, unlike those in commercial supplements.

Table 19.1 Biological functions and food sources of vitamins C, D and E

Vitamin	Biological functions	Food sources
C	Antioxidant because of reducing power	Citrus fruits, tomato, potato, broccoli, strawberry and spinach
	Required for the biosynthesis of collagen, carnitine, neurotransmitters and connective tissue components	
	Free radical scavenger	
D	Participates in bone health, and facilitates the intestinal absorption of calcium and phosphorus	Fatty fish, fish liver oils, chicken eggs, fortified milk, margarine, breakfast cereals and fruit juices
	Acts as an antiproliferation and prodifferentiation hormone	
E	Antioxidant that prevents the spread of free radicals, scavenges peroxide radicals, and protects polyunsaturated fatty acids in membranes and plasma lipoproteins	Vegetable oils, unprocessed cereals, nuts, fruits, vegetables and fatty meats
	Inhibits cellular protein kinase C activity in smooth muscle, platelets and monocytes	

3 Vitamins C, D and E in Cancer Care

Plasma vitamin C concentrations are higher in healthy adults than in cancer patients. Therefore, vitamin C deficiency increases the risk of cancer mortality (Ngo et al. 2019).

Despite controversies regarding vitamin C therapies, in recent decades, vitamin C continues to be applied orally or intravenously due to its potential anticancer effects. Jacobs et al. (2015) conducted a systematic review to identify the toxicity and antitumor effects of vitamin C. After reviewing approximately 60 studies, the authors found antitumor effects only in case reports and observational studies. Due to the applicability of this therapy, it is necessary to conduct randomized controlled trials.

Based on biological evidence, extracellular vitamin C in large amounts can increase the efficacy of chemotherapy and decrease its toxicity. Hoffer et al. (2015) reported the application of intravenous vitamin C as a therapy for advanced cancer without finding side effects. However, treatment with vitamin C alone does not act as an anticancer therapy when the disease is incurable and several previous treatments have been attempted.

In patients with cancer, vitamin C deficiency has been reported due to poor oral intake, infection, inflammation and treatments such as surgery, radiation and chemotherapy. Based on an exploratory systematic review, Klimant et al. (2018) reported that intravenous vitamin C decreases inflammation and the effects caused by treatments that reduce quality of life. Additionally, they propose the combined administration of intravenous and oral vitamin C, which is safe and inexpensive, to cancer patients as supportive therapy before and after chemotherapy.

Epidemiological studies suggest an inverse association between the concentration of 1,25-dihydroxyvitamin, 25D, in the blood and the incidence and mortality of colorectal cancer. In addition, randomized trials involving high doses of vitamin D allow assessing vitamin D supplementation (Dou et al. 2016). In patients diagnosed with cancer and with low vitamin D levels, supplementation is a therapy with long-term benefits, although the doses should be individualized (Calmarza et al. 2018).

Postmenopausal women present vitamin D deficiency and have a higher risk of breast cancer. Gonzalez-Fisher et al. (2016) reported that, during aging, the production of cholecalciferol in the skin decreases and that estrogen deficiency reduces the metabolic activation of vitamin D, providing evidence of an association between breast cancer and vitamin D.

Mokbel and Mokbel (2019) reviewed various epidemiological studies to relate breast cancer to serum vitamin levels. Among the vitamins with protective action is D3, as well as folates, vitamin B6 and beta-carotene. These vitamins inhibit various molecular events and signaling pathways related to breast carcinogenesis. Women at risk for breast cancer should consume supplements or foods with these nutrients to prevent disease onset. According to Calmarza et al. (2018), the adequate dose of vitamin D is one that, together with adequate intake and correct sun exposure, allows reaching levels of 25 (OH) vitamin D above 75 nmol /L.

Because α-tocopherol eliminates reactive oxygen species (ROS), tocopherol deficiencies have been linked to cancer and cardiovascular diseases, but additional clinical trials are required to obtain consistent data (Schubert et al. 2018).

α-Tocopheryl succinate is the most studied α-tocopherol analog. This analog has strong *in vitro* cytotoxic activity and *in vivo* anticancer activity in animal trials. Koudelka et al. (2015) documented evidence of anticancer analogs of vitamin E, such as α-tocopheryl succinate, being used as delivery systems for anticancer drugs in liposomal formulations. In addition, they found that proapoptotic vitamin E analogs have little toxicity to healthy cells and that their activity targets cancer cells. To enhance the efficacy and bioavailability of vitamin E analogs, the development of new formulations in the form of liposomes, nanoparticles or phospholipids complexes is required (Kanchi et al. 2017).

Guo et al. (2015) found, in a case-control study, that the dietary intake of antioxidant vitamins such as C and E can prevent uterine cancer in Chinese women. The beneficial effect was similar for vitamins C and E. Specifically, in this study it was found that the cases had lower intakes of vitamin C (80 mg/d) than the controls (112 mg/day). It was also reported that serum concentrations of vitamin E were lower in cases (0.81 μg/day) than in controls (1.01 μg/day).

3.1 Mode of Action that Relates Vitamins to Cancer

In recent decades, there has been an increase in clinical trials documenting the benefits of vitamin C, D and E supplementation for cancer patients. However, work to explain why some cancer cell types are more sensitive or resistant to certain chemopreventive agents continues.

Raymond et al. (2016) indicate that vitamin C therapy can be used to modulate the inflammation presented by cancer patients because vitamin C suppresses the expression of cyclooxygenase-2 (COX-2). In addition, in the extracellular fluid, vitamin C in high concentrations is converted into ascorbate radicals, which reduce ferric ions to ferrous ions. Then, ferrous ions react with oxygen molecules, producing superoxide anions, which react with hydrogen to form hydrogen peroxide. This series of reactions causes the accumulation of hydrogen peroxide in tumor cells and promotes apoptosis.

Cancer therapies result in the production of ROS, which attack cells and healthy tissues and produce side effects. According to Lee et al. (2019), tumor cells store more vitamin C than do normal cells to protect themselves from ROS. Therefore, in clinical practice, it is common to use vitamin C to protect normal cells during chemotherapy. The same authors noted that high doses of vitamin C affect the proliferation of breast cancer cells; this occurs because vitamin C causes tumor cells to store large amounts of iron, and with this, hydrogen peroxide is generated, acting as a pro-oxidant and destroying cancer cells.

Vitamin E, vitamin C, α-carotene and β-carotene prevent cervical carcinogenesis through various pathways (Guo et al. 2015). These antioxidant vitamins eliminate free radicals and oxidants to prevent DNA damage. With the neutralization of free radicals and oxidants, inflammatory processes are prevented from damaging DNA and proteins. In addition, these vitamins can protect the immune system from oxidative damage and inhibit insulin-like growth factors (IGFs).

Ngo et al. (2019) reported that vitamin C affects the essential systems that facilitate the survival and growth of tumor cells. Specifically, vitamin C affects redox imbalances, epigenetic reprogramming, and oxygen-sensing regulation in cancer cells.

In some studies with vitamin C applied intravenously, the concentration in blood has reached 20 millimolar. When the vitamin is ingested orally, the maximum concentration reached is 300 micromolar. In some studies, high vitamin C concentrations kill cells (Unlu et al. 2016). For this reason, intravenous vitamin C therapy is preferred by professionals of complementary or alternative medicine.

In breast cancer treatment, vitamin D3 suppresses cancer cell growth and favors natural cell death. Therefore, it has been reported that vitamin D3 has two effects: antiproliferative and proapoptotic (Mokbel and Mokbel 2019).

Vitamin D deficiency induces the development and progression of cancer and is prevalent in cancer patients. It has been shown that inflammatory states favor the onset of cancer and that vitamin D exerts its antiinflammatory activity by inhibiting prostaglandins. Calmarza et al. (2018) indicated that prostaglandins favor oncogenesis by promoting cell growth, inhibiting apoptosis and inducing the expression of oncogenes. Vitamin D3 decreases the production of prostaglandins because it regulates the genes that direct their production by decreasing the expression of cyclooxygenase-2 (COX-2) and by increasing the expression of prostaglandin-catalyzing enzyme (15-PDGH), thus reducing the amount of prostaglandins in the tumor environment.

Clinical studies have not supported the association of α-tocopherol with cancer prevention (Jiang 2019). However, in preclinical studies, it has been found that γ-tocopherol, δ-tocopherol, γ-tocotrienol and δ-tocotrienol destroy different types of cancer cells by reducing the membrane potential of the outer membrane of mitochondria. The anticancer activities of γ-tocopherol, δ-tocopherol, γ-tocotrienol and δ-tocotrienol are attributed to blocking key signaling molecules or mediators involved in cell death and tumor progression, such as phospholipids, eicosanoids, NF-kB (nuclear factor kappa B) and STAT3 (Signal transducer and activator of transcription 3).

3.2 Clinical Evidence Associating Vitamins C, D and E with Cancer

In recent years, clinical trials of various types have associated vitamins C, D and E with cancer, and in some of those studies, the results are contradictory. Therefore, researchers recommend increasing the number of trials, improving the methodological variables and expanding the range of cancer types to confirm the therapeutic action of these vitamins. Table 19.2 provides a list of some human clinical trials that associate cancer with vitamins C, D and E.

The application of intravenous vitamin C is used as an alternative and complementary treatment for cancer, but it is necessary to investigate in more detail the efficacy of vitamin C in the treatment of specific types of cancer and in chemotherapy regimens. Hoffer et al. (2015) designed a clinical study to investigate the tolerability, pharmacokinetics and efficacy of intravenous vitamin C in cancer patients during chemotherapy. Intravenous vitamin C was safe, and vitamin C uptake increased after cancer treatment. When monitoring its effects on quality of life, some patients showed an increase in energy and functional improvement.

For vitamin C to be considered an alternative therapy in Singapore, controlled clinical trials are required. However, most cancer patients receive alternative treatments, such as macrobiotic diets, raw food diets, acupuncture, and medicinal herbs. Raymond et al. (2016) reported a case series documenting the effectiveness of intravenous vitamin C in cancer patients in Singapore. In the trial, patients received vitamin C treatment and supplements that included laetrile, pancreatic enzymes, probiotics, antioxidants, and antiinflammatory drugs. In addition, the participants followed the Gerson diet, i.e., low consumption of sugar, carbohydrates and meat and high consumption of fruit and vegetable juices. According to the authors, intravenous vitamin C therapy improves the quality of life of patients, does not modify the response to conventional chemotherapy and appears to have antitumor activity.

Drisko et al. (2018) studied the application of intravenous ascorbic acid as the only oncological treatment in a patient with pancreatic ductal adenocarcinoma. As a result, they reported the control of tumor progression, and the patient felt good and active; therefore, ascorbic acid can be a complement to conventional chemotherapy regimens.

Gonzalez-Fisher et al. (2016) confirmed the association of breast cancer with vitamin D deficiency in women living in regions with large amounts of UVB radiation throughout the year. These authors found that the association was independent of body mass index (BMI), skin type, sun exposure, menopausal status and vitamin D consumption.

According to Calmarza et al. (2018), the incidence of prostate, colon and breast cancer in newly diagnosed patients is related to vitamin D deficiency caused by lower sun exposure and higher latitude. Therefore, for oncological patients to reach an optimal vitamin D level, they must improve their dietary intake, expose themselves to sunlight and start supplementation immediately.

Table 19.2 Clinical studies with humans that associate cancer with vitamins C, D and E

Objective	Study/Participants	Results	References
Document the safety and kinetics of vitamin C in combination with chemotherapy	14 patients (47–76 years old)	Six patients showed symptom improvement	Hoffer et al. (2015)
	Dose of 1.5 g/kg body weight two or three times per week	Six patients with colorectal cancer had disappointing experiences	
	Cancer types: Lung, colon, rectal, bladder, ovarian, cervical, bile duct, breast, and tonsillar	The application of intravenous vitamin C together with chemotherapy is not toxic, but unfavorable incidents can occur	
Study the effect of intravenous vitamin C therapy in cancer patients in Singapore	Nine patients with cancer	Greater survival than prognosis, better quality of life, and increased tolerance to conventional therapy were observed	Raymond et al. (2016)
	Dose of 25–100 g/day intravenous vitamin C		
	Oral nutritional supplements and modified diet		
Report the use of intravenous ascorbic acid as the only cancer treatment	68-year-old male patient with metastatic pancreatic ductal adenocarcinoma	The expected survival of the patient was 6 months, and he survived 4 years	Drisko et al. (2018)
	Doses of 75 and 125 g, two to three times per week	The patient felt good, his body weight recovered, and liver lesions decreased	
		Primary tumor remained but smaller in size	
Report the intravenous application of vitamin C during chemotherapy and radiotherapy	55-year-old patient with a high-grade malignant brain tumor with a poor prognosis	The patient lived more than 4 years with a good quality of life	Baillie et al. (2018)
	Dose of 85 g/infusion, three times per week for 1 year	Vitamin C acts as supportive therapy for cancer patients	
	Dose of 85 g/infusion, twice per week for 3 years		

(continued)

Table 19.2 (continued)

Objective	Study/Participants	Results	References
Associate the concentration of 25(OH) vitamin D with different types of cancer	Patients with a recent diagnosis of cancer	79.71% had vitamin D insufficiency or deficiency	Calmarza et al. (2018)
	25(OH) vitamin D was quantified, and a basic biochemical analysis was performed	Vitamin D concentration is not associated with sociodemographic factors	
	71 patients with urological cancer, 27 with colorectal cancer, 35 with head and neck cancer, and six with other types of cancer	Vitamin D concentration is related to the season of the year	
		In colorectal and head and neck cancer, vitamin D deficiency is present	
		In urological cancer, vitamin D insufficiency is present	
Relate appropriate vitamin D concentrations to breast cancer prevention	Case-control study with 76 women	The risk of breast cancer in women is 1.48-fold higher when sun exposure is <30 min	Gonzalez-Fisher et al. (2016)
	24 patients (22–79 years old) with breast cancer	4.09-fold higher when vitamin D intake is deficient	
	52 women (24–67 years old) in the control group	4.42-fold higher when an individual is obese or overweight	
		Ninefold higher when vitamin D level is less than 20 ng/mL	
Investigate the frequency of vitamin D deficiency in Egyptian women with breast cancer	Prospective study with 50 women (25–80 years old) with primary invasive, nonmetastatic cancer for 18–48 months	30% of the patients had vitamin D deficiency, and this deficiency was associated with larger tumors	Ismail et al. (2018)
Determine the relationship between the incidence of cancer and high-dose vitamin D supplementation without calcium	Randomized and double-blind trial with 5108 adults (50–84 years old)	In the vitamin D group, the incidence of cancer was 6.5%, and in the placebo group, the incidence was 6.4%	Scragg et al. (2018)
	Initial dose of 200,000 IU of vitamin D3, then monthly doses of 100,000 IU for 2.5–4.2 years	Supplementation with high-dose vitamin D without calcium is not associated with cancer prevention	

(continued)

Table 19.2 (continued)

Objective	Study/Participants	Results	References
Determine the association between antioxidant vitamin concentration and risk of uterine cancer	Case-control trial in a hospital 458 women with cervical cancer and 742 women as controls Food frequency questionnaire was used to estimate the daily intake of antioxidant vitamins	High plasma concentrations of vitamin E, vitamin C, β-carotene and α-carotene are associated with a lower risk of cervical cancer	Guo et al. (2015)

In some epidemiological studies, it has been reported that vitamin D deficiency is related to the onset and progression of breast cancer, while in others, no association has been found. Ismail et al. (2018) designed a trial with Egyptian women of different menopausal statuses to identify the possible association between vitamin D deficiency and breast cancer. They found that vitamin D deficiency has negative effects on disease-free survival. This deficiency was positively related to factors such as tumor size, lymph node status and HER2/neu receptor expression.

It has been noted in epidemiological studies that vitamin D supplementation is not associated with cancer prevention. Scragg et al. (2018) designed a randomized clinical trial to investigate the link between high-dose vitamin D supplementation and cancer incidence in adults in New Zealand. The authors concluded that vitamin D should not be used to prevent cancer.

4 Conclusions

Based on the information examined, vitamins C, D and E have vital functions that contribute to the maintenance of human health. Over the last decade, epidemiological studies have confirmed their therapeutic potential for the prevention and treatment of cancer; however, some results are not fully optimistic. Further clinical methodological studies are required to understand the mechanisms of action of vitamins C, D and E and to provide data on the supplementation regimens that lead to the success of chemopreventive treatments.

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Chapter 20
Minerals (Namely Selenium) and Cancer

Antoni Sureda, Xavier Capó, and Silvia Tejada

Abstract Selenium is an essential trace element in the human body associated with its biological roles, principally in the maintenance of the oxidation-reduction status as a component of selenoproteins. The pharmacological effect and toxicity of selenium is mostly dependent on the concentration, redox state and the type of selenium compound used. Several studies have reported the safety and non-toxicity levels for human administration. Moreover, diverse studies have revealed the existence of an inverse association between selenium exposure and cancer risk, mainly in murine models and *in vitro* assays. Some clinical trials point out the improvement of different types of cancer, such as cervical intraepithelial neoplasias in women or prostate in men or colorectal cancer. There are different observational, case-control and clinical trials evaluating the potential to prevent the development of several kinds of cancer taking into account the selenium intake, indicating a protection in front of them. However, some other randomized controlled trials indicated that selenium supplementation does not seem to decrease the risk of cancer, but could favour some of them such as prostate or skin cancer. This chapter focuses on reviewing the available information regarding Se and its preventive or therapeutic capacity against cancer.

Keywords Selenium · Prostate cancer · Colorectal cancer · Cancer risk

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1 Introduction

Cancer is a main concern worldwide being the second leading cause of death. In this sense, in 2018 around 18.1 million of new cases of cancer were diagnosed and 9.6 million of deaths were consequence of this disease (Ferlay et al. 2019). Among different factors, cancer can be developed by unhealthy factors, such as tobacco, alcohol, physical inactivity and an unhealthy diet. Cancer implies several physiological and biochemical alterations, including oxidative stress, inflammation, or specific metabolic pathway (Reuter et al. 2010). The risk factors and cancer prevalence are higher in developed countries since the population have changed their lifestyle, habits, and the life expectancy has increased. The main treatments used to fight cancer include surgery, chemotherapy, radiotherapy or specific drugs; however, they imply many side effects that diminish the life quality of the patients. Although cancer can be cured mainly in the first states, the research for achieving new effective treatments with fewer side effects is one of the first lines of investigation.

Several minerals are essential nutrients for the activity of proteins being important their ingestion though the diet following the Recommended Dietary Allowance (RDA). In fact, dietary supplements in a multivitamin format are frequently ingested by population in order to improve health status and prevent illnesses (Radimer et al. 2004). Knowing the risk factors can help to diminish several diseases, and different studies have indicated a relation between some minerals and health status. For instance, in a prospective study with more than 60,000 women it was reported that the risk of suffering from type 2 diabetes was inversely related to calcium and magnesium intakes (Villegas et al. 2009). Among different minerals, selenium (Se) is a trace mineral that was firstly considered as toxic for humans when it is ingested at high levels even in sub-toxic amounts it can induce some negative effects (Steinbrenner et al. 2011). However, Se has also been described to exert different positive biological effects. In fact, selenium (Se) has lately risen as a possible agent to treat several kinds of cancer, since Se has been shown to be related to antioxidant defence and redox state regulation (Sanmartín et al. 2012) and to modify inflammatory tumorigenesis in murine models (Barrett et al. 2015). Se can be found in different food or as supplements, but its bioavailability is different taking into account the origin being preferable the organic form (Gupta and Gupta 2016). Enough amounts of Se are important for different human functions that involve several systems, including the central nervous system, the endocrine system, the cardiovascular system, or immunity (Roman et al. 2014). Nevertheless, a great limitation to understand the effects of selenium in human diseases, and specifically in cancer, is the few epidemiologic and variable data available in the literature. This is related to the several levels of Se that can be found in foods according the different regions and the supplementation uses and quantities given to population. The objective of this chapter is to present the information available on Se and its relationship with cancer, dealing with nutritional aspects and sources of origin, types of Se compounds, and their potential preventive and / or therapeutic capacity.

2 Nutritional Aspects

Se is an essential trace element for humans (Fan and Kizer 1990). However, Se nutritional requirements depend on different aspects, mainly age and the physiological status. In this sense, it is established that the an adequate Se intake in healthy adults is 70 μg/day for males and 60 μg/day for females, but these values are lightly higher in the case of lactating women (Kipp et al. 2015; Xia et al. 2010). In the case of children, it is considered that the adequate values of Se intake mainly depends on the age (from 15 μg/day in children between 1 and 4 years to 60 μg/day in children between 13 and 15 years) (Kipp et al. 2015). An adequate Se intake is important, since low Se intake is frequently associated with several disorders as cardiopathies, cancer, hypertensive and thyroid disorders; in contrast, too high Se ingestion causes toxicity problems. Se toxicity is related to nervous system and skin disorders, paralysis, or hair and nails loss (Rayman 2008, 2012).

Se content in natural foods is very variable, since the content of Se in food depends on presence of this mineral in the environment. For this reason, Se intake widely changes throughout the world from deficiencies to toxic concentrations. Table 20.1 summarises the differential intake of Se in several countries (Rayman 2008).

Despite the great variability of this micronutrient, the main dietary sources of Se are constituted by vegetables, mainly cruciferous (as broccoli and cabbage), bulb vegetables (garlic and onion), mushrooms, asparagus and legumes (soy, lentils, beans). However, as it has been indicated above, the content of Se in these foods depends on the total of the nutrient available in the environment, mainly taking into

Table 20.1 Se intake in several countries. Modified from Rayman 2008

Country	Selenium intake (μg/person day)
Australia	57–87
Belgium	28–61
Brazil	28–37
Canada	98–224
China	7–4990
France	29–43
Egypt	29
Germany	35
India	27–48
Japan	104–199
New Zeeland	55–80
Spain	35
Sweden	38
Switzerland	70
UK	29–39
USA	106
Venezuela	200–350

account the soil and water characteristics. In fact, it is evidenced that vegetables from countries with soils poor in Se present lower levels of Se (Kipp et al. 2015). In general, it is documented that plants-based foods present higher amounts of selenomethionine than the animal foods; for instance, organic Se compounds -as selenocysteine or selenomethionine - constitute half of the Se present in cereals. In animal foods, Se forms are very variable but they can contribute by providing selenomethionine, selenocysteine, selenotrisulfide, selenopersulfide, and metallic selenide (Barceloux 1999); this animal source could be an alternative for Se intake since the animals can accumulate Se if they have been fed with plants presenting high amounts of Se (Adadi et al. 2019), so that organ meats or seafoods can be a food source of Se.

Apart from the Se amounts in different diets, another point to take into account is the capacity of the organisms to use this nutrient. Bioavailability of Se depends on several aspects as physicochemical properties or dietary effects; for example, the formation of metals complexes has been reported to significantly lessen the bioavailability of Se. In general, Se forms with better bioavailability are selenium compounds which contain amino groups as Selenomethionine, Selenocystine, Selenocysteine, Se-methylselenocystein (Kieliszek and Blazejak 2013). Nevertheless, it has been described that the bioavailability of selenium forms from vegetables is high, while Se from animal origin foods (especially fish) is low or moderate (Barceloux 1999). In addition, Se assimilation could increase with diets rich in low molecular weight proteins, and in vitamin B, C and D (Kieliszek and Blazejak 2013).

Dietary Se is absorbed in the intestine and it is reduced to selenide (H_2Se), this conversion supposes several redox reactions, where glutathione plays an important role generating an important intermediary, selenodiglutathione. H_2Se is converted in selenophosphate, which generates selenocysteyl-tRNA that are involved in the synthesis of selenoproteins. Dietary and endogenous selenoaminoacids are incorporated to the body proteins. When an over intake of Se occurs, the excess of H_2Se could be converted in selenosugars and trimethyl selenoium ion which are eliminated in the urine, and in dimethyl diselenide that is eliminated by breathing and is the responsible of the garlic breath, one of the symptoms of toxicity by Se (Fairweather-Tait et al. 2011; Kieliszek and Blazejak 2013; Skalickova et al. 2017).

3 Selenium Compounds

Se is a non-metallic compound that belongs to the Group VIa of the periodic table. Se can be found in the environment by forming several inorganic compounds, such as elemental selenium (Se), selenium dioxide (SeO_2), selenodiglutathione, hydrogen selenide (H_2Se), sodium selenide (Na_2Se), sodium selenite (Na_2SeO_3), sodium selenate (Na_2SeO_4), selenious acid (H_2SeO_3), and selenic acid (H_2SeO_4) (Barceloux 1999). Despite Se compounds are very similar to sulphur ones, they are less stable than the corresponding sulphur compounds (Skalickova et al. 2017). Se can also be

Table 20.2 Most abundant selenium forms and compounds. Modified from Kieliszek and Blazejak 2013

Forms	Compounds
Inorganic species	Elemental Selenium, Selenide, Selenate, Selenite
Simple organic species	Methylselenol, Dimethylselenide, Dimethyldiselenide, Trimethyl selenonium, Dimethylselenone, Dimethylselenoxide Methylseninic acid, Selenocyanate, Selenourea
Amino acids and low molecular compounds	Selenocystine, Selenocysteine, Se-methylselenocysteine, Selenocystein acid, Selenomethionine, Se-methylselnomethionine, S-(methylseleno)cysteine, Selenomethionine selenoxide hydrate, Selenohomocysteine γ-Glutamyl-Se-methylselenocysteine, Selenocholine, Se-adenosylselenohomocysteine, Selenobetaine, Selenoglutathione
Other compounds	Selenopeptides, Selenoproteins, Selenoenzymes, Se-metal metallothionines, Selenosugars

found being part of several organic or biogenic complexes as methylated Se species, selenoamino acids, selenoproteins, selenoenzymes, selenoaminocarboxylic acids, selenium peptides (Skalickova et al. 2017). The most important organic selenium peptides are selenocysteine which is essential by the synthesis of glutathione peroxidases, and selenomethionine that is the most assimilable Se form for humans and the most used Se form in nutritional complements (Kieliszek and Blazejak 2013). Table 20.2 shows most abundant Se compounds.

4 Relation of Selenium and Cancer

The relation between Se and cancer was evidenced for first time in 1969, when a study found an inverse association between Se exposure (Se levels in food and in blood) and cancer mortality, suggesting a protective role of Se against this disease (Shamberger and Frost 1969). Afterwards, more studies have found evidences of the beneficial effects of Se in the risk of suffering several kinds of cancer, such as prostate, liver, colorectal and thyroid cancers (Etminan et al. 2005; Glattre et al. 1989; Peters and Takata 2008; Yu et al. 1999).

The Nutritional Prevention of Cancer (NPC) trial carried out at the Nutritional Arizona Cancer Centre was the first study which evaluated the protective effects of Se against cancer (Shamberger and Frost 1969). This study demonstrated that the administration of 200 μg/day of organic Se (mainly in the form of selenomethionine) significantly diminished the incidence of cancer in humans. Following studies demonstrated that the supplementation with Se significantly reduced the incidence of colorectal, prostate and lung cancers (Duffield-Lillico et al. 2002; Reid et al. 2002). However other studies performed with the results of this trial showed an increase in the incidence of non-melanoma skin cancer (Duffield-Lillico et al. 2003). It was described that the protective activity of Se against cancer development depends on Se plasma levels; it was demonstrated that levels above 84 μg/L of Se in

plasma are enough to reduce the risk of suffering from oesophageal and gastric cancer in the Chinese population (Wei et al. 2004), although another study evidenced that levels of 147 μg of Se/L of plasma is the minimum necessary to decrease the risk of prostate cancer in Hawaiian population (Nomura et al. 2000). Specifically, the minimum Se intake to reach these plasma Se levels is 140 μg/day of Se compounds (Duffield-Lillico et al. 2003; Rayman 2008).

Most studies performed with Se supplementation used inorganic forms of Se including selenite, selenium dioxide or selenite; more recent studies used Se in its organic forms as selenocysteine and selenocystine and it was shown differences in their protective effects (Neve 2002). In fact, it has been suggested that both L-selenomethionine and L-Se-methylselenocysteine are the Se forms which would be the most protective ones in experimental models (Finley et al. 2001; Medina et al. 2001).

The mechanisms through Se exerts its protective effects against cancer are not yet well defined (Neve 2002). However, it is postulated that Se protective effects are mediated by metabolites which could induce several redox changes in the cells. In this sense it is thought that selenodiglutathione, methylselenol and hydrogen selenide could be the key compounds in the protective anticarcinogenic effects of Se (Fleming et al. 2001; Medina et al. 2001). The protective effects of Se against cancer may be mediated by several mechanisms, mainly by induction of reactive oxygen species (ROS) which, in turn, can exert cytotoxic effects on the cancer cells (Yan and Spallholz 1993); thiol interactions and/or chromatin binding that alter transcription and signalling pathways, as it was reported in a model of human breast cancer cell line (Tobe et al. 2015). However, it is thought that the protective effect of Se against cancer is related to:

- The increase in the glutathione peroxidase activity and other selenoproteins; this fact could be also related with the protective effects of Se against other pathologies as cardiovascular diseases (Fairweather-Tait et al. 2011).
- The overproduction of ROS in carcinogenic cells. It has been reported that seleno cystine can increase the production of ROS and induce DNA damage, resulting in mitochondrial mediated apoptosis related to p53 phosphorylation in cancer cells (Fan et al. 2013).
- The enhancement of immune function; it was evidenced that Se supplementation increases immune function (Neve 2002; Peretz et al. 1991), although molecular mechanism remains still unknown.
- The elimination of carcinogenic compounds via activation of glutathione-s-transferase; this response could be mediated by methylselenol (Fairweather-Tait et al. 2011; Neve 2002).
- The inhibition of the histone deacetylation (Fairweather-Tait et al. 2011). Histone deacetylases are key enzymes in the epigenetic regulation, in this sense it is suggested that histone deacetylases could play a central role in cancer treatment. It is evidenced that some Se compounds such as bis(5-phenylcarbamoylpentyl) diselenide or 5-phenylcarbamoylpentyl selenocyanide can prevent histone

deacetylase activity which caused cytotoxicity in melanoma (Gowda et al. 2012) and lung cancer cell lines (Karelia et al. 2010).
– The inhibition of cell proliferation and an increase in apoptosis. This process could be regulated by selenodiglutathione, hydrogen selenide and methylselenol (Fleming et al. 2001).
– The regulation of angiogenesis of the tumour. This effect could be regulated by methylselenol and Se-methylselenocysteine, which are able to reduce the expression of vascular endothelial growth factor in *in vitro* assays (Fairweather-Tait et al. 2011; Medina et al. 2001).

5 Selenium and Cancer Prevention

Adequate trace levels of Se are critical for the proper functioning of the organism, while excessive or low Se intake can lead to adverse health effects. Mainly due to its antioxidant action, Se is related to protective and preventive effects against various types of diseases characterized by the presence of oxidative damage, including cancer, cardiovascular or infectious diseases (Rayman 2012). In this sense, in the face of a pro-oxidant situation, an increase in selenoprotein levels and activities has been observed as a compensatory response. However, although the health effects of deficient and excessive levels of Se are possibly extensive and may include cancer and cardiovascular, metabolic, and neurological disorders, the results are not yet conclusive (Fig. 20.1). This controversy is made by the fact that there is no clear and standardized consensus on dietary recommendations for Se worldwide (Vinceti et al. 2017).

There are numerous *in vivo* studies using animal models that have shown how dietary supplementation with Se reduces the incidence of a wide diversity of cancer typologies. However, in the case of human studies, the relationship with total cancer risk or specific types of cancer has been somewhat controversial. The most promising effects of Se are focused above all on gastrointestinal cancer and prostate cancer. Among the epidemiological studies that seek to assess the potential protective activity of Se on the incidence of cancer, there are first studies with ecological design in the 70s. From these initial studies, then, many observational studies appeared, including case-control and cohort designs, and randomized clinical trials.

Shamberger and Frost (1969) published the first epidemiological study in which they suggested the existence of an inverse association between the consumption of Se and cancer death rates, reporting a Pearson correlation coefficient of $r^1 = -096$. This study opened the door to think about the potential benefits of high Se intakes for cancer prevention. It was carried out considering the population of the United States and Canada, and was followed by other studies analysing other areas of the world. Another interesting study observed the existence of an inverse relationship between Se intake, estimated from information on food intake in 27 countries, and mortality from different types of cancer, such as colon prostate, lung, breast, ovary, or leukaemia (Schrauzer et al. 1977). Other subsequent studies have found inverse

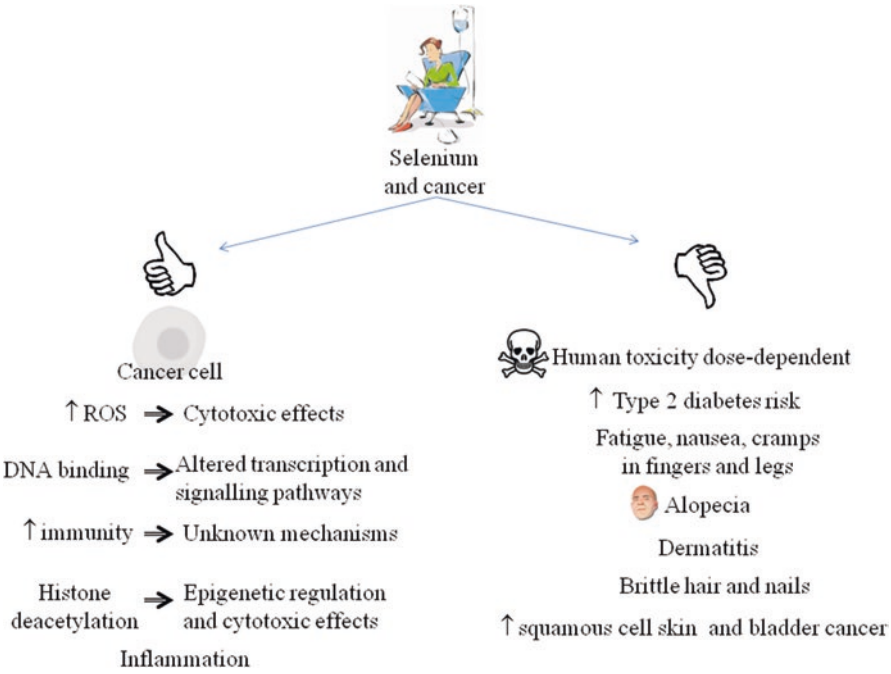

Fig. 20.1 Main effects of selenium in cancerous cells and side effects described

associations between Se consumption and mortality with some types of cancer, although not always in the same types of cancer or in the same intensity, which reduced the impact of the results (Chen et al. 1992; Clark et al. 1991; Kneller et al. 1992; Schaafsma et al. 2015; Guo et al. 1994b). Moreover, some studies did not observe any relation between Se and the aetiology of cancer or even observe a positive relationship (Guo et al. 1994a; Keshavarzi et al. 2012). These differences derive from different ways of collecting data, methods of determining Se levels, and the impossibility of considering it as a single individual level.

When longitudinal studies are analysed, most of them present a case-control design and have evaluated the relationship between the occurrence of cancer and basal levels of Se in serum, toenails, or diet, and in some cases, the levels of plasma selenoproteins. In this sense, a meta-analysis investigated the association between the baseline Se status and the risk of cancer from a total of 70 observational studies that included more than 2,300,000 subjects analysed (Vinceti et al. 2018). The analysis of the different observational studies did not show clear evidence on the possible preventive dose-response effect of baseline Se levels and the incidence of cancer. However, when the authors combined the results obtained in the different studies, they observed an inverse association between Se and the subsequent risk of all causes of cancer and some types of cancer such as prostate cancer and colon cancer. A subsequent meta-analysis specifically investigated the protective effects of Se in prostate cancer patients (Sayehmiri et al. 2018). The analysis of 30 observational

studies seems to indicate the existence of an inverse relationship between the risk of prostate cancer and Se exposure with an estimated relative risk of 0.89 (95% CI: 0.80–1.00) for case-control designs and 0.77 (95% CI: 0.52–1.14) for cohort designs. This study, unlike a previous study where 20 epidemiological studies were analysed, did not show an increase in the risk of prostate cancer in a low Se state (Brinkman et al. 2006). The authors concluded the need to carry out more studies considering other environmental factors such as other trace elements, economic and social situation, differences in Se levels between populations or a possible threshold effect. In relation to colorectal cancer, various studies have observed an inverse relationship between Se levels and the risk of developing this type of cancer. Thus, it has been evidenced that subjects with Se levels below the mean (<128 μg/L) had a higher probability and a greater number of adenomatous intestinal polyps (Clark et al. 1993). Similarly, other studies have also observed a robust association between low Se levels in blood serum and the colorectal cancer risk, while high values have been associated with a diminished prevalence of colorectal adenoma (Connelly-Frost et al. 2006; Lener et al. 2013). The European Prospective Investigation into Cancer and Nutrition (EPIC) study, comprising more than half a million participants recruited in European countries found that Se values below 80 μg increased the risk of colorectal cancer and hepatobiliary cancer (Hughes et al. 2016; Hughes et al. 2015). Finally, another study using a random-effects model examined in 26,397 subjects with colorectal cancer and 41,481 healthy subjects the association between diverse risk factors and colorectal cancer (Cornish et al. 2020). The results evidenced an association between elevated concentrations of Se in the blood and a decrease in the risk of colorectal cancer (estimated risk 0.85 (95% CI 0.75–0.96). In a meta-analysis evaluating epidemiological data on Se levels and the risk of lung cancer, it was concluded that potentially protective effects are only observed when high Se levels are compared in populations where average Se levels are low (Zhuo et al. 2004). In a post-study, the risk of lung cancer in smoking subjects based on Se status was found to vary depending on the presence of different polymorphisms, which shows the complexity to evidence conclusive results (Jablonska et al. 2008). Furthermore, a cohort study (the Copenhagen Male Study) where 3333 male subjects were followed for 16 years did not show that low baseline serum Se was an independent risk factor for lung cancer (Suadicani et al. 2012). However, a recent prospective study reported that elevated levels of Se increased overall survival in patients with stage I lung cancer at diagnosis (Pietrzak et al. 2019).

Moving on to the large-scale human clinical trials, the results are not totally conclusive either, there are studies in which it is observed that supplementation with Se reduces the risk of cancer while in others the results are not so clear. The Nutritional Prevention of Cancer (NPC) trial started in 1983 as the first clinical trial focused on Se (200 μg/day organic Se) as a preventive for non-melanoma skin cancer in a population at high-risk (Clark et al. 1996; Duffield-Lillico et al. 2002). Besides to this first outcome, secondary endpoints comprised overall cancer mortality and occurrence of other specific types of cancer. In a preliminary data published in 1996, the results reported little evidence of Se supplementation on the primary outcome (incidence of non-melanoma skin cancer) (Clark et al. 1996). However, a substantial

decrease in overall cancer occurrence and mortality was observed with a notable reduction in the incidence of prostate, colorectal, and lung cancer. These effects were maintained when the final results of the study were published (Duffield-Lillico et al. 2002; Reid et al. 2002), although at this point, a higher incidence of non-melanoma skin cancer was observed in the supplemented subjects contradicting the initial hypothesis of the trial (Duffield-Lillico et al. 2003). Furthermore, an additional examination of the NPC cohort revealed an elevated risk of diabetes in Se-treated patients (Stranges et al. 2007).

From the results obtained regarding prostate cancer, the Se and vitamin E cancer prevention trial (SELECT) was developed. Participants in the study were enrolled in four groups: placebo, selenomethionine (200 μg Se/day), vitamin E, or a combination of Se and vitamin E with a median follow up of 5.4 years (Lippman et al. 2005). However, the results did not show a consistent relationship between higher Se concentrations and a lower incidence of all-cause cancer -prostate, colorectal or lung cancer- whereas a minor increase in bladder cancer incidence was found (Klein et al. 2011; Lance et al. 2017; Lippman et al. 2009; Lotan et al. 2012). Furthermore, a troubling result from the data analysis was the fact that the risk of aggressive prostate cancer increased in the supplemented group, especially in those subjects with higher basal concentrations of Se in the toenails (Klein et al. 2011; Kristal et al. 2014). Also, through regression models, it was evidenced that the CC genotype at rs11781886, which is related to a reduced expression of the androgen-regulated prostate tumour suppressor protein NK3 homeobox 1 (NKX3.1) elevated the risk of prostate cancer when combined with Se (Martinez et al. 2014).

Other studies have also been focused in prostate cancer or others with similar results. In an interesting double-blind, randomized, placebo-controlled trial, 423 men characterized by a high-grade prostatic intraepithelial neoplasia were enrolled. They received selenomethionine (200 mg/day, follow up 3 years) as preventive treatment to develop prostate cancer. However, the authors found no protection of the Se in front of prostate cancer appearance (35.6% in Se group vs. 36.6% in control) (Marshall et al. 2011). Similar results were observed in a phase 3 randomized, double-blind, placebo-controlled clinical trial where 699 men were allocated to control group or Se yeast (200 or 400 μg/day, orally) groups. Participants had risk for prostate cancer but with a negative biopsy and followed until 5 years with controls every six months. Again no effects on the prostate cancer incidence were reported; moreover the prostate specific antigen also did no change between groups (Algotar et al. 2013). Another clinical trial with a phase III, randomized, placebo-controlled design (NCT00078897) included patients who suffered from a colonoscopic removal of one or more colorectal adenomas within six months prior the beginning of the study. Patients received 200 g/day of Se yeast (n = 685) compared to placebo (n = 689) and were followed-up for 6 or more months (if clinical experts required) when a colonoscopy was done. As other reported clinical trials, not only Se supplementation did not prevent colorectal adenoma recurrence but alto it increased other adverse events. Some problems that appeared were an increase in the risk of suffering from type 2 diabetes (mainly in older participants), brittle hair and nails, and squamous cell skin cancer (Thompson et al. 2016). Patients with

organ transplant (n = 184) were included in a randomised, multicentre, placebo-controlled, parallel group study to assess the effects of Se (200 μg/day, 3 years; follow-up of 5 years from the inclusion) on the skin cancers and warts and keratoses appearance. Although no adverse effects were reported by Se, there were no differences between groups (Dreno et al. 2007). Breast or ovarian cancers have been also evaluated with the Se supplementation. For instance, a double-blind trial used sodium selenite (250 g/day during an average time of 35 months) in women with BRCA1 mutations; however, no relation was observed with breast or ovarian cancer. In a second part, associations of different variables showed low risk of cancer, being glutathione peroxidase 4 the one with strong relation between Se levels and non-TT variant (OR 0.32, p: 0.0009), between Se levels and TT variant (OR 0.10, p: 0.047), and without adnexectomy and with TT variant (OR 0.038, p: 0.014) (Lubinski et al. 2011). A randomized, double-blind, placebo-controlled, phase III trial included subjects presenting resected non–small-cell lung cancer to determine the protection of Se in front of a second primary tumour. Patients received Se yeast (200 μg/day, n = 1040) during 4 years and compliance assessment was done every 3 months; however, in a preliminary analysis the second primary tumour was non-statistically higher in the Se group than in control. In addition, the recurrence of lung cancer (74.4% in Se vs. 79.6% in control) and time to death after 5 years was similar between groups without significant differences. Although increases in diabetes mellitus or skin cancer were not detected, results showed no prevention to develop a second lung cancer. In fact, the committee that followed the study recommended finishing the trial (Karp et al. 2013).

Other clinical trials aimed to evaluate supplementation with diverse supplements, among them, Se is included. In a clinical trial (Procomb trial, ISRCTN78639965), 209 patients with lower urinary tract symptoms, but without evidence of prostate cancer and with values of prostate specific antigen below 4 ng/ml were included in the study (Morgia et al. 2017). Patients were supplemented with Se (50 μg)/lycopene (5 mg) or placebo with a 2-years of follow up. No evidence of significant differences in the prostate specific antigen were observed between the groups. Also, supplementation did not imply changes in the risk of prostate cancer compared to the control group, which suggests the absence of positive effects associated with Se supplementation. In another study, long-term Se supplementation effects on mortality were evaluated in a population of rather low status of Se (The Denmark PRECISE study, NCT01819649) (Rayman et al. 2018). A total of 491 volunteers were randomly treated during 5 years with Se-enriched-yeast daily/100, 200, or 300 μg) or placebo and followed for another 10 years. Participants who took 300 μg of Se evidenced a non-significant increase in all-cause mortality, cancer or cardiovascular mortality after 5 years that became significant after the 10-years of follow-up. The patients from the 100 and 200 μg Se groups evidenced a non-significant decrease in mortality at 5 years, which was even lost after the cessation of supplementation. The authors concluded that supplements with high Se levels should be avoided, particularly in countries with adequate background Se intake. In a randomized clinical trial (Linxian General Population Nutrition Intervention Trial, NCT00342654), 29,584 subjects from Linxian (China) received different supplementations including a

mixture of Se (50 μg) + vitamin E (30 mg) + β-carotene (15 mg) during 5.25 years, and followed for 25 years (Wang et al. 2018). The effects on total mortality in this group were reduced during the supplementation period, and especially the stomach cancer although the effect disappeared 10 years post-intervention (Blot et al. 1993; Qiao et al. 2009). At the conclusion of the trial, no significant effects were observed in the Se group on mortality from all types of cancer or from oesophageal or gastric cancer. In this sense, supplementation could decrease mortality in the short term, but is unlikely to exert a significant long-term effect once supplementation ends. Another interesting clinical trial (the Shandong Intervention Trial, NCT00339768) investigated the effects of *Helicobacter pylori* treatment (amoxicillin and omeprazole during 2 weeks), antioxidant supplementation (Se 37.5 μg, vitamin C 250 mg and vitamin E 100 IU), and garlic as prevention of gastric cancer for 7.3 years in 3365 subjects (Li et al. 2019; Ma et al. 2012). Preventive treatment for *H. pylori* infection reduced the risk of gastric cancer, while the incidence decreased even more with antioxidant vitamins supplement but not with garlic supplements. After more than 22 years of follow-up, the results showed a substantial reduction in the risk of death related to gastric cancer in the groups supplemented with vitamins or garlic.

The lack of clear results associated with clinical trials have been reported in a meta-analysis carried out by Vinceti et al. (2018) where after analysing 10 high-quality clinical trials, they did not observe remarkable effects of Se on reducing the general risk of cancer or specific cancers, such as prostate cancer. Another recent meta-analysis has also not observed preventive effects of Se supplementation (or vitamins A, C and E) on the incidence or mortality from lung cancer (Cortes-Jofre et al. 2020). In addition, some notable adverse effects were evidenced in some of them, like an increase in the risk of type 2 diabetes and high-grade prostate cancer or dermatological disorders.

6 Selenium in Cancer Therapy

Preclinical studies have pointed out the anticancer properties of Se compounds, opening a strategy to combine this mineral with used cancer treatments. However, it should be noted that large ingested amounts of Se could be toxic (Rayman et al. 2018), and different works reported positive and negative results, maybe related to the existence of more than twenty selenoproteins with different actions. Anyway, different studies performed in animal models have pointed out benefits in several diseases, and a synergistic interaction between Se and drugs used for cancer treatments (Caffrey and Frenkel 2013; Evans et al. 2017), it could be related to an interference in the processes of drug resistance and/or an increase in the therapeutic effect of the used drugs. Some previous studies reported the anti-apoptotic and antioxidant effects of Se (100 nM – 1 μM) in CCL-97 (R2C) tumour Leydig cells on cell viability, oxidative stress, calcium concentration, and apoptosis (Defo Deeh

et al. 2019). But experiments with animals also showed some promising results. For instance, in a study with a model of colorectal cancer (induced by 1,2-dimethylhydrazin) in rats (Abedi et al. 2018), Se-enriched yeast administrated by gavage improved the histological findings with less number and size of aberrant crypt foci, diminished the angiogenesis of tumour cells, increased the P53 protein expression as the most important suppressors of tumours, and inhibits the growth of damaged cells. In a most recent study (Saeed Ali et al. 2019), Se was administrated in the drinking water in a colorectal adenocarcinoma mice model, observing reduced the tumour size and located tumours compared to the multiple plaque masses in controls; this histological findings were related to the observed inflammatory reaction, lymphoplasmocytic reaction, lymphoid hyperplasia, and necrosis in the Se group. In addition, they reported a pro-oxidant effect and an induction of apoptosis of the Se in the cancer cells describing a diminished Caudal Type Homeobox 2 (CDX-2) expression level (as marker of the colorectal cancer), reduced glutathione (GSH) levels, glutathione peroxidase activity, and an increased lipid peroxidation, and increased caspase-3 expression. Finally, Se also had an anti-angiogenic effect since a reduction of the Vascular Endothelial Growth Factor (VEGF) expression was observed. The latest animal experiments try to reduce the Se toxicity looking for a vehicle that allows better anticancer results with fewer side effects. In this sense, nanocarriers are another line of interest. For example, Se-methylselenocysteine and L-selenomethionine were compared to selenium nanoparticles (Sonkusre 2019) in a human prostate epithelial carcinoma cell line and mice were used to evaluate the toxicity. The authors reported diminished tumour cell viability but not related to apoptosis or necrosis since no changes were observed in lactate dehydrogenase as indicator of the loss of membrane integrity. In contrast, a dose-dependent overexpression of genes (tumour necrotic factor and interferon regulatory factor) related to necroptosis was reported. Moreover, the effect of the Se nanoparticles did not produce deleterious effects on human red blood cells integrity or death in mice; but the Se-nanoparticles orally administrated to animals showed toxicity since an increase of alanine aminotransferase (ALT) levels was observed. Other works use the Se as a carrier to load different drugs addressed to the efficacy of cancer therapies, such as in lung (Zou et al. 2019), colorectal (Xia et al. 2020a), or cervical (Xia et al. 2020b) carcinoma. For instance, a recent study with colorectal cancer cells (HT-29) and mice using selenium nanoparticles with short interfering RNA reported the suppression of the proliferation, migration and invasion of the tumour cells and the *in vivo* growth in mice (Xia et al. 2020a). Attending to these results, there is a need to perform more studies to evaluate the possibilities of using nanocarriers. Interestingly, in a study using selenoether and diselenide bonds (among others), to create a delivery system for paclitaxel-citronellol conjugates as treatment for cancer, showed influence on the drug delivery as well as a cytotoxic effect of the drugs by the ROS production thanks to the selenoether and diselenide bonds in a ubiquitous KERATIN-forming tumour cell line HeLa (KB cells), a human alveolar basal epithelial cell carcinoma (A549 cells), and breast cancer (4T1 cells) (Sun et al. 2019).

In humans, diverse Se assays have been designed to test the pharmacokinetics of the cancer treatment (Fakih et al. 2006; Mix et al. 2015). Some of these works,

reported some acute, reversible side effects after a daily dose of selenite combined with the first line of chemotherapy (Brodin et al. 2015), that include nausea, fatigue, and cramps in fingers and legs, but without systemic toxicity. Other examples related to pharmacokinetics are a phase I clinical study with 45 patients that present gynaecologic malignancy (Song et al. 2018), in which selenious acid were administrated (50–5000 μg/dose) followed by paclitaxel and carboplatin as chemotherapy. Authors reported that Se did not alter the drugs pharmacokinetics and was safe and well tolerated by the patients with all the tested Se amounts. In another phase I randomised double-blinded study (Evans et al. 2019), 24 patients with solid malignancies or chronic lymphocytic leukaemia received 400 μg of sodium selenite, Se-methylselenocysteine and seleno-L-methionine during 8 weeks (ACTRN12613000118707). Again, safety and well tolerance of Se without toxicity were reported.

Other reports have reported the positive effects of Se in ameliorating some complications of cancer or in the recurrence or regression of different cancers. In a randomized, double-blind, placebo-controlled clinical trial (NCT01432873) carried out in 74 patients with lymphomas (Daeian et al. 2014), the effect of 200 μg of Se (twice a day for 2 weeks, orally) was tested to assess the complications related to the hematopoietic stem cell transplantation after receiving chemotherapy. Although the authors observed changes in the plasma pro-inflammatory cytokine levels due to the transplantation, no changes were observed between control and treated groups during the supplementation arguing that it could be related to an improvement in the antioxidant status of patients from the Se group. In another phase II, randomized, double-blind, crossover trial with children (Vieira et al. 2015), the quality of life and renal and liver functions of patients suffering leukaemia and lymphomas and solid tumours and receiving chemotherapy were evaluated. The study reported that the Se supplementation (80% plus dietary reference intake, 30 days and 1-year follow-up) induced a reduction of the side effects associated with the chemotherapy since symptoms –such as fatigue, nausea, or physical functions- were ameliorated, as well renal (creatinine serum and urea levels) and liver (aspartate aminotransferase (AST) and ALT serum levels) functions improved but not always with significant differences. The same group using the same protocol study with 36 children (Rocha et al. 2016) also evaluated the effects of Se in neutropenia as one of the most common side effects of chemotherapy. They reported an improvement in the patients with solid tumours since the circulating neutrophils increased, while children with leukaemia and lymphomas did not report significant changes but a tendency to improve; what it is more, the solid tumour patients had higher IgA and IgG levels when compared with the other patients. Thyroid cancer patients were submitted to a total thyroidectomy and received ^{131}I radiation, the effect of 300 μg of selenium orally for 10 days on salivary glands was evaluated. The authors reported less damage of these glands so reducing the side effects of radiation (Son et al. 2017). Finally, nutritional status is another factor that is highly altered when chemotherapy is in process. In a recent study, 48 patients were randomly assigned to control or supplemented group to assess the effects of the whey protein, the supplement also included Se (0.76 mg/day, follow-up 6 and 12 weeks) as a snack. Authors reported a better protein status,

and a recovery of the GSH and IgG levels that were both reduced with the chemotherapy; it was interpreted as a better nutritional status, oxidative stress level and better immune function by the supplement, relating the Se enrichment with the improvement in the immune system and the oxidative stress (Bumrungpert et al. 2018).

Focusing the attention in the cancer development, some years ago it was reported a significant difference between the Se levels in plasma of patients that developed some kinds of cancer when compared to controls, especially in breast and gastrointestinal tract cancer, and these levels continued decreasing with the recurrence or the cancer progression (Gupta et al. 1994). Likewise, in a Finnish study the Se concentration found in women toenail with breast cancer was slightly lower than controls (Mannisto et al. 2000), but other works did not report differences (Hunter et al. 1990). Some genes have also been associated with cancer, for example, the BRCA1 mutations –as mentioned above, related to the breast and ovarian cancer development- allows an increase in 8-oxodG as product of cellular DNA damage. In a randomized, double blind, placebo-control study, women with and without adnexectomy that present mutations in this gene were given sodium selenite (300 μg/day orally). The authors reported lower 8-oxodG concentrations in the supplemented groups with adnexectomy (n = 18 in healthy BRCA1 mutations, n = 16 in cancer patients with the mutation), although without statistical differences (Dziaman et al. 2009). In a double-blind, phase III, randomized, placebo-controlled trial, the combination of Se (200 μg) with other compounds (zinc, vitamins A, C and E) was orally administrated during 5 years to 164 patients with respect to 166 placebo group, all of them had at least one adenoma endoscopically removed. A reduction up to 39% of colorectal recurrent adenomas was observed in both small tubular and advanced adenomas; however, a great limitation is that it is difficult to assess if the results are attributable to only one or some of the combined compounds administrated (Bonelli et al. 2013). More lately, in a randomised, double-blind, placebo-controlled trial (IRCT201412215623N32) comprising 58 female suffering from cervical intraepithelial neoplasia grade 1 (Karamali et al. 2015), 200 μg of Se yeast was daily administrated as supplement for 6 months; the authors reported a significant regression of the grade 1 cancer (88% vs. 56% with respect to placebo) and improved the insulin and lipid profile when compared with the placebo group. The supplementation with Se yeast (300 μg/day during 5 weeks) in a randomized, placebo-controlled study (NCT00446901) with men suspected to have a possible prostate cancer revealed a down-regulation of the genes related to the epithelial-to-mesenchymal transition in the prostate when compared to controls; this is a process that is not complete in cancer cells inducing multiple transitional states in the prostate and that is involved in inflammation and wound healing (Kok et al. 2017). Patients with breast cancer-related lymphedema were included in a randomized, double-blind, controlled trial. Sodium selenite was intravenously administrated (500 μg, 5 sessions in 2 weeks, n = 14 vs. n = 12 controls) observing a significant change of the lymphedema grade from III to II, although no changes were observed in the antioxidant status. For that reason, the authors pointed out that Se may act though other mechanisms, such as its anti-inflammatory properties (Han et al. 2019). Conversely, other investigations

have reported that Se did not reduce the recurrence to suffer from cancer, such as in a placebo-controlled, double-blind, trial with resected non–small-cell lung cancer patients, in which the occurrence of second primary tumours was evaluated after the supplementation with Se yeast (200 μg/day, 48 months). In this case, the authors reported no benefits of Se in the appearance of the new lung tumours (Karp et al. 2013). Similarly, in a phase III placebo-controlled, double-blind, multicenter, randomised clinical trial (NCT00729287) in which oral selenium yeast 200 μg daily was given to bladder cancer patients during 3 years (Goossens et al. 2016).

7 Conclusions

Cancer is a main concern all around the world, but currently used treatments to fight them are still hard for patients and with many side effects derived for chemotherapy or radiotherapy. For that reason, looking for new strategies that affect the development and recurrence of cancer or ameliorating the side effects of the actual treatments are of interest. Among them, the use of compounds found in diet is one option. Se has been observed as a possible tool to prevent the development of several types of cancer, including colorectal, breast, prostate, or lung. This is derived for the *in vitro* and animal models in which Se has modify the mechanisms related to the development of cancer, including oxidative properties that can increase the cytotoxic effects of the tumour cells, the anti-inflammation properties or the increase of the immunity. However, the clinical trials are limited since most of them have studied the association between the levels of Se in volunteers and the development of cancer in long periods of follow-up of years. So that, a risk to develop cancer has been reported in several studies not without controversial results, since some of them establish a positive and others a negative association. Other studies have reported non-toxic effects at concrete doses of administrated Se giving a start to apply different safety concentrations of this compound to be used in human. It should be taking into account that it has been also listed some side effects due to the use of Se, such as an increase in the probability to suffer from type 2 diabetes, some cancers, or the appearance of fatigue or nausea, among others. Finally, there are studies and clinical trials in humans that reported a positive effect of Se supplementation in the prevention of developing cancer in humans, mainly colorectal carcinoma. In contrast, clinical trials evaluating the direct effect as anti-tumour agent are scarce and with no clear results to reduce the cancer stages. What is more, there are controversial and opposite results related to selenium intake and cancer. However, it seems that individuals that present low Se levels at baseline, mainly because of the low ingestion though the diet in some countries, can obtain benefits from the Se supplementation in front of developing cancer; in contrast, population living in places where the Se intake is relatively high seem not to need Se supplementation since no advantages have been described. For that reasons, the need of more controlled and prospective studies evaluating the effects of selenium in specific cancers

should be addressed to know the real effect and the mechanisms by which this trace element exerts its effects.

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Chapter 21
Dietary Fibers/Beta-Glucan and Cancer

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Abstract Cancer is a multicomplex disease brought about uncontrolled proliferation of cells with contribution of hereditary and environmental factors. Although there are more than 100 known types of cancer and standard approaches have been developed for certain types of cancer, cancer is also an individual disease. With the advances in technology, new therapeutic strategies are being developed in addition to available treatments. The pathways involved in signal transduction consist of protein kinases, MAP kinases, Ras/Raf/MEK/ERK, PI-3 kinase/protein kinase B and STAT proteins. Beta glucans are considered important activators of cellular immune function and macrophages which are the most important biological targets. The protective effects of beta glucan have been demonstrated in different infections caused by parasites, fungal and bacterial. Preclinic studies have shown substantial antitumor activity of glucans in different tumors. Moreover, it was demonstrated

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that beta-glucan has a powerful effect on the antibodies which naturally occurring in cancer. Beta-glucans have been used an adjunct therapy in clinical studies, primarily in the Far East and shown positive effects on patient survival and quality of life. In near future beta-glucan and the other nutraceuticals might be new vision on agriculture in the global economy.

Keywords Cancer · Dietary fibers · Signaling · Mechanisms

1 Introduction

At the present, cancer is one of the most complex diseases caused by accelerating division of cells, inhibiting normal controls on the biosystem, excessive DNA damage and DNA repair defects (Torgovnick and Schumacher 2015). With the scientific advancement, new therapies are being developed using natural compounds which have demonstrated to be benefitial in addition to the treatments available. Beta-glucan are one of these natural compounds and they derive from bacteria, yeast, seaweed and fungal (Table 21.1) (Bashir and Choi 2017; Synytsya and Novak 2014; Kobayashi et al. 1995; Menaga et al. 2012; Kogan 2000). Beta-glucans may be found with different types polysaccharides in several fibers Beta glucans are important modulators of the immune system. Beta-glucans may be able to stimulate the immune system, immune response and inflammation (Błaszczyk et al. 2019). Beta-glucans may have a function in drug delivery systems. Beta-glucans either drug carrier, an adjuvant, or in combination with different materials to form suitable drug delivery systems. Thus; which may be immunoadjuvant features and talent to stabilize drug formulations, facilitate drug delivery. Beta-glucans may also have new roles in cancer chemotherapy. For example, Beta-glucans are showing through an evolving understanding which is involved in a concept denominated innate immune memory. Geller et al. emphasized that the hollow, porous beta-glucan microparticles have been associated to utilize particulate beta-glucan as a delivery vesicle. With these new roles, Beta-glucans may play an important role to cancer prevention. Thus, we believe that we will see the different uses of beta-glucans in the future. In this chapter, we emphasized the latest findings about beta glucans on cancer and their possible molecular mechanisms.

2 Beta-Glucans

Increasing population and decreasing agricultural resources have prompted scientists to work on the production of fast growing, natural and alternative dietary supplements of high quality. Cereals are the primary source of dietary fibre. Oat and barley are regarded as an ideal and natural source of cereal-based functional foods.

Table 21.1 Common bioactive beta glucans, their sources, structure and molecular weight (Bashir and Choi 2017; Synytsya and Novak 2014; Kobayashi et al. 1995; Menaga et al. 2012; Kogan 2000)

Beta Glucan	Source	Beta Glucan	Polysaccharide Structure	Molecular Weight
AM-ASN	*Amanita muscaria*	Fungal	(1 → 3), (1 → 6)-β-D-glucan	260.000 Da
Beta-glucan I (AAG)	*Auricularia auricula-judae*	Fungal	(1 → 4), (1 → 6)- β-D-glucan	nd
Flammulin	*Flammulina velutipes*	Fungal	α-(1 → 4)- β-d-glucan	18.900 Da
Glomerellan	*Glomerella Cingulata*	Fungal	Branched (1,3;1,6) β-glucan	681.000 Da
Grifolan	*Grifola frondosa*	Fungal	(1 → 3) (1 → 6)-β-d-glucan	1.200.000 Da
LNT (Lentinan)	*Lentinus (Lentinula edode)*	Fungal	(1 → 3) (1 → 6)-β-d-glucan	1.000,000 Da
G-A	*Ganoderma Japonicum*	Fungal	(1 → 3) (1 → 6)-β-d-glucan	82.000 Da
SPG (Sonifilan/ schizophyllan)	*Schizophyllum commune*		(1 → 3) (1 → 6)-β-d-glucan	1.640.000 Da
SR (Scleroglucan)	*Sclerotium rolfsii or S. glucanicum*	Fungal	Linear (1,3;1,6) β-glucan	1.400.000 Da
SSG (Sclerotinia sclerotiorum glucan)	*S. Sclerotiorum (ascomycotina)*	Fungal	Linear (1,3;1,6) β-glucan	nd
Krestin PSK	*Trametes versicolor*	Fungal	β-1,4 main chain / β-1,3 and β-1,6 side chain	100,000 Da (Błaszczyk et al. 2019)
Pestolotan	*Pestalotia sp. 815*	Fungal	(1–6)-branched (1–3)-beta-D-glucan	nd
Epiglucan	*Epicoccum nigrum*	Fungal	(1→3;1→6)-beta-glucan	nd
Pleuran	*Pleurotus ostreatus*	Fungal	(β-(1,3/1,6)-D-glucan)	nd
Skleroglucan	*Sclerotinum glucanicum*	Fungal	(1 → 3) (1 → 6)-β-d-glucan	1.400.000 Da
Tylopilan	*Tylopilus felleus*	Fungal	Branched (1,3;1,6) β-glucan	Nd
CI-6P	*Cordyceps Cicadea*	Fungal	Branched (1,3;1,6) β-glucan	21.000 Da
Glucan phosphate (GluP)	*Saccharomyces cerevisiae*	Yeast	Beta-1,3-Glucan phosphate	nd
PGG (betafectin)	*Saccharomyces cerevisiae*	Yeast	Branched (1,3;1,6) β-glucan	nd
Saccharomyces cerevisiae	*Saccharomyces cerevisiae*	Yeast	Branched (1,3;1,6) β-glucan	nd

(continued)

Table 21.1 (continued)

Beta Glucan	Source	Beta Glucan	Polysaccharide Structure	Molecular Weight
WGP-glucan (whole glucan particle)	*Saccharomyces cerevisiae*	Yeast	Branched (1,3;1,6) β-glucan	nd
Zymocel	*Saccharomyces cerevisiae*	Yeast	Poly-(1-6)-beta-D-glucopyranosyl-(1-3)-beta-D-glucopyranose	nd
Zymozan	*Saccharomyces cerevisiae*	Yeast	Branched (1,3;1,6) β-glucan	nd
Chrysolaminarin/ leucosin	*Chaetoceros mülleri*	Algal/ seaweed	(1 → 3), (1 → 6)-β-D-glucan	nd
LAM (Laminarin/ Laminaran)	*Laminaria species (e.g. digitata)*	Algal/ seaweed	β-1,3- and β-1,6-glucan	5000 Da
Curdlan	*Alcaligenes faecalis*	Bacterial	Beta-1,3-glucan	2,000,000 Da

Beta glucans are polysaccharides composed of D-glucose monomers linked by β-1,3 and β-1,4 glycosidic bonds or a backbone of β-1,3-linked D-glucopyranosyl units. Frequency of beta-glucan side branches, and β -(1-6)-linked side chains length may play a role in immunomodulation and differences in molecular mass, solubility, viscosity, and structure of beta-glucans dictate the differences in biological activity (Baldassano et al. 2017). Oat and barley are two of the richest sources of beta-glucans. On the other hand, other cereals such as rye and wheat have a lower beta-glucan concentration (Peterson 1991). There are many sources of beta-glucan, both in the sea and on land. Seaweed, yeast and mushrooms are among these sources. Particularly, seaweed is among the natural and fast-growing alternative nutrition sources which also consumed as nutritional content in different countries. It has a rich beta glucan content as it is a fast growing resource (Yang et al. 2019).

2.1 Pharmacodynamics and Pharmacokinetics of Beta Glucans

Many beta-glucans are classified as naturally non-digestible carbohydrates which processed by the microbiota at varying degrees (Knudsen et al. 1993; Wang et al. 2008). Hence, it has been suggested that their immunomodulatory acitivities might be related to microbiota dependent action. Beta-glucans may bind with high affinity to specific receptors of immune cells where can be improved potent an immunomodulatory effect (Vos et al. 2007).

Beta-glucan is a ligand by receptors located in cell membranes of immune cells. Major beta-glucan receptors are Dectin-1 and Complement receptor 3 (CD11b / CD18) and the other receptors include toll like receptor (Toll-2), lactosylceramide,

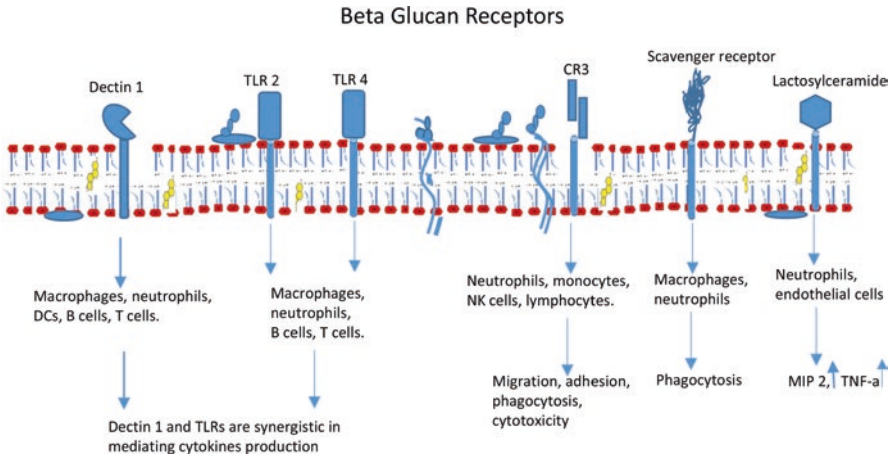

Fig. 21.1 Beta glucan receptors and immunity induced by β-glucan (This figure adapted from; Novak and Vetvicka 2008; Chan et al. 2009; Vetvicka et al. 2007; Lehne et al. 2006; Yoon et al. 2013). *TNF* tumor necrosis factor, *MIP-2* Macrophage inflammatory protein-2, *TLR* Toll-like receptor, *CXCL* C-X-C motif ligand

and scavenger receptor family (Fig. 21.1) (Novak and Vetvicka 2008). Regarding the pharmacodynamic and pharmacokinetic features of beta-glucan, there are several studies in humans and animal models (Chan et al. 2009). In a study that assessed absorption and tissue distribution of enterally administered radiolabeled beta-glucan in a rat model, beta glucan leads to humoral and cellular immune responses in gastrointestinal system (Vetvicka et al. 2007). In a Phase I study suggested that different doses intake of beta-glucans (100 mg/day, 200 mg/day or 400 mg/day) were given for four consecutive days and adverse effects were not found. However, in the same study, the immunoglobulin A concentration in saliva markedly increased in the 400 mg/day arm, suggesting a systemic immune response was induced (Lehne et al. 2006).

2.2 Beta-Glucan as an Immunomodulating Agent

Owing to their physicochemical properties, beta-glucans have a wide range of biological activities in cancer cells such as induction of differentiation, free radical scavenging activity and immunomodulatory effects. The major biological roles of beta-glucans have been included not only the immunity concerning cancer treatment but also immunity to infection, and regeneration of bone marrow (Yoon et al. 2013; Prasad et al. 2012).

Clinical studies have shown that beta-glucans boost the immune response by activating the important cells of the immune system cells enabling them to act more quickly in recognizing and destroying harmful foreign cells. This activation triggers the responses of acquired immune system cells including MHC I and II molecules, antigen presentation via CD4 + or CD8 + T cells and B cells, resulting in inhibition of tumor growth and metastasis (Yoon et al. 2013). Beta-glucans have potent pleiotropic effects on different parts of the defense apparatus, consisting of cytokine production (Schepetkin and Quinn 2006; Vetvicka and Vetvickova 2007). Beta-glucans may be activated and thus B cells may secrete some proinflammatory lymphokines (e.g.IL-8) (Chan et al. 2016).

Beta-glucans are considered powerful activators of cell-mediated immunity and macrophages are the strongest efficient biological targets. The protective effects of beta-glucan have been also demonstrated in parasites, fungal and bacterial infections caused by Leishmania major and donovani, Candida albicans, Toxoplasma gondii, Staphylococcus aureus, Escherichia coli, Mesocestoides corti, Trypanosoma cruzi and Bacillus anthracis (Al Tuwaijri et al. 1987; Cook and Holbrook 1983).

3 Beta Glucans and Cancer

3.1 Cancer

Cancer is a multicomplex disease caused by uncontrolled division and proliferation of cells with contibution of genetic and environmental factors. Although there are different types of cancer and standard approaches have been developed for certain types of cancer, cancer is also an individual disease (Fitzmaurice et al. 2015). With the advances in technology, new therapeutic strategies are being developed in addition to available treatments (Pavlopoulou et al. 2015).

It is well known from both clinical and experimental studies that glucans improve the effectiveness of anti-cancer features and immunotherapy. Beta-glucans found in soluble dietary fibres forms a gel and tight structure by holding water and slows down the intestinal transit. These fibres cannot be digested but are converted to short-chain fatty acids through fermentation and modulate gut microbiota by altering intestinal pH. Beta-glucans are also effective in lowering serum cholesterol levels and reducing the absorption of glucose in the intestine and the amount of insulin in the body. Aside from these properties, beta-glucans are important antioxidant and immunomodulating agents. Cereals and seafoods contain a significant amount of beta-glucans.

More than 250 genes associated with cancer have been identified and several mechanisms are involved in human cancers. While some cancer pathways are linked with a specific type of cancer, others may have critical roles in a wide range of malignant tumors (Schulz 2007).

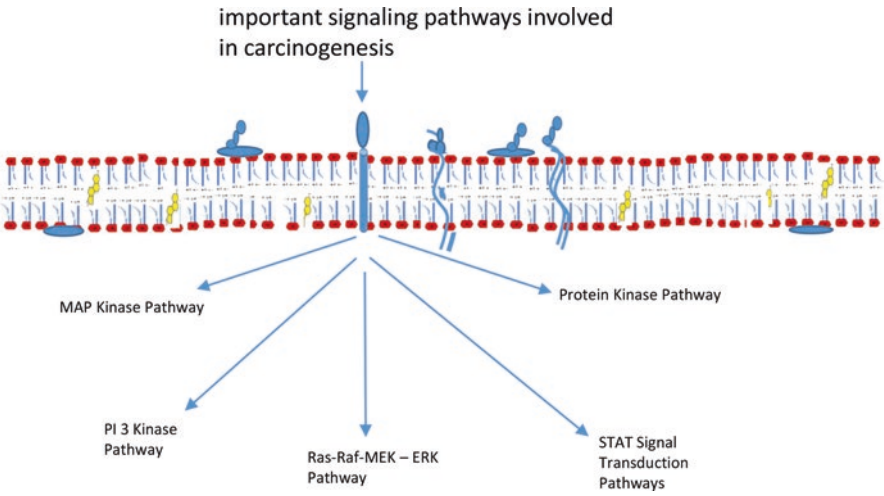

Fig. 21.2 Major signaling pathways involved in carcinogensis (This figure adapted from; Blume-Jensen and Hunter 2001; Pawson et al. 2002; Pawson 2002; Liem et al. 2002; Bowman et al. 2000)

Basically, carcinogenesis involves gradual accumulation of mutations that affect the biological events such as cell survival, control of cell growth and differentiation (Doğan and Güç 2004). Thus, the first mechanism affected in cancers is usually the signal transduction pathways within the cell, and perturbation of these pathways gives rise to different diseases, mainly cancer (Blume-Jensen and Hunter 2001).

3.1.1 Major Signaling Pathways Involved in Carcinogenesis

The pathways involved in signal transduction consist of protein kinases, MAP kinases, Ras/Raf/MEK/ERK, PI-3 kinase/protein kinase B and STAT proteins (Fig. 21.2) (Blume-Jensen and Hunter 2001; Pawson et al. 2002; Pawson 2002; Liem et al. 2002; Bowman et al. 2000).

3.1.2 Protein Kinases and Signal Transduction

Protein kinases are activated by phosphorylation which in turn activates a series of events leading to phosphorylation of different amino acids. Protein kinases are broadly divided into two main groups: membrane-associated and cytoplasmic protein kinases. These proteins are also classified as tyrosine and serine/threonine kinases based on their catalytical properties (the type of aminoacid undergoing phosporylation) (Pawson et al. 2002; Pawson 2002).

Protein kinases can lead to oncogenic transformation through four mechanisms (Blume-Jensen and Hunter 2001):

1. Retroviral transduction of proto-oncogene,
2. Genomic rearrangements,
3. "Gain of function (GOF)" mutations,
4. Overexpression of protein kinase.

3.1.3 MAP Kinase Signal Transduction Pathway

MAP kinases belong to the superfamily of "Mitogen-activated protein kinases" (Liem et al. 2002). They are present in all eukaryotic cells and play a crucial role in transferring the information from the cell membrane to the nucleus. These signal transduction cascades are involved in the regulation of embryogenesis, cell survival, proliferation and differentiation and apoptosis. MAP kinases are divided into three main groups (Platanias 2003):

1. p38 MAP kinase family,
2. "Extracellular signal regulated kinase (ERK)" family,
3. "c-Jun NH2- terminal kinase (JNK)" family

3.1.4 Ras/Raf/MEK/ERK Signal Transduction Pathway

Hormones, growth factors, differentiation factors and tumor-promoting substances use this signal transduction pathway. This pathway is initiated by Ras activation and the kinase cascade proceeds with Raf MAPK kinase kinase (MAPKKK), MEK MAPK kinase (MAPKK) and Erk (MAPK) proteins. Ras and Raf are protooncogenes.

3.1.5 PI-3 Kinase/Protein Kinase B Signal Transduction Pathway

The phosphoinositide-3 kinase (PI-3K) family are proteins responsible for the transduction of cell growth and survival signals (Chang et al. 2003). Following the stimulation of the receptor, PI-3K catalyzes phosphorylation of inositol phsopholipids in the cell membrane. Phosphatidylinositol triphosphate (PIP3) is a lipid mediator that is formed by this pathway. PIP3 is involved in the activation of PIP3-dependent kinases (PDK) and protein kinase B (PKB) (Blume-Jensen and Hunter 2001).

3.1.6 STAT Signal Transduction Pathways

"Signal transducer and activator of transcription (STAT)" proteins were firstly identified in interferon (IFN)-regulated gene transcription in the 1990s. Nowadays, different cytokines have been recognized to activate some STAT proteins. Seven members of the STAT proteins family were recognized in mammalian cells, designated as STAT1-4, STAT5a, STAT5b and STAT6 (Bowman et al. 2000) (Fig. 21.2).

Although success has been achieved in the destruction of tumors using surgical methods and radiotherapy in cancer treatment, residual cancer cells and disease recurrence due to metastasis remain a significant challenge. Therefore, chemotherapy in combination with aforementioned modalities is also used in the treatment process. However, given the damage caused by chemotherapy to healthy cells of the body as well as cancer cells, there is a growing interest in individually-tailored, tumor-specific therapies (Wayteck et al. 2014). Immunotherapy is a specific therapy, meaning that it only targets cancer cells and not normal cells in their surroundings (Yang 2015).

Beta-glucan Cell surface receptors Dectin-1 and complement receptor type 3 (CR3) on innate immune cells including have been described the subject of detailed reviews. Also Dectin-1 signaling has been shown in molecular features. Dectin-1 signaling after beta-glucan ligation has been shown to result in activation of phospholipase C (PLC) and different kinases such as; Src family kinases (SFKs), spleen tyrosine kinase (Syk), phosphatidylinositol-3 kinase (PI3K), Akt, mitogen activated protein kinase (MAPK) (Brown 2005; Brown and Williams 2009; Goodridge et al. 2011; Marakalala et al. 2011; Netea and Maródi 2010).

However CR3-mediated pathways in response to several Ligands have been the focus of an intensive study, beta-glucan-mediated CR3- pathways downstream of lectin domain ligation are less characterized. Syk / PI3K and p38 Mitogen-Activated Protein Kinase (p38 MAPK) activation has been determined in human neutrophils following beta-glucan ligation of CR3 (Berton and Lowell 1999; Li et al. 2006).

3.2 Anti-Cancer Effects of Beta-Glucans

Currently, there is a growing interest in functional components and antioxidant content of foods due to global health problems coupled with increased awareness of healthy nutrition and changes in life expectancy. To this end, a significant portion of high value studies in the field of biotechnology have recently focused on the use of antioxidant sources of functional products. Many studies in humans and animals have shown substantial antitumor activity of beta-glucans in a number of tumors (Hong et al. 2003, 2004; Malyarenko et al. 2019; Bouike et al. 2011; Ross et al. 1999). Moreover, it was demonstrated that beta-glucan has an important synergy with the antibodies which naturally occur in cancer (Hong et al. 2004; Xu et al. 2017; Fortin et al. 2018).

Beta-glucans have been applied as adjuvant therapy in clinical studies, mainly in the Far East and emphasized positive effects on health-related quality of life and patient survival (Vetvicka 2013).

Chan et al. reviewed the literature on the pre clinic studies of beta-glucans, particulatly on their immune and anti-cancer mechanisms and concluded that the intrinsic differences of beta-glucans can induce immune and anti-cancer responses (Chan et al. 2009).

Anti-cancer activity of beta glucans may involve control of inflammation via immunostimulatory patterns on one hand (Shear et al. 1943; Mo et al. 2017) and their potential effects on the control of intestinal hormones (Mo et al. 2017; Huang 2015).

The significance of the immune system in preventing tumor formation has been demonstrated in vivo studies and is shown by epidemiological evidence, such as the increased frequency of certain types of cancer in immunocompromised individuals (Maccalli et al. 2017; Asgari et al. 2017). Macrophages of the innate immune system play a central role in all stages of host defense by releasing inflammatory mediators. Beta-glucans stimulate arachidonic acid release and expression of cyclooxygenase via Dectin-1 receptor (Chan et al. 2009).

Furthermore, beta-glucans have been found to induce maturation of monocyte-derived dendritic cells with considerable cytokine production and enhance proliferation of human peripheral blood mononuclear cells (Chan et al. 2007). In preclinic studies determined that systemic beta-glucan therapy promotes migration of neutrophils to the site of inflammation and improves antimicrobial effect (LeBlanc et al. 2006). Additionally, it was found that beta-glucans increase the production of oxygen radicals and cytokines by neutrophils and macrophages to increase antimicrobial activity via CR3 activated by focal adhesion kinase (FAK) (Novak and Vetvicka 2009). Demir et al. (Demir et al. 2007) reported that oral beta-glucan therapy appears to trigger activation and modification of peripheral blood monocytes in vivo in patients with metastatic breast cancer.

Mo et al. (Mo et al. 2017) investigated the effects of (1 → 3)-Beta-Glucan on the tumor volume in S180 tumor-bearing mice by administering (1 → 3)-Beta- Glucan over 16 days by intragastric route and showed that different doses of (1 → 3)-Beta-Glucan suppressed tumor growth in a dose-dependent manner and increased the tumor inhibition rate in treated mice compared to the control group ($P < 0.01$). Different scientists reported that beta-glucans can actively be used as adjuvant cancer therapy (Thomas et al. 2017; Zhang et al. 2015). A glucan based siRNA carrier system (BG34-10-Re-I) was developed that can effectively assemble siRNA into uniformly distributed nanoparticles that are delivered to macrophages in Balb/c mice animal model (Zhang et al. 2015). Kushner et al. (Kushner et al. 2014) treatment also included the immunostimulant beta glucan in neuroblastoma patients. The present results suggest that could have a major impact on treatment of high-risk neuroblastoma patients. According to the sources provided by ClinicalTrials.gov, the US National Library of Medicine, the clinical studies conducted with current bata glucan and the details about these studies are shown in Table 21.2 (https://clinicaltrials.gov/ct2/results?cond=&term=%CE%B2-Glucan+&type n.d.).

Table 21.2 ClinicalTrials.gov Search Results 09/03/2020 (https://clinicaltrials.gov/ct2/results?cond=&term=%CE%B2-Glucan+&type n.d.)

Title	Status	n	Study Results	Conditions	Interventions
The effect of Oral β-glucans supplement on appetite and insulin resistance in non alcoholic fatty liver disease	Unknown status	48 participants	No results available	• Non alcoholic fatty liver disease	• Dietary supplement: Beta glucan • Dietary supplement: Maltodextrin
Nebulized resveratrol plus Carboxymethyl- Beta Glucan for reducing IL-5 in children with allergic rhinitis	Completed	88 participants	No results available	• Allergic rhinitis	• Drug: Resveratrol plus Carboxymethyl-Beta Glucan • Drug: Placebo
Bivalent vaccine with escalating doses of the immunological adjuvant OPT-821, in combination with Oral Beta glucan for High-risk Neuroblastoma	Recruiting	296 participants	No results available	• Neuroblastoma	• Biological: adjuvant OPT-821 in a vaccine containing two antigens (GD2L and GD3L) covalently linked to KLH • Biological: Oral Beta glucan
Beta Glucans and the metabolic syndrome - a human intervention study under BEST	Completed	16 participants	No results available	• Healthy	• Other: no Beta glucan • Other: oat Beta glucan • Other: barley Beta glucan • Other: mutant-barley Beta glucan
Myeloid-derived suppressor cells (MDSCs) in OSCC patients	Active, not recruiting	130 participants	No results available	• Squamous cell carcinoma of the oral cavity	• Dietary supplement: Beta glucan
The effect of a breakfast meal containing oat Beta glucan on food intake at a subsequent meal in Normal-weight and Overweight subjects	Completed	28 participants	No results available	• Satiety	• Other: Oatmeal + OatWell28CF Int 1 • Other: Oatmeal + OatWell28CF Int 2 • Other: Oatmeal + OatWell28CF Int 3 • Other: Cream of Rice

(continued)

Table 21.2 (continued)

Title	Status	n	Study Results	Conditions	Interventions
To study the effect of Beta glucans on wound healing	Completed	30 participants	No results available	• Burns	• Dietary supplement: Oral β-glucans
					• Drug: Oral sugar powder
Effect of 6 weeks daily consumption of a cereal-based juice beverage on gastrointestinal health	Completed	56 participants	No results available	• Irritable bowel	• Dietary supplement: Barley β-glucans
					• Dietary supplement: Control
Host modulatory effects of Beta glucan on localized aggressive periodontitis	Completed	30 participants	Has results	• Localized aggressive periodontitis	• Drug: Beta1,3/1,6-D-glucan
					• Other: Placebo
Effect of the molecular weight of oat Beta glucan on its ability to	Completed	367 participants	No results available	• Hypercholesterolemia	• Dietary supplement: Wheat bran
Lower serum cholesterol					• Dietary supplement: 3g high MW
					• Dietary supplement: 4g medium MW
					• Dietary supplement: 4g low MW
Oat Beta glucan as a supplement in Chilean type 2 diabetics	Completed	37 participants	No results available	• Type2 diabetes	• Dietary supplement: Oat Beta glucan as a supplement in type 2 diabetics
Effect of immune-enhancing enteral nutrition on immunomodulation in critically ill patients	Completed	30 participants	No results available	• Critical illness	• Dietary supplement: Standard enteral nutrition
					• Dietary supplement: High-protein enteral nutrition of immune modulating nutrients enriched with Beta glucan
					• Dietary supplement: High-protein enteral nutrition of immune modulating nutrients without Beta glucan

Effects of barley on glucose control	Active, not recruiting	24 participants	No results available	• Healthy	• Dietary supplement: 0g barley β-glucans no fibre
					• Dietary supplement: 2g barley β-glucans
					• Dietary supplement: 4g barley β-glucans
					• Dietary supplement: 6g barley β-glucans
					• Dietary supplement: 0g barley β-glucans
					With fibre
Barley and Rice mixture effects on blood glucose	Completed	39 participants	No results available	• Blood glucose	• Other: 100% steel cut barley
				• Dietary Fiber	• Other: 100% barley flakes
				• Hunger	• Other: 50/50 rice-barley mix
					• Other: 80/20 rice-barley mix
					• Other: 100% rice
					• Other: White bread
Effect of serving size and addition of sugar on the glycemic response elicited by oatmeal	Completed	38 participants	No results available	• Diabetes mellitus	• Other: Classic Quaker quick oats
					• Other: Cream of Rice cereal
Lung Cancer vaccine plus Oral dietary supplement	Completed	5 participants	No results available	• Lung Cancer	• Biological: Vaccine 1650-G
Beta glucan and monoclonal antibody 3F8 in treating patients with metastatic Neuroblastoma	Active, not recruiting	45 participants	No results available	• Neuroblastoma	• Biological: Beta glucan
					• Biological: Monoclonal antibody 3F8
					• Other: Immunohistochemistry staining method
					• Other: Laboratory biomarker analysis

(continued)

Table 21.2 (continued)

Title	Status	n	Study Results	Conditions	Interventions
The glycemic response elicited by Beta glucans of different physical properties and form	Completed	15 participants	No results available	• Type 2 diabetes	• Dietary supplement: Oat Beta glucan
					• Dietary supplement: Placebo
Effect of Beta glucan on cholesterol lowering	Completed	45 participants	Has results	• Hypercholesterolemia	• Dietary supplement: Control
					• Dietary supplement: 3g LMW β-glucans
					• Dietary supplement: 5g LMW β-glucans
					• Dietary supplement: 3g HMW β-glucans
A trial comparing a diet including products aimed at targeting satiety	Completed	298 participants	No results available	• Weight	• Other: Active diet products
				• Appetite	• Other: Placebo diet products
Effect of consuming oat bran mixed in water before a meal on glycemic responses in healthy humans - a pilot study	Completed	10 participants	No results available	• Glucose intolerance	• Other: 0 grams oat bran
					• Other: 4.5 grams oat bran
					• Other: 13.6 grams oat bran
					• Other: 27.3 grams oat bran
A study of the effect of oats on post prandial glucose response	Completed	24 participants	No results available	• Normoglycemic	• Other: Oatmeal
				• Normal body weight	
Four-hour glycemic kinetic response following 13C-enriched	Completed	15 participants	No results available	• Glycemic and Insulinemic response	• Other: Oatmeal
Oatmeal breakfast compared to hot corn grits					• other: Corn grits

Healthy effects of an innovative probiotic pasta	Completed	40 participants	No results available	• Obesity • Inflammation • Dyslipidemia • Constipation	• Dietary supplement: Whole grain pasta
Glycemic impact of oatmeal plus OatWellXF28	Completed	40 participants	No results available	• Glycemic responses	• Other: Oatmeal + OatWell28XF • Other: Oatmeal • Other: Hot cereal
The effect of dietary Fiber on food liking	Terminated	12 participants	No results available	• Different fermentable Fiber • Satiation	• Other: Eating
Efficacy and safety of fermented barley on decrement of body fat in obese subjects	Completed	80 participants	Has results	• Overweight; hyperlipidemia	• Dietary supplement: Barley β-glucans (3.0g) • Dietary supplement: Placebo
Effects of Lentinula Edodes bars on dyslipidemia and Oxidative stress in cholesterol individuals: Randomized study	Completed	68 participants	No results available	• Dyslipidemias	• Other: Shiitake cereal bar
A 12-week human trial to compare the efficacy and safety of Polycan on bone metabolism	Completed	60 participants	Has results	• Bone health in Perimenopausal women	• Dietary supplement: Polycan • Dietary supplement: Placebo
Portfolio 5 - multicentre dietary advice on serum lipids in hyperlipidemia	Completed	351 participants	No results available	• Hyperlipidemia • Cardiovascular disease	• Dietary supplement: Dietary portfolio – intensive • Dietary supplement: Dietary portfolio - routine • Dietary supplement: Control (low saturated fat therapeutic diet)
Effect of dietary fibre and whole grain on the metabolic syndrome	Completed	15 participants	No results available	• Metabolic syndrome	• Other: Bread types

(continued)

Table 21.2 (continued)

Title	Status	n	Study Results	Conditions	Interventions
Impact of DHA/oat on metabolic health in gestational diabetes mellitus	Unknown status	80 participants	No results available	• Gestational diabetes mellitus in pregnancy	• Dietary supplement: DHA
					• Dietary supplement: Oat grains
SATIN: Satiety innovation. Study 2-university of Aberdeen	Completed	40 participants	No results available	• Overweight and obesity	• Other: Arabinoxylan
					• Other: Beta Glucan
Magnetic resonance imaging-portfolio diet study #7	Withdrawn	–	No results available	• Cardiovascular diseases	• Behavioral: Portfolio plus diet
				• Hypercholesterolemia	• Behavioral: DASH-like (high fibre) dietary advice
				• Diabetes	
				• Metabolic syndrome	
				• Obesity	
Clinical trial to evaluate Papilocare® gel efficacy into Repairment of cervical lesions caused by HPV	Recruiting	200 participants	No results available	• HPV infection	• Device: PAPILOCARE
				• Lesion cervix	• Device: PLACEBO
Enhanced multicenter dietary portfolio study	Withdrawn	–	No results available	• Cardiovascular diseases	• Behavioral: Enhanced portfolio plus
				• Type 2 diabetes	Structured exercise
				• Hypercholesterolemia	• Behavioral: High fiber diet plus routine exercise
Diet for the maintenance of weight loss and metabolic health in obese postmenopausal women	Recruiting	150 participants	No results available	• Metabolic syndrome	• Other: The MED
				• Diet modification	• Other: The DASH
				• Postmenopause	• Other: Control diet

The combined portfolio diet and exercise study	Recruiting	200 participants	No results available	• Cardiovascular diseases	• Behavioral: Portfolio diet and structured exercise
				• Hypercholesterolemia	• Behavioral: DASH-like diet and structured exercise
				• Type 2 diabetes	• Behavioral: Portfolio diet and routine exercise
				• Metabolic syndrome	• Behavioral: DASH-like diet and routine exercise
Canola oil, fibre and DHA enhanced clinical trial	Completed	30 participants	No results available	• Metabolic syndrome	• Dietary supplement: Butter, sunflower and safflower oil
					• Dietary supplement: High oleic canola oil and DHA (HOCO-DHA)
					• Dietary supplement: Barley β-glucans
					• Dietary supplement: HOCO-DHA and barley Beta glucan
Phase 2 study of Imprime PGG & Pembrolizumab in subjects with Adv SCCHN who failed Pembro Monotherapy or experiencing SD	Terminated	1 participant	No results available	• Squamous cell carcinoma of the head and neck	• Biological: Imprime PGG
					• Drug: Pembrolizumab
Study of Imprime PGG and Pembrolizumab in advanced melanoma and triple negative breast Cancer	Active, not recruiting	64 participants	No results available	• Advanced melanoma	• Biological: Imprime PGG
				• Triple-negative breast Cancer	• Drug: Pembrolizumab

4 Conclusion and Future Perspectives

Beta-glucans may be used with different aims due to their metabolic activity in the cell. However, considerable structural differences in molecular basis, branching frequency and natural glucan groups linked with chitins and mannoproteins render the clinical uses of natural glucans have been interesting potential research topics. Small synthetic oligosaccharides might be use prepared from natural glucans in the future. Some of these natural oligosaccharides have demonstrated superior biological activities than natural glucans such as induction of phagocytosis, modulation of gene expression and anti-anemia. Latest studies strongly suggest the possibility of making small chemical changes in the structure of these oligoglucans to enhance their biological activity. Different studies has shown that beta-glucans can be present anti-tumor (Chaung et al. 2009; Liu et al. 2009), anti-infectious (Shimizu et al. 2009) and anti-inflammatory (Zhou et al. 2009) activities through the modulation of the immune system (Queiroz et al. 2010). Therefore, beta- glucan-containing agents which are produced by mushrooms, algae and fungi, such as krestin (Sugiyama et al. 2010), picibanil (Oh-hashi et al. 1978), lentinan (Mashiba et al. 1979), and sizofiran (Chihara et al. 1970), are also actually used as anti-cancer drugs. A black yeast, *Aureobasidium pullulans* (AFO-202) produces a beta-glucan in extracelllar environment. The production of beta-glucan by *A. pullulans* is approved as a preservative for food, and is consumed as a healthy food in Thailand. Muramatsu et al. shown that oral use of *A. pullulans*-cultured fluid (AP-CF) decreases mortality of mice after a lethal influenza A virus infection (Muramatsu et al. 2012).

At the same time, beta-glucans might be also an important as therapeutic effect on Covid 19. Beta- glucan might be strong immune stimulator which can be activate macrophages and have powerful immune effects on B-lymphocytes, natural killer cells, and suppressor T cells in the immune system. Dectin-1 is a type II transmembrane receptor which having beta-glucan seceptor involved in innate and adaptive immune responses to foreign antigens and pathogens. Dectin-1 interaction with pattern-recognition receptors (PRRs; the so-called Pathogen-Associated Molecular Patterns—PAMPs) and Toll-like receptors (TLRs; a class of proteins that play a key role in the innate immune system.) in the innate immune responses to beta-glucan recognition. Beta-glucan could activate suppressor cells in human lymphocyte—in particular, regulatory T cells (Treg)—and also induce the production of suppressive cytokines (Ikewaki et al. 2007) which may be helpful in suppressing the cytokine storm observed in Coronavirus Disease 2019. The immunological actions of the *Aureobasidium pullulans* (AFO-202) beta-glucan could have an important role against SARS- CoV 2 viruse infection by immunosuppressing pro-inflammatory cytokines (Rao et al. 2020).

Thus; which may be immunoadjuvant features and talent to stabilize drug formulations, facilitate drug delivery (Vetvicka and Vetvickova 2012). Beta-glucans may also have new roles in cancer therapy. For example Beta- glucans are showing through an evolving understanding which is involved in a concept denominated innate immune memory (Geller et al. 2019). As mention above beta-glucans may

benefit as value-added important molecules with the bioeconomic potential and advanced technology in different countries. For this reason, we must need to more focusing on bioeconomic strategy.

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Chapter 22
Omega-3 Polyunsaturated Fatty Acids and Cancer

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Abstract Naturally occurring fatty acids can be classified into three categories based on the number of double bonds in side-chain. These are saturated fatty acids, (no double bonds), monounsaturated fatty acids (MUFAs, a single double bond), and polyunsaturated fatty acids (PUFAs, ≥2 double bonds). Long-chain-PUFAs contain 13–19 carbon atoms whereas very-long-chain fatty acids contain 20–28 carbon atoms. PUFAs are divided into two families, named as omega-3 and omega-6 fatty acids. Alpha linoleic acid and linoleic acid which are classified as long chain-PUFAs are essential fatty acids which cannot be synthesized by the human body. Omega-3 fatty acids have an anti-inflammatory effect, while omega-6 fatty acids show proinflammatory activity. Modern farming and western diets contain extreme amounts of omega-6 PUFAs while containing very low amounts of omega-3 PUFAs, resulting in an omega-6/omega-3 ratio varying from 1:1 to approximately 20:1. Therefore, we have to take omega-3 Fatty Acid through food or supplements to maintain the balance between omega-3 and omega-6. Clinical studies have shown that omega-3 has a preventive and therapeutic effect for multiple sclerosis, anxiety, depression, dyslipidemia, coronary heart disease, metabolic syndrome, diabetes and

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cancer related complications. In this section, the data obtained as a result of clinical and molecular studies conducted on the preventive and therapeutic effects of omega-3 on some cancers and cancer-related complications will be discussed.

Keywords Omega-3 · Omega-6 · EPA · DHA · Cancer · Clinical Trials · Meta-analysis

1 Introduction

Cancer is one of the most important health problems for humanity at the present which is a complex disease caused by uncontrolled division of cells and under the influence of environmental conditions. With the advancement of technology, new treatment approaches and beneficial natural sources are being demonstrated in addition to the available treatments (Hanahan and Weinberg 2011). Omega 3 fatty acids are present in natural sources such as algae, oils, fish, flaxseed plants. Main Omega-3 fatty acids (FAs) are alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Rodriguez-Leyva et al. 2010). Omega-3 FAs are important modulators of the immune system. They are considered biological response regulators that reduce inflammation and with beneficial effects on inflammation-related disorders including cancer. Omega-3 FAs can regulate cell membrane properties including membrane fluidity and phospholipid composition. Omega-3 FAs can also function as signal transduction molecules inside the cell (Gutiérrez et al. 2019). In this review, we emphasized the latest findings about omega-3 FAs on cancer and their possible molecular mechanisms.

2 Omega-3 and Omega-6 Fatty Acid Metabolism in Human

Naturally occurring FAs basically grouped into three classes based on the number of double bonds in side chains as follows: (1) Saturated FAs (SFAs), no double bonds), (2) Monounsaturated FAs (MUFAs, a single double bond), and (3) Polyunsaturated FAs (PUFAs, ≥2 double bonds) (Orsavova et al. 2015). Long-chain (LC)-PUFAs contain 13–19 carbon atoms and Very-Long-Chain (VLC)-FAs contain 20–28 carbon atoms (Kassab et al. 2019). PUFAs are classified in two main families named as omega-3 (ω-3) and omega-6 (ω-6) FAs. Omega-3 FAs have a carbon-carbon double bond in the omega 3 position, while this double bond is in the omega six position in omega-6 FAs. (Balić et al. 2020). α-Linolenic Acid (ALA) and Linoleic Acid (LA) which are classified as LC-PUFAs cannot be synthesized by the human body (Simopoulos 2016). The same elongases and desaturases are used

to metabolize LA and ALA, resulting in a competitive environment for converting LA and ALA to LC-PUFAs. Also, Δ-5 and Δ-6 desaturases have higher affinity for ALA-derived metabolites compared to LA-derived metabolites. For this reason, Omega-3 and Omega-6 taken with foods must be balanced (Husted and Bouzinova 2016). In animals, Δ6-desaturase creates a double bond at the sixth Carbon-Carbon bond position from the -COOH ends of LA and ALA and generate Gamma-Linolenic Acid (GLA) and Stearidonic Acid (SDA), respectively. Since LA and ALA are competitors for the same enzyme, this step is considered as the rate limiting step for metabolites to be synthesized in the next steps. Two-carbon elongation of GLA and SDA by Elongation of Very Long Chain Fatty Acids Protein 2 and 5 (ELOVL2 and 5) enzymes converts them to Dihomo-γ-Linolenic Acid (DGLA) and Eicosatetraenoic Acid (ETA) respectively. Δ5-desaturase creates a double bond at the fifth Carbon-Carbon bond of the DGLA and ETA and produce Arachidonic acid (ARA) and Eicosapentaenoic acid (EPA), respectively (Delarue and Guriec 2014). After the two successive elongation cycles of EPA, Docosapentaenoic acid (DPA) and tetracosanolpentaenoic acid are synthesized. Tetracosahexaenoic acid (THA) is produced by Δ6-desaturation. β-oxidation of the THA results in the formation of Docosahexaenoic Acid (DHA) which is the final VLC-PUFA product of this pathway (Lenihan-Geels et al. 2013a, b). There is a difference between genders in terms of the rate at which ALA is metabolized to EPA and DHA, and this rate is higher in women than in men. The conversion rate of ALA to EPA and DHA in men is 8% and 4%, respectively, while in women these rates are around 21% and 9% (Akerele and Cheema 2016). In the LA pathway, first Docosatetraenoic acid (DTA) and second Tetracosatetraenoic acid (TTA) are formed from ARA with two consecutive elongation steps. Δ6-desaturase activity and β-oxidation converts it to tetracosapentaenoic acid (BTPA) and Docosapentaenoic acid (BDPA), respectively (Balić et al. 2020). In the pathway of LA, ARA can be converted to Epoxyeicosatrienoic acids (EETs) and Dihydroxyeicosatrienoic Acids (DHETs) by Cytochrome P450 (CYP) epoxygenase and Epoxide Hydrolase (EPHX) related methabolisms, Series 2 prostaglandins (PGA2, PGE2, PGI2, and thromboxane A2) by Cyclooxygenases-2 (COX-2) and Series 4 leukotrienes (LTB4, LTC4, and LTE4) by lipoxygenases (5-LOX). In the path of ALA, EPA can be converted to Series 5 leukotrienes (LTB5, LTC5, LTD6) though 5-LOX and Series 3 prostaglandins (PGB3, PGD3, PGE3, PGI3, and thromboxane A3) through COX-2. DHA can also be converted to D-Series Resolvins (RvD1 to RvD6), Protectins (Neuroprotectin D1), and Maresins (MaR1 and MaR2) (Saini and Keum 2018). Omega-3 and Omega-6 FAs have an opposite effect on the immune system, and omega-3 FAs show anti-inflammatory effects while omega-6 FAs have a proinflammatory effect (Kumar et al. 2019).

3 Recommended Daily Doses of Omega-3 Fatty Acids

Modern farming and western diets contain extreme levels of omega-6 PUFAs while containing very low levels of omega-3 PUFAs, resulting in an omega-6/omega-3 ratio varying from 1:1 to approximately 20:1, which is appropriate for human physiology. In order to restore this balance, omega-3 FA supplements must be made from outside (Simopoulos 2016). While the omega-3 FAs obtained from fish and algae have DHA and EPA content, omega-3 FAs from terrestrial plants are mainly in the form of ALA. Some studies show that taking the omega-3 in the form of DHA and EPA is more effective than taking it in the form of ALA (Hong et al. 2019). Omega-3 LC-PUFAs may be in Ethyl Ester (EE), Triacyl Glycerol (TAG), or phospholipid bound form. Phospholipid bonded omega-3 LC-PUFAs (EPA, DPA, and DHA) have some advantages over their EE or TAG linked forms. Phospholipid-bound omega-3 LC-PUFAs are more stable than TAG and EE-bound forms, more resistant to oxidation, more bioavailable, and can cross the blood brain barrier (Ahmmed et al. 2020). SDA effectively increase EPA and DHA levels compared to ALA in humans. Therefore, taking omega-3 in the form of SDA instead of ALA may be more effective in skipping the rate limiting enzyme step and increasing the levels of EPA and DHA in the blood (Ha and Kim 2018). Until now, different organizations have made recommendations for the daily recommended intake of omega-3 FA doses, and these recommendations vary depending on the gender, pregnancy and lactation status, age of the individuals and for what purpose they use them. According to National Health and Medical Research Council (NHMRC) recommendations, daily intake of 1.3 g/day ALA or 160 mg/day EPA + DPA + DHA is sufficient for men, and 0.8 g/day ALA or a total of 90 g/day EPA + DPA + DHA is sufficient for women. NHMRC also suggested that the dietary omega-3 LC_PUFAs, 610 mg/day for men and 430 mg/day for women, would help reduce the risk of chronic disease (National Health and Medical Research Council [NHMRC] 2006). Food and Agriculture Organization (FAO) and World Health Organization (WHO) recommended daily intake of EPA plus DHA at 250 mg for adult males and non-pregnant or non-lactating adult females, and at 300 mg for pregnant or lactating females in order to reduce the risk of coronary heart disease. American Heart Association (AHA) recommended intake a total of EPA + DHA as 2–4 g/day in the case of hypertriglyceridemia treatment (WHO\FAO 2010; American Heart Association Nutrition Committee et al. 2006). The European Food Safety Agency (EFSA) recommended to intake at least 250 mg/day EPA + DHA for adults to protection from cardiovascular diseases (EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) 2010). The International Society for the Study of Fatty Acids and Lipids (ISSFAL) recommended 0.7% of energy per day should come from ALA in healthy adults (International Society for the Study of Fatty Acids and Lipids (ISSFAL) 2004). United Kingdom Food Standards Agency and Department of Health recommended that people should take 0.2 g/day omega-3 (Bates et al. 2010).

4 Sources and Bioavailability of Omega-3

Marine fishing is the most important source for omega-3 FAs for human needed. Tuna, anchovy, chinook, sockeye, rainbow, sardine, herring, Atlantic salmon, horse mackerel, arctic charr, sockeye salmon and cod are rich sources for EPA and DHA (Delarue and Guriec 2014; Amjad Khan et al. 2017). However, as a result of overfishing in order to meet this need, marine fish stocks for many fish species have significantly decreased. In order to avoid this situation, the stage of producing fish in fish farms has started (Adarme-Vega et al. 2014). When fish farms were first established, fish feeds and fish oils were used to feed the fishes. However, the increase in the number of fish farms and the amount of production resulted in the need to investigate terrestrial alternatives that could replace it, due to the insufficient amount of fish oil used as fish food (Tan et al. 2020). This situation resulted in the tendency to use plant resources as fish food. While marine fishes are fed with omega-3 FA rich phytoplankton and zooplankton in their natural environment, farmed fish are fed with vegetable oils rich in omega-6 FAs. Therefore, farmed fish accumulate lower amounts of EPA and DHA compared to fish living in natural environment. It is also known that fishes living in cold waters have a higher omega-3 FA accumulation compared to fishes living in warm waters. This situation causes people who consume farmed fish not to take the essential omega-3 FAs in sufficient amounts and the nutritional value of the fish to decrease (Saini and Keum 2018).

Terrestrial plants and nuts that are rich in ALA and used as supplements in the nutrition of fish raised in farms are: almonds, chia seeds, butternuts, flax seeds, soybean kernels, walnuts, vegetables including; beans, canola, broccoli, cauliflower, lettuce, spinach, leeks, purslane, radish seed, soybeans, avocado, raspberry, strawberry (Baker et al. 2016; Amjad Khan et al. 2017). Chinese snake gourd (*Trichosanthes kirilowii*) and paprika (*Capsicum annuum*) species are rich in ALA content. Although the EPA ratio in plants is very low, plants such as *Undaria pinnati* (13%) and *Rhododendron sochadzeae* (2%) contain a significant amount of EPA. *Diospyros mespiliformis* was found to be contain DHA (4.65 g FA per 100 g oil). Members of the Primulaceae and Boraginaceae have mean amounts of SDA as 4.73% and 4.99% of total FA in seeds, respectively. *Hippophae rhamnoides* seeds and *Echium humile ssp. pycnanthum* seeds contain 0.36% and 16.2% SDA of their total FAs respectively (Cholewski et al. 2018). Specifically, *Echium plantagineum* and *Buglossoides arvensis* species belonging to Boraginaceae, show the highest amount of up to 12.5% and 20% SDA relative to total fatty acids, respectively (Lenihan-Geels et al. 2013a, b). In recent years, it has been trying to develop resources that can replace fish feed and fish oil by increasing the EPA and DHA content of plants with genetic modifications. *Camelina sativa* and *Brassica napus* (canola) stand out in these studies. Ruiz-Lopez et al. generated transgenic *C. sativa* lines transferring *omega-3 desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ12-desaturase, Δ5 and Δ6-FA elongase* genes of some algae and oomycets including; *Emiliania huxleyi Ostreococcus tauri, Physcomitrella patens, Phytophthora sojae, Thraustochytrium sp.,Phytophthora infestans,*. They showed

that two different transgenic *C. sativa* lines in which their seeds contain EPA levels of up to 31% and in which their seeds contain up to 12% and 14% EPA and DHA respectively (Ruiz-Lopez et al. 2014). Victor et al., investigated the effects of fish oil, wild-type Camelina oil (WCO) and EPA-Camelina oil (ECO) used in the nutrition of juvenile Atlantic salmon fish on the accumulation of EPA, DHA and DPA in muscle tissue, and total omega-3. They determined that, these molecules accumulation rate in the ECO group was 23.8% and was 29.3% in the fish oil group (Betancor et al. 2015). Betancor et al. suggested that genetically engineered Camelina oils are a potent source of omega-3 LC-PUFAs and are candidates to replace fish oil in sea bream diets (Betancor et al. 2016). In a recent double-blind, postprandial cross-over trial it was showed that, postprandial consumption of 450 mg EPA + DHA from *Camelina sativa* oil and commercial blended fish oil in healthy humans resulted no difference in plasma triacylglycerol, phosphatidylcholine or non-esterified fatty acids, Very Low-Density Lipoproteins (VLDL), Low-Density Lipoproteins (LDL), High-Density Lipoproteins (HDL), Tumor Necrosis Factor-α (TNFα), Interleukin 6 and 10 (IL-1 and 6) and soluble Intercellular Cell Adhesion Molecule-1 (ICAM-1) concentrations (West et al. 2019). Transfection of microalga *Schizochytrium sp.* genes into Canola resulted in DHA and EPA synthesis in Canola seeds (Walsh et al. 2016). In a 28-day repeated dose toxicity study showed that, genetically modified Canola oil at 3 mL or (2.76 g)/kg bw/day administered through oral gavage to rats resulted in no signs of toxicity and no adverse effects in clinical, pathological, or histopathological observations (Andre et al. 2019). Genetically modified *Arabidopsis thaliana* with *Δ6-desaturase* gene from *Muraenesox cinereus* and *ELOVL5* from *Acanthopagrus schlegelii*, and a *Δ5-desaturase* gene from *Phaeodactylum tricornutum* resulted in synthesis of EPA, DSA and DPA in Arabidopsis seeds (Kim et al. 2015a, b).

In the marine food chain, the primary producers of Omega-3 FAs are microalgae and they are transported to humans through zooplankton and fish that feed on them (Sayanova and Napier 2016). Photosynthetic microalgae tend to synthesize higher amounts of EPA than heterotrophic ones. *Nitzschia hassall, Nannochloropsis hibberd, Phaeodactylum bohlin, and Porphyridium nägeli* can present a high percentage of EPA in total FAs, Nannochloropsis genus is currently the main source of marketed oils due to its potential to produce high EPA. Among the heterotrophic microalgae; *Schizochytrium sp., Crypthecodinium cohnii Javornick, Ulkenia sp., Gaertner thraustochytrium sp., and Aurantiochytrium sp.* are rich in DHA and supplements derived from some of these are in commercial use for human consumption and fish feeding (Delarue and Guriec 2014; Harwood 2019; Tocher et al. 2019). As a result of recent studies, it has been reported that EPA obtained from *Nannochloropsis sp., Phaeodactylum tricornutum, and Porphyridium cruentum* has protective effects against cardiovascular diseases and atherosclerosis, supports mental development and have anti-inflammatory properties. It has been reported that DHA obtained from *Crypthecodinium cohnii, Schizochytrium spp., Ulkenia spp.* is also important for protection from cardiovascular diseases and supporting the health and development of the nervous system (Barkia et al. 2019). When fish oils and algal oils are compared, it has been determined that algal oils have some superior properties

compared to fish oils. The quality of fish oils varies according to the type of fish, season and the region where it was caught. In addition, fish oils have an unwelcome odor and taste by users and may also be contaminated with polychlorobiphen and heavy metals. On the other hand, microalgae are suitable for the production of high value added products by being reproduced very quickly with little cost in bioreactors, regardless of seasonal and climatic conditions (Lopes da Silva et al. 2019). They can also be used by vegan individuals (Craddock et al. 2017).

Another way of taking EPA and DHA as a food supplement is by using krill oil. Antarctic krills *Euphasia superba* are small crustaceans living in the Antarctic ocean with an estimated total biomass of 300,000–500,000 million metric tons (Kwantes and Grundmann 2015). They do not contain heavy metal pesticides and dioxins as they live in cold and clean seas such as the southern (Antarctic) and northern (Arctic) polar seas (Ursoniu et al. 2017). The omega-3 FA content of the krill oils is comparable to the omega-3 FA content of fish oils. The EPA and DHA in the krill oil are not found in TAG form like fish oil. The omega-3 FAs found in krill oil are in the form of EPA and DHA as in fish oil (Andraka et al. 2019). The phospholipid-bound omega-3 FAs in krill oil make it easier to absorb than the TAG bound forms in fish oil and ensures higher bioavailability (Ulven and Holven 2015). Another superiority of krill oil compared to fish oil is that, it contains a fat-soluble carotenoid astaxanthin which have antioxidant properties (Liu et al. 2020). Krill oils have already taken their place in the markets under different brands as a food supplement (Kleiner et al. 2015).

In addition to these, there are also studies to make fish oil, seed oil or algae oil supplements into animal foods in order to increase the EPA and DHA ratios in the edible tissues and milks of ruminants and to ensure their bioavailability from human (Nguyen et al. 2018). Similarly, supplements can be added to chicken feeds to obtain Omega-3 FA enriched eggs (Alagawany et al. 2019; Ehr et al. 2017). In addition to these, there are also studies that marine bacteria and bivalves and also fungi can be considered as alternative natural sources of EPA and DHA (Alagarsamy et al. 2019; Carboni et al. 2019; Gemperlein et al. 2019).

5 Health Benefits of Omega-3

Omega-3 FA supplementation has been shown to have protective activity in the development and progression of some diseases through their intermediate and/or final metabolites produced in the associated pathway, some of which are listed below. A meta-analysis applied by Su et al. showed that anxiety symptoms decreased faster in patients who took Omega-3 FA supplements compared to patients who did not use them (Su et al. 2018). As a result of a systematic review applied by al Ammar et al., the authors found that omega-3 FA or fish oil supplementation in multiple sclerosis patients, has positive effects on recurrence rate, Quality of Life (QOL) and disease progression regulating the levels of inflammatory markers including TNF-alpha, IL-1B, Interferon Gamma (INFG), and IL-6 (Al Ammar et al.

2019). A meta-analysis applied by Choi and Chae, showed that the combined application of statin and Omega-3 FA is more effective in reducing total cholesterol / HDL cholesterol than statin alone in patients with dyslipidemia (Choi and Chae 2018). A meta-analysis applied by Langlois et al. in patients undergoing cardiac surgery, it was reported that, oral/enteral and parenteral supplementation of Omega-3 FA reduces the length of stay in the Intensive Care Unit (ICU) and postoperative atrial fibrillation rate (Langlois et al. 2017). In a meta-analysis applied by Abdelhamid et al., in cases having low dose and high dose supplementation of Omega-3 FA, it has been showed that Omega-3 FA supplementation slightly reduces the risks of coronary heart disease, cardiovascular disease events, stroke and mortality (Abdelhamid et al. 2018). In another meta-analysis of randomized controlled trials applied by Zhong and Wang indicated that Omega-3 FA supplementation in gestational diabetes significantly reduces fasting plasma glucose, Homeostatic model assessment (HOMA) of insulin resistance and C-Reactive Protein (CRP) (Zhong and Wang 2019). In a meta-analysis of randomized controlled trials applied by Asbaghi et al., it has been suggested that co-supplementation of Omega-3 FA and Vitamin E significantly reduce the serum concentrations of triglycerides and LDL of overweight patients with metabolic syndrome (Asbaghi et al. 2019). After the meta-analysis of randomized controlled trials, Chewcharat et al., suggested that Omega-3 FA supplementation in diabetic nephropathy patients reduce the proteinuria in type 2 diabetes mellitus (type 2 DM) patients (Chewcharat et al. 2020). In addition to these, there are lots of studies showing that Omega-3 FA supplementation may have preventive/therapeutic effects on the development/progression of some cancers or may have therapeutic effects on some cancer related complications. These studies will be discussed in the next section for each cancer types.

6 Cancer and Omega-3

Clinical and molecular studies conducted to date have shown that Omega-3 has a preventive effect in terms of the development of some cancer types, and also has a therapeutic effect for some cancer types. Nabavi et al. (2015) published a comprehensive review examining the relationship between Omega-3 and cancer in 2015 (Nabavi et al. 2015). It may be helpful to have a pre-reading of this review before reading the next section. In this section, the relationship between colorectal, prostate, gastric pancreatic, breast, lung, head and neck cancers and lekemia and Omega-3 will be discussed in detail under subheadings.

6.1 Colorectal Cancers and Omega-3

In a randomized clinical trial conducted in China it was determined that the total Omega-3, EPA and DHA levels in the blood of patients with colorectal cancer were lower compared to control individuals (Wang et al. 2015a, b). Seven double-blind placebo controlled randomized clinical trials were reported between 2015 and 2020 years. In a multi-center study conducted in high risk patients for adenoma randomized to placebo, EPA (2 g/day), aspirin (300 mg/day), and to EPA plus aspirin groups. As a result of this study, it was determined that neither aspirin nor EPA had protective properties in terms of the development of at least one adenoma, but the EPA + Aspirin group developed less colorectal adenomas compared to other groups (Hull et al. 2018). As a result of another study performed in patients who had undergone nonmetastatic colon resection and who had undergone Omega-3 or salt infusion in the perioperative period, it was showed that more infectious events were observed in omega-3 FA group compared to salt infusion group (Bakker et al. 2020). Third study was performed in colorectal cancer patients which have been treated with XELOX (capecitabine plus oxaliplatin) chemotherapy regimen. Half of the patients administered with 30 billion CFUs per sachet daily for 4 weeks and 2 g Omega-3 FA daily for 8 weeks, in addition to the chemotherapy regimen, while the other group administered with placebo preparations. As a result of this study, higher Quality of Life (QOL) reduction, reduced chemotherapy related side effects and IL-6 level is detected in the intervention group in comparison to the placebo group (Golkhalkhali et al. 2018). Fourth study was a multi-center phase III trial which was conducted by the North Central Cancer Treatment Group (NCCTG) in patients with resected stage III colon cancer and having treatment with infusional FOLFOX regimen (fluorouracil, leucovorin, and oxaliplatin) with or without adjuvant cetuximab. Modified Block Brief food frequency questionnaire (FFQ) containing 68 questions was applied to the participants. Results of this study indicated that, higher Omega-3 FA supplementation and fish intake is associated with better 3-year Disease Free Survival (DFS) in patients with tumors having wild type KRAS and also associated with better DFS in patients with tumors having mismatch repair deficiency (Song et al. 2019). Fifth study was a prospective study conducted in gastric and colorectal cancer patients undergoing elective surgery and having seven day parenteral nutrition regimen after the surgery indicated that, Omega-3 FA supplementation did not show any statistically significant effect on pro-inflammatory factors including IL-6, CRP, TNF-α, and Procalcitonin (Ma et al. 2015). In the sixth study which is a Phase II study conducted in colorectal cancer patients undergoing metastatic liver resection surgery it was shown that 2 g/day EPA supplementation resulted in Overall Survival (OS) benefit compared the control group (Watson et al. 2016). The results of the last study showed that, preoperative EPA treatment in colorectal cancer patients undergoing metastatic liver resection surgery reduce plasma C-C Motif Chemokine Ligand 2 (CCL2) level and results in improved overall survival (Volpato et al. 2016).

Two meta-analysis were reported about Omega-3 FA and colorectal cancers in the 2015–2020 period. Results of a meta-analysis of prospective studies conducted by Chen et al., showed that, higher intake of Omega-3 FA is associated with reduced risk for proximal colon cancer (Chen et al. 2015). In another meta-analyse indicated that, 0.2 g/kg fish oil supplementation at least 7 days on postoperative period reduce IL-6 level in the cases undergoing chemotherapy, the Omega-3 FA supplementation (0.6 g/day for 9 week) decrease CRP levels (Mocellin et al. 2016).

In addition to clinical studies on the protective efficacy of Omega-3 FAs in the initiation and progression of colorectal cancer, further studies are also conducted to investigate their effects on colorectal cancer cell lines. *In vitro* and *in vivo* analysis indicated that EPA and DHA treatment of colorectal cancer cell lines suppress proliferation and induce apoptosis of colorectal cancer cells through activation of Free Fatty Acid Receptor 4 (FFAR4), Free Fatty Acid Receptor 1 (FFAR1), Gαs/cAMP-dependent protein kinase A (PKA)/ Macrophage Stimulating 1 (MST1/2), Large Tumor Suppressor Kinase 1 (LATS) and phosphorylation and cytoplasmic translocation of Yes1 Associated Transcriptional Regulator (YAP). This activation cascade depends on FFAR1/FFAR4 and results in a decrease in Connective Tissue Growth Factor (CTGF), Cellular Communication Network Factor 1 (CCN1), Early Growth Response 3 (EGR3), Amphiregulin (AREG), NLR Family Apoptosis Inhibitory Protein (NAIP), Survivin (BIRC5), BIRC7, and Myeloid Cell Leukemia 1 (MCL1) expression (Zhang et al. 2016a, b). Proteomic analysis of human colorectal adenocarcinoma cell line HT-29 showed that, DHA treatment results in; Caspase-3 activation, down-regulated proteasome pathway and anti-angiogenic and anti-apoptotic proteins while up-regulation of pro-apoptotic factors and tumour-suppressor proteins. These findings show that Omega-3 FAs are effective not only on apoptosis and cell growth but also on the proteosomal and angiogenesis pathways (Ortea et al. 2018). Treatment of DNA mismatch repair-deficient and KRAS-mutated colorectal cancer cell line LS174T with DHA, disrupts cell connections, decrease the expression levels of B Lymphoma Mo-MLV Insertion Region 1 Homolog (Bmi-1), SRY-Box Transcription Factor 2 (Sox-2), Octamer-Binding Protein 4 (Oct-4) and Nanog pluripotency genes and induce Caspase-3 activation indicating that Omega-3 may also negatively regulate the pluripotency and stemness feature of colorectal cancer cells (Mahmoudi et al. 2020). EPA reduces expression levels of Vascular Endothelial Growth Factor (VEGF) and IL-6 and reduces VEGF secretion from cancer associated fibroblasts suppressing Extracellular Signal Regulated Kinase (ERK) phosphorylation in colorectal cancer cells. This shows that Omega-3 also affects the interaction between cancer cells and their microenvironment (Ando et al. 2019). EPA treatment counteracts inflammatory-driven NOTCH1 activation, decrease Matrix Metallopeptidase 9 (MMP9) activity and reduce the inflammatory-driven Epithelial to Mesenchymal Transition (EMT) in proinflammatuar conditioned medium treated colorectal cancer cells pointing out the effect of Omega-3 FA against to invasive properties of colorectal cancer cells (Fazio et al. 2016). DHA plus radiation treatment in HT-29 cells reduced proliferation, Cyclin D1 (CCND1) expression and Glycogen Synthase Kinase 3 Beta (GSK3β) phosphorylation while increased G0/G1 phase cell cycle arrest.

In addition, it has been showed that DHA attenuate the radiation induced nuclear β-catenin (CTNNB1) activity (Murad et al. 2019). There are also studies examining the individual effects of metabolites generated during the metabolism of Omega-3 FFAs on colorectal cancer cell lines. Resolvin D1 (RvD1) is a metabolite synthesized from DHA. RvD1 inhibits TNFα stimulated c-Myc expression in colon cancer cells and human colorectal carcinoma cell line HCT 116 which constitutively overexpress c-Myc protein. RvD1 achieves this effect by stimulating the proteasomal degradation of c-Myc and direct interaction with the ALX/ Formyl Peptide Receptor 2 (FPR2) receptor (Zhong et al. 2018). Omega-3 FA attenuates N-Methyl-N-nitrosourea (MNU) induced colorectal cancer in experimental rat models, inhibits colony formation, proliferation, and invasion whereas induces apoptosis in colorectal cancer cells through inhibiting Phosphoinositide-3-Kinase (PI3K)/AKT Serine/Threonine Kinase 1 (AKT)/ B-Cell CLL/Lymphoma 2 (Bcl-2) signaling pathway (Huang et al. 2020). Omega-3 FA rich diet inhibits growth of C57BL6 murine colon adenocarcinoma cell line MC38 in mice, significantly elevate the Epoxydecosapentaenosoic acid (EDP) via CYP450, EDPs reduces expressions of several genes related to Wnt pathway, such as Axin2, and C-jun, c-Myc (Wang et al. 2017).

6.2 Prostate Cancer and Omega-3

There are a limited number of clinic trials on prostate cancer and Omega-3 FA. In such a study, serum EPA and DHA were found to be inversely associated with intraprostatic inflammation while LA was found to be positively associated it in prostate cancer (Nash et al. 2015). Dietary intake of DHA and blood EPA concentration was found to be associated with reduced prostate cancer risk. 0.2% increase in blood DPA concentration was also found to be associated with a 3% reduction in risk for the development of prostate cancer. Also, dietary ALA was inversely associated with prostate cancer risk (Alexander et al. 2015). In Caucasians, a negative association was observed between EPA level and disease aggressiveness in peri-prostatic adipose tissue who underwent radical prostatectomy (Figiel et al. 2018). 1.9 g EPA + DHA daily supplementation for 90 days in patients with elevated Prostate Specific Antigen (PSA), a suspicious finding in digital rectal exam or transrectal ultrasound do not have a significant effect on Ki-67 and TNF Receptor Superfamily, Member 6 (FAS) expression in prostatic tissue biopsies (Zhang et al. 2016a, b). In line with these limited results, we can say that Omega-3 supplementation may have protective properties for prostate cancer, but more clinical research is needed to determine this precisely by considering the subgroups of prostate cancer.

Studies examining the effects of Omega-3 FAs on intracellular signal transduction in prostate cancer cell lines are discussed below. Treatment of human prostate carcinoma cell line DU145 with 20 μM EPA results in a decreased number of viable cells at 48 and 72 h. Pretreatment of the Lipoprotein A (LPA) induced DU145 cells with 20 μM EPA results in inhibition of LPA-induced Extracellular Signal-Regulated

Kinase 2 (ERK) and Focal adhesion kinase (FAK) phosphorylation, activation of P70 S6 Kinase (p70S6K), CCN1 expression. This study suggests that EPA blocks the pathways that activate growth signals rather than inducing apoptosis in prostate cancer cells (Liu et al. 2015). DHA induce the expression of pro-apoptosis genes including Bcl-2 Associated X (BAX) and Bcl-2 Associated Agonist of Cell Death (BAD) and apoptosis, but inhibits the expression of antiapoptotic genes including Bcl-2 and survival in androgen independent prostate cancer cell lines. Also, binding DHA to its receptors FFAR1 and FFAR4 leads to activation of Gas and PKA, phosphorylation of MST1, activation of LATS1 kinase and YAP phosphorylation and cytoplasm accumulation and degradation of YAP. The results of this study show that while Omega-3 FAs activate apoptosis ralated pathways in prostate cancer cell lines, they block the synthesis of growth-related proteins by means of YAP degradation (Wang et al. 2018). Omega-3 treatment of DU145 and human metastatic prostate adenocarcinoma cell line PC-3 reduce Interferon gamma (IFN-γ) induced Interleukin 18 Binding Protein (IL-18BP) expression, and IFN-γ receptor expression. Also, it has been reported that this treatment reduces the phosphorylated Janus kinase 1 (JAK1), Signal Transducers and Activators of Transcription 1 (STAT1), ERK1/2, and P38 Mitogen Activated Protein Kinase (MAPK14) proteins. These findings suggest that Omega-3 FA treatment of prostate cancer cells makes these cells more susceptible to IL-18 mediated immune response (Wang et al. 2015a, b).

Treatment of PNT1A cells with DHA at 100 μM, decrease the mitochondrial bioenergetic reserve capacity and increased Reactive Oxygen Species (ROS) production while decreased AKT phosphorylation. This finding suggests that ROS may play a role in the drift of cells treated with DHA to apoptosis (Tamarindo et al. 2019). In another study, treatment of DU145 cells for 24 h with fish oil, EPA, or DHA significantly reduced the cell viability. DHA treatment for 24 h significantly upregulates the expressions of proapoptotic genes including; BAX, Caspases 1, 3 and 9, Cell Death Inducing DFFA Like Effector A (CIDEA), DNA Fragmentation Factor Subunit Alpha (DFFA), TNF, and Tumor Protein P53 (TP53), while downregulates the expressions of antiapoptotic proteins including; X-Linked Inhibitor Of Apoptosis (XIAP), Apoptosis Inducing Factor Mitochondria Associated 1 (AIFM1), AKT1, BH3 Interacting Domain Death Agonist (BID) and Baculoviral IAP Repeat Containing 6 (BIRC6) which are also associated with TP53, MAPK, TNF, PI3K/AKT, and nuclear factor-kappa B (NF-κB) signaling pathways (Sun et al. 2017).

Wild type mice dietary Omega-3 FA supplementation decrease allografted tumor growth whereas in experimental *FFAR4* gene knockout mice models Omega-3 FA had no antitumorigenic effect. Omega-3 FA treatment decrease the M2-like tumor associated macrophages number in tumor tissue in wild type mice but not in experimental *FFAR4* gene knockout mice model. Higher expression of stromal FFAR4 is correlated with a greater reduction in expression of the genes responsible for cell cycle progression in patients with prostate cancer consuming a high Omega-3 FA diet indicate that Omega-3 FAs exert their effect through FFARs (Liang et al. 2019). Another *in vivo* study showed that; Omega-3 FA supplementation in androgen sensitive mouse prostate cancer cell line MycCaP allografted tumors in immunocompetent FVB mice results in significantly smaller tumor volume, lower expression of

IL-6, TNF-alpha, IL-10 and CCL-2. Also, treatment of MycCap cells with fish oil and Omega-3 FA reduced protein expression of in the NFkB pathway transcription factors and their downstream target genes Bcl-2, BCL-XL, survivin and XIAP showing the activity of Omega-3 intervention on inflammatory pathways in prostate cancers (Liang et al. 2016).

6.3 Gastric Cancers and Omega-3

There were six randomized clinical trials that evaluate the Omega-3 FA and gastric cancer. First study was an open label longitudinal study conducted in gastric adenocarcinoma patients who were in pretreatment. A supplementation of a formula enriched with 3.2 g/day of EPA/DHA for 30 days given in the intervention group while a standard formula was given in the control group. As a result of this study an increase in weight gain and a reduction of IL-6 were observed in intervention group compared to control group (Feijó et al. 2019). Second research was a prospective study conducted in patients with gastric adenocarcinoma or gastric tumors who underwent elective radical subtotal or total gastrectomy. Ordinary diet plus 400 mL/day of the oral interventional supplement for 3–5 days were given before the surgery and an oral soft diet plus 1200 mL/day of the interventional diet for postoperative days 5–14 was administered to the patients. Interventional diet was composed of arginine, glutamine, and Omega-3 FA. Perioperative enteral nutrition enriched with immunomodulator compounds showed no significant immunomodulation effect compared with the standard enteral nutrition (Ma et al. 2018). Third study was conducted in surgically treated patients with stomach cancer. 0.15 g/100 ml EPA and 0.10 g/100 ml DHA for seven days was given to interventional group while standard enteral nutrition was given to control group. Results of this study showed that postoperative enteral nutrition enriched with EPA plus DHA provide short-term benefit for stage IV cancer patients however there is no effect on long-term survival in patients (Klek et al. 2017). Fourth study was a multi-centre, open-label, superiority phase III trial who underwent gastrectomy for gastric cancer. 600 kcal with 2·2 g/day of EPA was given for 21 days with oral intake. As a result, there were no significant effect of the interventional EPA supplementation on weight loss, postoperative complications and CRP level (Ida et al. 2017). Sixth clinical trial was conducted in patients with gastric cancer under chemotherapy. A chemotherapy including Cisplatin plus oral administration of PUFAs supplement in the scale of 3600 mg/day and in three courses were given to intervention group. Gene expression analysis in tumor biopsy samples showed that, expression levels of Caspase-3 and Caspase-9 genes and DNA damage was significantly increased in the intervention group compared to the control group (Dolatkhah et al. 2017). Last clinical trial was a Phase II study conducted in patients with advanced stage esophagealgastric adenocarcinoma having palliative EOX (epirubicin, oxaliplatin, and capecitabine) chemotherapy regimen coupled with weekly infusion of EPA + DHA. Results of this study showed that, Omega-3 FA supplementation reduced

chemotherapy associated toxicity and resulted in improvements in radiological responses and anti-inflammatory cytokine profile (Eltweri et al. 2019).

There were three meta-analysis of previous clinical trials were reported between 2015 and 2020 years. A meta-analysis evaluating the inflammatory and immune function of Omega-3 FA enriched adjuvant parenteral nutrition in patients undergoing surgery for gastrointestinal malignancy showed that; postoperative CD3+ and CD4+ cell counts, CD4+/CD8+ cell ratio, lymphocyte count, immunoglobulins A, M and G are increased in response to Omega-3 FA supplementation while CRP, IL-6 and TNF-α and and the incidence of infectious diseases are decreased (Zhao and Wang 2018). Another meta-analysis evaluating the effects of Omega-3 FA supplementation in gastric cancer on circulating biomarkers related to inflammatory response indicated that a significant decrease in the expression of IL-6 and TNF in the intervention group (Mocellin et al. 2018). Third meta-analysis evaluating the effects of preoperative oral or enteral immune modulating nutrition containing Omega-3 FA in patients undergoing tumor resection surgery for gastrointestinal cancer showed that preoperative immune modulating nutrition containing Omega-3 FA in gastrointestinal cancer patients undergoing surgery decreased infectious complications and length of stay in hospital however did not affected overall survival of the patients (Adiamah et al. 2019).

There is only one *in vitro* study evaluating the effect of omega-3 FA on gastric cancer cell lines. In that study, DHA treatment induced transcriptional activitiy of Peroxisome Proliferator-Activated Receptor Gamma (PPARγ) in human gastric cancer cell line AGS. Activated PPARγ induce the expression of Suppressor of Cytokine Signaling 3 (SOCS3) which is one of the negative regulators of STAT3. SOCS3 suppress the phosphorylation by JAK and nuclear translocation of STAT3 which results in decreased expression of oncogenic protein c-Myc (Ji et al. 2016).

6.4 Leukemia and Omega-3

Few studies have examined the usability of Omega-3 FAs in different stages of leukemia treatment and their effectiveness on leukemia cells *in vitro*. In a double-blind, randomized, placebo-controlled trial which evaluate the effect of supplementation of fish oil on chemotherapy related hepatotoxicity in children and adolescent Acute Lymphocytic Leukemia (ALL) patients in the maintenance phase, oral Omega-3 FA capsules containing 80 mg EPA and 120 mg DHA given daily for 6 months was given to patients in addition to MTX treatment in the intervention group. Results of this study showed that, patients in the intervention group had significantly lower levels of liver enzymes and malondialdehyde (MDA) with higher antioxidant levels compared to control cases (Elbarbary et al. 2016). Second study was a randomized clinical trial which evaluated the effect of oral supplementation of fish oil for 9 weeks on inflammatory nutritional risks in patients with a haematological malignancy at the beginning of chemotherapy. 2 g/day (367 mg/day of EPA and 243 mg/day of DHA) of fish oil for 9 weeks was given to the patients with leukaemia or

lymphoma enrolled to the study. Results of this study showed that CRP and CRP/albumin ratio was reduced in the intervention group. Also, it was shown that, overall long-term survival is higher in the patients of intervention group (Chagas et al. 2017).

In an *in vitro* study, six Acute Myeolgenous Leukemia (AML) cell lines including HL-60, KG1a, ML-2, MOLM13, THP-1 and U937 were treated with DHA (from 0 to 50 μM) or EPA (from 0 to 150 μM) for 48 h. DHA and EPA showed inhibitory effect on cell proliferation in all cell lines in a dose dependent manner and propagated the apoptosis. Treatment of the U937 cell line with the fish oil (0 to 100 μg lipids/mL) for 24 h/ 48 h and treatment of primary AML cell with 50 μg/mL for 48 h resulted also in reduced cell viability. The treatment of HL-60, MOLM-13 and U937, cell lines with DHA, fish oil and EPA and DHA supplemented seed oil resulted in dose-dependent decrease in mitochondrial respiration and an increase in glycolysis. Transcriptomic analysis of U937 cells treated with 50 μg/mL fish oil for 6 h or 24 h revealed that dysregulated ROS pathway, increased expression of Heme Oxygenase 1 (HMOX1) and NAD(P)H Quinone Dehydrogenase 1 (NQO1) which are the downstream targets for NF-E2-Related Factor 2 (Nrf2). Immunofluorescent analyses showed that Nrf2 has been translocated to the nucleus after the fish oil treatment. Incubation of the primary leucoblasts obtained from AML patients with a combination of AraC with fish oil (50 μg/mL) for 48 h resulted in reduced cell viability compared to cells incubated with AraC alone. These findings show that Omega-3 FA supplementation activates Nrf2 protein, which acts as a receptor for endogenous and exogenous stress factors, migrating to the nucleus and stimulates the expression of antioxidant and antitumor genes (Picou et al. 2018).

6.5 Pancreatic Cancer and Omega-3

There were five randomized double-blind, controlled clinical trials that evaluate the Omega-3 FA and pancreatic cancer. In the first study comparing the effects of Omega-3 FA (300 mg/day) either given as marine phospholipids and Fish oil formulation on body weight, QOL and on the plasma fatty acid profiles in pancreatic cancer patients 0.3 g/day Omega-3 FA for 6 weeks was given the patients. As a result, there were no significant difference in appetite, weight stabilization, and QOL observed between the two groups (Werner et al. 2017). Second study was a prospective trial evaluating the effects of nutritional intervention with an EPA-enriched supplement on the nutritional status of pancreatic cancer patients that receiving gemcitabine-based neoadjuvant chemoradiation therapy. Two bottles of 440 ml/day (560 kcal/day) EPA-enriched nutritional supplement during the irradiation component of treatment was administered to patients in intervention group for 5 weeks. Patients that consumed 50% of the EPA-enriched formula was found to have a higher skeletal muscle mass ratios compared the patients in the control group. Also, the psoas major muscle area ratio was higher in the nutritional intervention group compared to control group (Akita et al. 2019). Third clinical trial evaluated the effects of intravenous Omega-3 FA in advanced pancreatic adenocarcinoma

patients receiving gemcitabine chemotherapy. Proteomic analysis indicated the increase the expression of the genes implicated in apoptotic pathways and lipoprotein remodelling whereas decrease in the genes expression implicated in inflammatory response, PI3/Akt pathway, TGF-beta and angiogenesis pathways. In addition to these differential expressions in the genes associated with the epigenetic regulation are also observed (Runau et al. 2020). Fourth study evaluated the effects of intravenous Omega-3 FA on the antitumor activity of gemcitabine and QOL in patients with advanced stage pancreatic cancer. Patients were administered gemcitabine 1000 mg/m^3 weekly followed by up to 100 g intravenous Omega-3 FA enriched supplement for 3 weeks. Results of this study showed that, combination of Omega-3 FA enriched supplement and gemcitabine is safe and may improve the QOL in patients with advanced stage pancreatic cancer (Arshad et al. 2017). Last study was a prospective trial investigating the effects of preoperative enteral diets enriched with EPA on the incidence of hypercytokinemia after pancreatoduodenectomy. Patients in the intervention group received 2.0 g/day EPA orally for 7 days before the surgery. Results of this study showed that there was no significant difference in the incidence of infectious complications, serum concentration of IL-6, serum albumin, prealbumin, transferrin, serum IL-1beta, TNF-alpha, or CD4/8T lymphocyte ratio between the intervention and control group (Ashida et al. 2019).

Two human pancreatic cancer cell lines (Panc-1 and Capan-1) and Pancreatic Stellate Cells (PSC) were treated with DHA, EPA and Lipidemin in combination with gemcitabine. Gemcitabine plus Omega-3 FA treatment inhibited proliferation and invasion of these pancreatic cancer cell lines and PSCs and reduced Platelet-derived growth factor (PDGF-BB) secretion from these cell lines (Haqq et al. 2016). In another study, treatment of the human pancreatic cancer cell line MIA PaCa-2 with different concentrations of Omega-3 FA (ALA) decreased cell viability depending on the time and increased the expression of Wnt Inhibitory Factor-1 (WIF1) which is a Wnt antagonist (Rahmani et al. 2019). Treatment of human pancreatic cancer cell line PANC-1 with DHA decrease cell viability, antiapoptotic Bcl-2 expression, DNA binding activity of NF-κB, phosphorylated IκBα level, phosphorylated STAT3 level, phosphorylation of Epidermal Growth Factor Receptor (EGFR), STAT3 DNA-binding activity, and CCND1 and survivin expression. In contrast DHA treatment of these cells increases DNA fragmentation, cleaved Caspase-3, apoptosis and proapoptotic Bax expression. In addition to these, DHA treatment results in exclusion of EGFR receptors from Lipid Rafts disrupting its downstream signaling in the cytoplasm (Park et al. 2018).

Studies in experimental animals have also supported the findings that Omega-3 FAs have anti-inflammatory, growth-slowing and proapoptotic effects for pancreatic cancer cells. Treatment of human pancreatic ductal epithelial cells and human pancreatic nestin-expressing cells and KRAS mutated forms of these cells with the Omega-3 FA resulted in reduced cell viability and proliferation while increase in cell cycle arrest and apoptosis. DHA and EPA treatment reduced total and phosphorylated forms of AKT, phosphorylated Forkhead Box O3 (FOXO3a) and phosphorylation and subsequent degradation of apoptotic signal BAD, whereas elevated levels of cleaved Caspase3. Feeding of KRAS B6 mice with Omega-3 FA enriched

diet resulted in less aggressive pancreatic cancers, reduced size of neoplastic lesions, decreased cell proliferation in these animals (Ding et al. 2018). In another study, suppression of mouse pancreatic carcinoma cell line PK03 growth in mice after the Omega-3 FA supplementation together with either soluble epoxide hydrolase inhibition or knockout indicated that Omega-3 FA epoxy metabolites play an important role in inhibiting pancreatic carcinogenesis. Therefore, authors suggested that, use of Omega-3 FA together with soluble epoxide hydrolase inhibition may be a highly effective approach for inhibiting pancreatic cancer (Xia et al. 2019).

6.6 Lung Cancer and Omega-3

Studies on the interaction between Omega-3 FA and lung cancer are predominantly based on *in vitro* research. Treatment of human Non-Small-Cell Lung Carcinoma cell lines A549 and H1299, and mouse lung cancer cell line LLC with 100 μM DHA for 24 h decrease the viability of these cells. Transfection of LLC cells with *mfat-1* gene which generate Omega-3 FAs from Omega-6 FAs results in a reduction in cell viability, invasion and metastasis. *In vivo* tumor xenograft models with these cells have significantly longer survival time and partial tumor regression. Among the DHA-derived eicosanoids, RvD1 is significantly elevated in exogenous DHA treatment and after the transfection with *mfat-1* gene in these cells. Treatment ofA549 cells with RvD1 elevates the expression level of miR-138-5p while downregulates Forkhead Box C1 (FOXC1) expression, Akt and Erk1/2 phosphorylation. This relationship between Omega-3, miR-138-5p and FOXC1 shows that epigenetic changes are also effective in the effects of Omega-3 FAs on cancer cells (Bai et al. 2019). Treatment of A459 cells with different concentrations of DHA for 24 h reduce cell proliferation, migration, invasion, growth, colony formation, Bcl-2, MMP9, Human Enhancer of Filamentation 1 (HEF1), VEGF, PI3K and catalase expressions and phosphorylated Akt level while increased the apoptotic rate, cleaved poly-ADP-ribose polymerase (PARP), Caspase-3 and Bax expression and ROS generation. These findings, as we have discussed in some previous publications, suggest that ROS derivatives may have important effectsin the process of Omega-3 FA leading cancer cells to death (Yin et al. 2017).

In another study, treatment of A549 cell line with 50 μg/ml DHA or 60 μg/ml EPA for 24 h decreased cell proliferation and autophagy, Beclin 1 (BECN1) expression, total Akt and mammalian Target of Rapamycin (mTOR) level while increased the Laktat Dehidrogenaz (LDH) activity, LC3-II/LC3-I ratio, p62 expression, p-Akt and p-mTOR (Yao et al. 2015). Treatment of A549 cells and H1299 cell lines with DHA reduced the viability, phospho-mTOR, phospho- Ribosomal Protein S6 Kinase B1 (S6K1) and phospho-Eukaryotic Translation Initiation Factor 4E Binding Protein 1 (4E-BP1) levels and inhibit the PI3K/Akt pathway through disrupting EGFR phosphorylation while increase Annexin V and TUNEL positivity, cleaved PARP level, sub-G1 arrest, LC3-II and p27 expression, phospho- AMP-Activated Protein Kinase (AMPK), phospho Eukaryotic Translation Initiation Factor 2,

Subunit 1 (EIF2S1) levels (Kim et al. 2015a, b). The results of these two studies show that the autophagy also plays an important role in the effect of Omega-3 on lung cancer cells. In the last study, treatment of A549, H1299 and human normal epithelial cell line BEAS-2B with increasing concentrations of MAG-DHA which contain 621 mg/g DHA, 107 mg/g DPA and 27 mg/g EPA for 48 h combined with Carboplatin resulted in inhibition of cell proliferation, invasiveness, phospho-EGFR and total EGFR level, ERK phosphorylation *in vitro*. Also, treatment with DHA inhibited the tumor growth in *in vivo* H1299 and A549 xenografts mice models (Morin and Fortin 2017).

6.7 Breast Cancer and Omega-3

Most randomized clinical studies related to Omega-3 FA intervention and cancer have been conducted on breast cancer. Some of the clinical trials of breast cancer and Omega-3 FA supplementation have been carried out for the manageability of side effects in patients receiving neoadjuvant aromatase inhibitor or chemotherapy. A prospective, multi-center, open-label, single-arm clinical trial evaluated the effects of hydroxytyrosol combined with Omega-3 FA and curcumin on CRP level and musculoskeletal symptoms in postmenopausal breast cancer patients receiving adjuvant hormonal therapy with elevated CRP levels. Three capsules containing EPA/DHA, hydroxytyrosol and curcumin, were administered to patients per day for 30 days. As a result, significant decrease in CRP level and basal pain severity index score, IFN-gamma levels and triglycerides were detected (Martínez et al. 2019). Examination of the effects of 4.3 g/day Omega-3 FA for 24 weeks on musculoskeletal pain in postmenopausal breast cancer patients receiving adjuvant Aromatase inhibitor therapy revealed that there is no significant difference in the mean pain severity scores between the study groups (Lustberg et al. 2018).

In a multi-center trial evaluated the effect of 3.3 g/day Omega-3 for 24 weeks on aromatase inhibitor induced musculoskeletal pain in postmenopausal women with hormone-sensitive breast cancer receiving adjuvant therapy for ≥90 days. Triglyceride levels of patients receiving Omega-3 FA supplementation was found to be decreased compared to control group. However, the authors reported that there is no difference between two groups in joint pain/stiffness score (Hershman et al. 2015). The effects of Omega-3 FA were evaluated in obese breast cancer patients with aromatase inhibitor-related arthralgia was evaluated in another trial. Intervention group was administered with 3.3 g/day (560 mg) EPA plus DHA for 24 weeks. Results of this study showed that significantly lower Brief Pain Inventory (BPI) pain scores and lower BPI average pain and pain interference scores were detected at 24 weeks in the intervention group compared with control group (Shen et al. 2018). 2.4 g/day Omega-3 supplementation in locally advanced breast cancer patients receiving neoadjuvant chemotherapy including adriamycin/cyclophosphamide followed by paclitaxel+/−trastuzumab resulted in an improvement in the xerostomia in intervention group (de la Rosa et al. 2019). Observation of the effect of

fatigue reduction diet rich in Omega-3 for 3-months on fatigue and sleep quality in breast cancer survivors indicated that improvements in the fatigue and sleep quality in the intervention group (Zick et al. 2017). Last trial evaluated the efficacy of low/high dose Omega-3 or Omega-6 FA for 6 weeks in reducing cancer associated fatigue in breast cancer survivors. Interestingly, compared with Omega-3-FA, Omega-6 FA supplementation led to decreased the expression levels of proinflammatory markers in the TNF-α signaling pathway including TNF Receptor Superfamily Member 1A and 1B (TNFRSF1A and 1B), TNF Alpha Induced Protein 2 (TNFAIP2), and Nuclear Factor Kappa B Subunit 2 (NFKB2).

Improvements in Brief Fatigue Inventory, Multidimensional Fatigue Symptom Inventory scores were also observed in the intervention group. However, Omega-3 FA group had a statistically significant decreases in IL-6, Prostaglandin E Synthase 2 (PTGES2), and IFNγ expression levels (Peppone et al. 2019). A systematic review of systematic reviews showed that Omega-3 FA significantly improve pain severity scores in aromatase inhibitor related arthralgia observed in breast cancer survivors (Kim et al. 2018). These studies show us that Omega-3 supplementation might be useful to improve the QOL of patients who receive chemotherapy or neoadjuvant aromatase inhibitor therapy and to manage the side effects of drugs such as fatigue, xerostomia, sleeping disorders and arthralgia.

An important area of breast cancer and omega-3 studies is the studies conducted to reduce breast density in high-risk patients. In a Phase II Pilot study two capsules each containing 465 mg/day EPA and 375 mg/day DHA were administered the premenopausal women without any invasive breast cancer history for 6 months. There was no significant changes in serum estradiol, progesterone, testosterone, CRP, adiponectin, leptin, insulin, TNF-α, resistin, Plasminogen Activator Inhibitor-1 (PAI-I), Hepatocyte Growth Factor (HGF), Nerve Growth Factor (NGF), and Monocyte Chemoattractant Protein-1 (MCP-1) tissue cytokines levels. Administration of EPA and DHA resulted in a decrease in Ki-67, and breast density. Proteomic analysis showed that a decrease in Bcl-2, Eukaryotic Initiation Factor 4E (EIF4E), fibronectin, Progesterone Receptor (PgR), Phosphorylated Proline-Rich Akt Substrate (PRAS40), Regulatory Associated Protein of mTOR (Raptor), Stearoyl-CoA Desaturase (SCD-1), and SMAD Family Member 3 (SMAD3) and an increase in phosphorylated 4E-BP1, Protein Kinase C alpha (PKC alpha), and Tuberous Sclerosis 2 (TSC2) in tumor tissue (Fabian et al. 2015). Oral administration of 4 g/day capsules (465 mg/g of EPA and 375 mg/g of DHA) to postmenopausal women with high breast density receiving or not Raloxifene 60 mg/day or 60 mg/day orally showed that increases in plasma DHA concentration is correlated with a decrease in absolute breast density in cases with BMI values over the 29 (Sandhu et al. 2016). Administration of 4 g/day capsules (1860 mg EPA + 1500 mg DHA) in women with high breast density receiving Raloxifene orally reduce Stearoyl-CoA Desaturase 16 and 18 (SCD-16 and 18) expression which has been found to be associated with breast density in obese women (Manni et al. 2017).

A meta-analysis of 13 studies examined the association of dietary Omega-3/Omega-6 ratio with breast cancer risk. This analysis showed that higher dietary intake Omega-3/Omega-6 FA ratio associated with lower breast cancer risk both in

Western and Asian countries (Nindrea et al. 2019). In another study the effect of Omega-3 on survival in locally advanced ductal invasive stage IIIB breast cancer, who receiving neoadjuvant cyclophosphamide, doxorubicin, and fluorouracil chemotherapy was evaluated. 1 g/day Omega-3 FA in an oil-fish capsule were given to intervention group for 51 days. As a result of this study it was shown that Ki-67 and VEGF expression was decreased while overall survival with disease-free survival was increased in the intervention group compared to control group (Darwito et al. 2019). Considering the results of these studies, it can be concluded that Omega-3 supplementation may have a protective effect in reducing the breast tissue density of high-risk patients and also may contribute to the prolongation of the survival of patients receiving chemotherapy.

Some of the breast cancer Omega-3 FA studies are studies conducted on the reduction of inflammation in patients. Patients were randomized to 4 six ounce servings of fish per week or ~ 1.68 g/day of EPA + DHA for three months. There is no significant difference of COX-2, CD68, and IL-6 expression in breast adipose tissues both groups (Straka et al. 2015). In another study, 2 g/day fish oil containing 1.8 g of Omega-3 FA was administered to treatment naïve breast cancer patients for 30 days in the intervention group. The percentage of CD4+ T lymphocytes and CRP levels were found to be stable in fish oil arm while there was an increase in CRP and a reduction in the percentage of CD4+ T lymphocytes were observed in control group. Also, there were no significant changes in serum proinflammatory cytokine and prostaglandin E2 levels between the two groups (Paixão et al. 2017). Administration of 1000 g/day DHA for 12 weeks did not altered the breast tissue levels of TNFα, COX-2, IL1β and aromatase in obese patients with breast cancer history or benign proliferative breast disease (Gucalp et al. 2018). The results of the limited number of published studies indicate that Omega-3 FA does not show the expected effect in reducing inflammation in breast cancer patients.

Depending on their receptor positivity, breast cancers can be classified as estrogen receptor positive (ER+), progesterone receptor positive (PR+) and HER2 positive and triple negative. Depending on the positivity for these receptors, breast cancer cells differ in terms of biological processes and serve as markers for the treatment to be applied (Zhang et al. 2018). Treatment of HER2-overexpressing human breast ductal carcinoma cell line BT-474 with DHA reduce cell growth with and without trastuzumab decreasing the Akt and MAPK phosphorylation (Mason et al. 2015). Epidermal growth factor (EGF) increase cell migration and invasion through phosphorylation of EGFR and HER-2 as well as of C-Jun N-Terminal Kinase 2 (JNK2), ERK1/2, and Akt, Plasminogen Activator, Urokinase (PLAU) and MMP-9 expression in HER2 overexpressing human mammary adenocarcinoma cells line SK-BR3 and these changes were reversed by pretreatment with DHA (Li et al. 2015). The results of these studies show that Omega-3 suppresses the expression of both growth factor-activated signaling pathways and genes associated with invasion and metastasis in HER2 overexpressing breast cancer cells.

Treatment of insulin induced ER+ metastatic human mammary adenocarcinoma cell line MCF-7 cells with Omega-3 resulted in attenuate cell proliferation. Erk1/2 and Akt phosphorylation reduced by EPA and DHA stimulation (Guo et al. 2017).

Treatment of MCF-7 cells with DHA inhibits expression of Sterol Regulatory Element-Binding Protein-1a (p-SREBP-1, m-SREBP-1), and Fatty Acid Synthase (FASN) incuced by estradiol and insulin induced and decrease pAkt/Akt and pS6/S6 ratios, inhibited MCF-7 cell proliferation. (Huang et al. 2017). Treatment of MCF-7 cells with DHA results in inhibition of the proliferation of the MCF-7 cell, increase early apoptotic cells through increasing the levels of cytoplasmic cytochrome c and Smac/Diablo, cleaved Caspases-8, −9 and −3, Bax, Fatty Acid Synthase (FASN), TNF Receptor Superfamily Member 10a (DR4), and TRAIL and reducing the Bcl-2 (Xue et al. 2017). DHA obtained from Antarctic krill increase the accumulation and interaction of CD95 and Caveolin-1 (CAV1), reduce migration and invasion of MCF-7 cells and down-regulates MMP2 expression through the FAK/SRC/PI3K/AKT signaling cascade (Zheng et al. 2018). DHA suppresses cell invasion and 12-O-tetradecanoylphorbol-13-acetate (TPA) induced MMP-9 activation through increasing PPAR-γ expression and modulation of MAPK signaling pathway in MCF-7 cells (Hwang et al. 2017). The findings of these studies show that Omega-3 administration in ER+ breast cancers suppresses ER-mediated FASN and PPAR synthesis, increases the expression of proapoptotic genes, increases the expression of antiapoptotic genes, and blocks cell proliferation-related signaling pathways.

Treatment of highly metastatic triple negative human mammary adenocarcinoma cell line MDA-MB-231 with DHA increase the subG1 cell population, decrease the cell growth, invasion and motility through triggering PARP cleavage, Caspase-3 activity and inhibiting Cyclo Oxygenase 2 (COX-2) and NF-κB signaling, translocation of β-catenin to the nucleus and MMP expression (Yun et al. 2016). DHA treatment of docetaxel-treated MDA-MB-231 cells shows that decreased PKCε and δ expression levels in cell membrane and nuclear fractions, resulting in diminished ERK1/2 phosphorylation and Akt pathway and increased the activity of docetaxel (Chauvin et al. 2016). Treatment of MDA-MB-231 cells with DHA decrease cell viability, pro-caspase-1 and pro-1IL-1b expression levels while induces pyroptosis programmed cell death increasing of NF-κB and High Mobility Group Box 1 (HMGB1) nuclear-cytoplasmic translocation Apoptosis-Associated Speck-Like (ASC) and Gasdermin D (GSDMD) expression, IL-β secretion (Pizato et al. 2018). Co-treatment of MDA-MB-231 cells with DHA and Delta-T3 reduce lipid droplet biogenesis and potentiate lipophagy and inhibit breast cancer malignancy (Pizato et al. 2019). These findings show that Omega-3 FA administration in triple negative breast cancers blocks the Wnt / beta-catenin signaling pathway, growth factor-activated signaling pathways and proinflammatory pathways, while activating pro-apoptotic pathways and lipolysis pathways.

Omega-3 FA ethyl esters inhibit survival both in the MCF-7 and MDA-MB231 breast cancer cell lines through inhibiting NF-kB signaling pathway (Chen et al. 2016). EPA, DHA eicosapentaenoyl-ethanolamine (EPEA) and docosahexaenoyl-ethanolamine (DHEA) treatment of breast cancer cell lines MCF-7 and MDA-MB-231 inhibited cell proliferation, migration and invasion through reducing the levels of phosphorylated forms of p38 MAPK, ERK and JNK, and the total levels of integrin β3 (ITGB3), MMP-1 and VEGF (Brown et al. 2020). MDA-MB-231

cells showed increased levels of alkaline phosphatase (ALP), RUNX Family Transcription Factor 2 (Runx2), and Osterix (OSX) and elevated ALP activity compared to less metastatic MCF-7 cells. DHA treatment blocks the expression of these osteoblast differentiation transcription factors in breast cancer cell lines (Sharma et al. 2020). Combination of retinoic acid and Omega-3 FAs caused an increase in autophagy increasing the LC3II/β-actin ratio and activating the FFAR1/Gαq/p38 MAPK signaling in breast cancer cell lines MCF-7, MDA-MB-231 and SKBR-3 (Zhu et al. 2017). DHA treatment induces cell death in highly transformed breast cell line SK-BR-3, through reducing ERK1/2 and STAT3 phosphorylation while only slightly affects these processes in MCF-7 breast cell line with lower degree transformation (Rescigno et al. 2016). These findings show that Omega-3 FA administration activates autophagy pathways while blocking vascular pathways in both ER + and triple negative cancer cell lines.

DHA increase the small RNA content of exosomes derived from breast cancer cell lines and increases exosome secretion from these cells. miR-23b, miR-27b, and miR-320b carried by exosomes have been recieved by endothelial cells and inhibits endothelial tube formation through reducing the expression of Angiomotin Like 1 (AMOTL1), ETS Proto-Oncogene 2, Transcription Factor (ETS2), Neuropilin 1 (NRP1) and PLAU expression. This finding shows that Omega-3 FA applied cells also trigger certain epigenetic changes (Hannafon et al. 2015). Treatment of MCF-7 and triple negative human mammary carcinoma cell line Hs578T cells with DHA increase ROS level, increase nuclear translocation of Nrf2 through PI3K/Akt signaling pathway and induce Oxidative Stress-induced Growth Inhibitor 1 (OSGIN1) expression, Bax/Bcl ratio and cytocrome-c release from mitochondria. This finding indicates that Omega-3 acts through FFAR receptors (Tsai et al. 2017). However, another study found that this effect may not be FFAR mediated. Knockdown of FFAR4 which is overexpressed in breast cancer cells reduce cell growth, supports Omega-3 FA induced cell growth inhibition and increase apoptosis indicating that Omega-3 FA also induce the apoptosis of breast cancer cells independently of FFAR4 (Zhu et al. 2018).

Combination of Omega-3 FAs and All-Trans Retinoic Acid (ATRA) exhibited synergistic inhibition of cell growth and increase of cell cycle arrest and cell apoptosis in three human breast subtype cancer cell lines (ER+ MCF7, HER2+ SK-BR-3, Triple negative HCC1806 and MDA-MB-231 cells) through decreasing Bcl-2 levels and increasing the cleaved-PARP level (Lin et al. 2017). Coadministration of fish oil with PD98059 or LY294002 to tamoxifen-resistant breast cancer cell lines MCF-7/TamR cooperatively reduce cell migration (Davison et al. 2018). These two studies show us that the administration of Omega-3 FA together with drugs that cause breast cancer cells to apoptosis can increase the effectiveness of drugs. DHA inhibits Gremlin 1 (GREM1) expression which is a BMP antagonist and GREM1-induced EMT via ERK activation in triple negative human mammary cancer cell line MDA-MB-453 and Hs578T (Sung et al. 2020). DHA also inhibits 12-O-tetradecanoylphorbol-13- acetate (TPA) induced Fascin-1 (FSCN1) related migration of breast cancer cells by suppressing the PKCδ- and Wnt-1/β-catenin-mediated cellular pathways (Lii et al. 2016).

Studies in experimental animal models also support the findings obtained as a result of studies in cell lines. In animal studies it was proposed that, maternal fish oil enriched diet or endogenously synthesized Omega-3 FAs promotes the apoptosis pathways and inhibits NF-κB and JAK-STAT signaling pathways and results in reduced risk mammary tumor of female offsprings (Li et al. 2018, 2019). Combined treatment of triple negative drug resistant breast cancer patient derived xenografts in experimental mice models with docetaxel plus DHA showed that, synergistic effects of two compounds in increasing the expression of proapoptotic proteins Receptor Interacting Serine/Threonine Kinase 1 (RIPK1) and BH3 Interacting Domain Death Agonist (BID), and decreasing the proliferation marker Ki67 and anti-apoptotic proteins Bcl-2, PARP and BIRC5 (Newell et al. 2019). The fish oil-fed rats adipokine profile is associated with reduced inflammation and the reduced M1/M2 macrophage ratio compared to the rats fed with the Western diet. Conditioned media of primary rat adipose tissue obtained from rats fed with the Western diet increased stem cell self-renewal compared to conditioned media of fish oil fed rat adipose tissue (Hill et al. 2019).

6.8 Head and Neck Cancers and Omega-3

In a multi-centre, Phase-II study 1500 mL immune modulating enteral nutrition formula containing 3.4 g/L EPA + DHA was infused to head and neck or esophageal cancer patients who underwent radiochemotherapy. ROS production were found to be increased in polymorphonuclear cells and pro-inflammatory prostaglandin-E2 production was found to be low in the patients administered with immune modulating enteral nutrition formula compared to standard formula. Transcriptomic analysis in this case showed an increase in the expression of genes encoding the immune receptors, antioxidant enzymes and NADPH oxidase subunits (Talvas et al. 2015). In another study, head and neck squamous cell cancer patients about to start any antineoplastic therapy were given 2 g/day EPA for 6 weeks, starting two weeks before the start of treatment. Results of this study showed that TNF-a, IL-1b, IL-6, and IFN-g, lymphocytes, triglycerides, and LDL concentrations decreased while HDL levels were decreased in the intervention group. Also, it was observed that the weight of the patients in the intervention group remained stable, but the patients in the control group lost weight (Solís-Martínez et al. 2018).

In the study conducted by Hanai et al., 480 ml/d EPA-enriched oral nutritional supplement for a total of 28 days, starting 14 days before surgery to head and neck cancer patient. There were no significant differences were observed in body weight, lean body mass, albumin and prealbumin postoperative complications, inflammatory marker level parameters between intervention and control groups (Hanai et al. 2018). Omega-3 enriched nutritional supplementation for a total of 15 days, starting 3 days before the administration of cisplatin-based chemotherapy to esophageal cancer patients given orally resulted significantly reduced stomatitis, aspartate aminotransferase and alanine aminotransferase levels in the intervention group

compared to control group (Miyata et al. 2017). In the study of Miyata et al., esophageal cancer patients supplemented with EPA, GLA and antioxidants enriched formula after surgery. Alterations in the body weight and lean body weight ratios were found to be significantly greater in the intervention group (Matsuda et al. 2017). However, EPA enriched supplementation for 4 weeks (2 g/440 ml) in oral cavity cancer patients who received antineoplastic pretreatment, did not resulted in a difference in terms of CRP and CRP/albumin ratio between the two groups (Carvalho et al. 2017). Treatment of oesophageal cancer cell lines OE19 and OE33 with EPA and DHA resulted in a decrease in cell growth, VEGF, IL-6, TNF-α and p53 protein expression while an increase in antiproliferative p21 expression (Eltweri et al. 2018). These studies show that Omega-3 FA supplements can be applied in minimizing weight loss and inflammation and treatment-induced toxic effects observed during treatment in head and neck cancer patients.

7 Clinical Trials

42 clinical trials were seen as a result of the screening performed with the terms 'omega-3' and 'cancer' at www.clinicaltrials.gov on 03.09.2020. The status of these works was: recruiting 24, 16 active but not recruiting and 2 enrolling by invitation. Phase levels of these studies were as follows; Early Phase 1 (3), Phase 1 (1), Phase 1 and 2 (3), Phase 2 (9), Phase 3 (4), Phase 4 (2). The most studied cancer types in the field of Omega-3 and cancer are as follows; colorectal cancer (6), breast cancer (13), prostate cancer (6), gastrointestinal system and pancreas (6), leukemia and lymphoma (2) and head and neck cancers (2). Details of ongoing clinical studies are shown in Table 22.1.

8 Conclusion

In this chapter, we have summarized in detail the Omega-3 relationship with head and neck cancers, lung, breast, pancreas, prostate, gastrointestinal system, colorectal cancers and leukemias. When we evaluate the results of clinical studies, it seems that Omega-3 can be used for the purposes of reduction of cancer risk in high risk patients, immune-modulation, reduction in infectious complications and chemotherapy-related toxicity and side effects, weight stabilization, improvement of the QOL, fatigue and sleep quality, overall survival, control of pain, effect of the chemotherapy in cancer patients. When we evaluate the molecular studies conducted in this field, we can see that Omega-3 has an effect on cellular processes such as apoptosis, cell cycle, growth factor signaling pathways, autophagy, invasion, migration, metastasis, pluripotency, vascularization, and inflammation in different cancer cell lines. In some studies, we see that these effects may differ from each other in histological or genetic subgroups of cancers that develop in a particular

Table 22.1 Active and recruiting clinical trials on omega-3 and cancer

NCT Number	Status	Study title	Conditions	Phase
NCT04268134	Recruiting	Altering lipids for tolerance of aromatase inhibitor therapy	Breast cancer	Phase 2
NCT04216251	Active, not recruiting	PRevention using EPA against coloREctal cancer	• Colorectal, adenoma, colorectal cancer, endoscopic surgery, eicosapentaenoic acid, gastrointestinal microbiome	Phase 1 Phase 2
NCT04209244	Recruiting	Effect of fish oil on hyperlipidemia and toxicities in children and young adults with acute lymphoblastic leukemia	Leukemia, acute lymphoblastic	–
NCT04184713	Active, not recruiting	Evaluation of the effect of an specific oral nutritional supplement on the nutritional status in cancer and malnutrition	• Malnutrition, cancer, nutrition, related cancer	–
NCT04027088	Recruiting	Effect of preoperative immunonutrition in upper digestive tract	• Immunonutrition, gastric cancer, esophageal cancer, pancreas cancer, surgery—complications	–
NCT04001543	Recruiting	Efficacy of an oral immunomodulatory nutrient on survival during postoperative concomitant chemoradiotherapy in head and neck cancer	Head and neck squamous cell carcinoma	–
NCT03994055	Recruiting	Effect of an anti-inflammatory diet on patients with cervical cancer	• Cervical cancer, uterine, cervical neoplasm, pelvic, inflammatory disease, radiation toxicity, diet modification	–
NCT03961685	Recruiting	Cancer activity and lifestyle measurement study	Breast cancer	–
NCT03831698	Recruiting	Omega 3 fatty acids in colorectal cancer (CRC) prevention in patients with lynch syndrome (COLYNE)	• Colorectal cancer Lynch syndrome	Phase 2

(continued)

Table 22.1 (continued)

NCT Number	Status	Study title	Conditions	Phase
NCT03831178	Recruiting	Docosahexaenoic Acid (DHA) for women with breast cancer in the neoadjuvant setting	Breast cancer	Phase 2
NCT03824652	Recruiting	WALNUTS for POWER: polyphenols, Omega-3 fatty acids, weight loss, and EneRgy	Prostate cancer	Phase 2
NCT03806426	Recruiting	Effect of EPA-FFA on polypectomy in familial adenomatous polyposis	Familial adenomatous polyposis	Phase 3
NCT03753334	Active, not recruiting	Effects of EPA in men with biochemical recurrence or progression of prostate cancer.	Prostate cancer	Phase 2
NCT03720158	Enrolling by invitation	Enteral omega 3 during radiotherapy to improve the quality of life and functionality of head and neck cancer patients	• Head and neck squamous cell carcinoma • Quality of life Radiotherapy; complications	–
NCT03665714	Recruiting	Effects of Immunonutrition (Impact Oral®) in patients undergoing surgery for gastrointestinal cancer	Gastrointestinal cancer	–
NCT03661047	Recruiting	OMega-3 fatty acid for the immune modulation of colorectal cancer	Colon cancer	Phase 2
NCT03598309	Recruiting	Phase II trial to modulate intermediate endpoint biomarkers in former smokers	• Lung diseases Lung cancer, Protection against	Phase 2
NCT03516253	Recruiting	Fish oil and EPO in breast cancer	Breast cancer	
NCT03428477	Recruiting	EPA for Metastasis Trial 2	• Liver metastasis Colon cancer	Phase 3
NCT03416777	Recruiting	Meat-based versus Pesco-vegetarian diet and colorectal cancer	• Nutrition aspect of cancer • Diet modification Cancer of colon	–
NCT03387098	Active, not recruiting	QUILT-3.070:pancreatic cancer vaccine: subjects with pancreatic cancer who have progressed on or after standard-of-care therapy	Pancreatic cancer	Phase 1 Phase 2

(continued)

Table 22.1 (continued)

NCT Number	Status	Study title	Conditions	Phase
NCT03383835	Active, not recruiting	Study of moderate dose omega 3 fatty acid supplement in premenopausal women at high risk for breast cancer	Breast cancer	–
NCT03329248	Active, not recruiting	QUILT-3.060: NANT Pancreatic Cancer Vaccine: molecularly informed integrated immunotherapy in subjects with pancreatic cancer who have progressed on or after standard-of-care therapy	Pancreatic cancer	Phase 1 Phase 2
NCT03260231	Recruiting	Dietary milled seed mix in patients with non-Hodgkin lymphoma	Non-hodgkin lymphoma	–
NCT03204266	Active, not recruiting	Oral supplementation to enhance recovery pilot study	Malignant neoplasms of urinary tract	Early Phase 1
NCT03151291	Recruiting	Effects of WB-EMS and specific dietary supplements on cancer patients	Cancer Cachexia Weight Loss Muscle Weakness	–
NCT03031821	Recruiting	Metformin in patients initiating ADT as prevention and intervention of metabolic syndrome	• Prostate cancer Metabolic syndrome	Phase 3
NCT02996240	Active, not recruiting	Breast, omega 3 free fatty acid, ph 0	Breast cancer	–
NCT02831582	Recruiting	Omega-3 supplementation in prevention of aromatase inhibitor-induced toxicity in patients with stage I–III breast cancer	• Arthralgia Breast neoplasms	Phase 2
NCT02681601	Recruiting	Nutrition support to improve outcomes in patients with unresectable pancreatic cancer	Cancer of pancreas	–
NCT02670161	Enrolling by invitation	Quality improvement and practice based research in neurology using the EMR	• Brain tumors • Epilepsy • Migraine	Phase 4
NCT02538484	Recruiting	Impact of omega 3 fatty acid supplementation on aromatase in obese subjects	Breast cancer	Early Phase 1

(continued)

Table 22.1 (continued)

NCT Number	Status	Study title	Conditions	Phase
NCT02516540	Recruiting	Efficacy of lifestyle intervention in BRCA1/2 mutation carriers	Hereditary breast and ovarian cancer	
NCT02333435	Active, not recruiting	Effects of EPA on prostate cancer cells proliferation and quality of life	Prostate cancer	Phase 2
NCT02295059	Active, not recruiting	Omega 3 fatty acids and ERPR(−)HER2(+/−) breast cancer prevention	Breast cancer	–
NCT02278965	Active, not recruiting	Metformin and omega-3 fatty acids in woman with a history of early stage breast cancer	• Stage 0 breast carcinoma • Stage I, Stage II, Stage III breast carcinoma	Phase 1
NCT02176902	Recruiting	Low-fat diet and fish oil in men on active surveillance for prostate cancer	• Adenocarcinoma of the prostate • Stage I, Stage IIA, Stage IIB prostate cancer	–
NCT01881048	Active, not recruiting	Window of opportunity study targeting the inflammatory Milieu	• Stage IA, Stage IB, Stage II, Stage IIIA, Stage IIIB, Stage IIIC, Stage IV Breast cancer	Early Phase 1
NCT01733147	Active, not recruiting	Modulation of esophageal inflammation in Barrett's Esophagus by omega-3 fatty acids	• Barrett's esophagus Obesity	Phase 4
NCT01653925	Active, not recruiting	Molecular mechanisms of Dutasteride and dietary interventions to prevent prostate cancer and reduce its progression	• Prostatic neoplasms Low grade prostate cancer	–
NCT01478477	Active, not recruiting	Omega-3 fatty acids in preventing joint symptoms in patients with stage I–III breast cancer receiving Anastrozole, Exemestane, or Letrozole	• Recurrent breast cancer • Stage IA, Stage IB, Stage II, Stage IIIA, Stage IIIB, Stage IIIC breast cancer	–
NCT01169259	Active, not recruiting	Vitamin D and Omega-3 Trial (VITAL)	• Cancer Cardiovascular disease	Phase 3

tissue. However, when we look at the clinical studies conducted, we see that in most of these studies, the genetic subgroups of cancers were ignored. The reason why contrasting results were obtained in clinical studies in some cancer types may be due to the fact that the patients included in the study were not very homogeneous. In addition, polymorphisms or haplotypes in genes that metabolize Omega-3 fatty acids may differ in different populations. From the perspective of individualized medicine, it may be useful to consider these parameters in future studies.

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