Evidence-based Anticancer Complementary and Alternative Medicine 5

William C.S. Cho *Editor*

Cancer Chemoprevention and Treatment by Diet Therapy

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Preface

Subsequent to our previous volume entitled *Evidence-based Non-pharmacological Therapies for Palliative Cancer Care*, this volume steps forward to gather leading oncologists, physicians, and scientists in the field to discuss the application of diet therapy for the prevention and treatment of cancer. This volume represents an extensive collection of researches on diet therapy for cancer. We focus on providing resources and ideas of good diet help in cancer prevention and treatment based on scientific evidences and clinical trials. A substantial proportion of the materials in this book are published and unpublished findings of the authors. Some parts are reviews derived from published studies of other clinicians and scientists. This book consists of ten chapters presenting the research evidence relevant to the application of a range of commonly used dietary natural compounds and foods in cancer prevention and treatment, including resveratrol, flavonoids from fruits and vegetables, flaxseed oil, green tea, soy food, lycopene-rich foods, antioxidant-rich foods, and Mediterranean diet. An overview of the modulation of proteasome pathways by nutraceuticals is also covered. In addition, the attenuation of multifocal cell survival signaling by bioactive phytochemicals in the prevention and therapy of cancer is included as well. Although written primarily for medical and scientific professionals, this book can be used as a useful reference to cancer patients and those who are interested in diet therapy for cancer. We hope that this book will provide a resource to advocate the best diet therapy for cancer patients. Our goal is that the ideas in this book will help support cancer treatment, while also nurturing the sense of cancer prevention for a lifetime.

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Chapter 1
Effect of Dietary Resveratrol in the Treatment of Cancer

Pragya Srivastava, Varun Vijay Prabhu, Neelu Yadav, Raghu Gogada, and Dhyan Chandra

Abstract Resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring phytoalexin, is found in natural foods and food products such as grapes, peanuts, berries, and red wine. In recent years, resveratrol has gained considerable attention for its anticancer activities across multiple cancers. The anticancer effects of resveratrol are multifaceted and involve regulation of diverse cellular signaling, including apoptotic and non-apoptotic cell death, cell survival, and cell cycle progression. Anticancer activities of resveratrol are associated with engagement of AKT/PKB pathway, NF-κB pathway, death-receptor and TRAIL-induced apoptosis, and induction of p21/p27, as well as downregulation of cyclins and cyclin-dependent kinases. Resveratrol has been shown to have synergistic effects with radiation therapy. Resveratrol increases the therapeutic index of anticancer agents by sensitizing cancer cells to apoptosis. In preclinical studies, evidence gathered from mouse models suggests that resveratrol has beneficial effects against several types of human cancers and has been shown to inhibit or attenuate the initiation and progression of skin, prostate, colon, and breast cancers. Although resveratrol is widely considered to be well-tolerated in clinical trials for the treatment of human cancers, a limited number of studies have so far been conducted in human population because of poor bioavailability. These preliminary studies in human clinical trials have yet to recapitulate the physiological benefits seen in mouse models. This chapter provides a comprehensive description of resveratrol-mediated modulation of cellular signaling pathways, which may be exploited to develop further strategies for cancer therapy.

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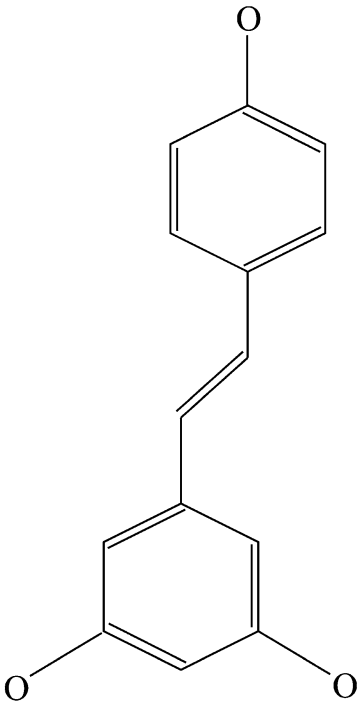

Fig. 1.1 Chemical structure of *trans*-resveratrol

1.1 Introduction

Although natural bioactive compounds in fruits and vegetables (Johnson 2007; Shanmugam et al. 2011) possess cancer preventive effects, there is an increasing interest to use them as potential cancer therapeutics. Multiple naturally bioactive compounds have been implicated in targeting different stages of carcinogenesis such as initiation, transformation, proliferation, invasion, metastasis and angiogenesis (Reddy et al. 2003; Dorai and Aggarwal 2004; Sarkar and Li 2006; Ichikawa et al. 2007). Resveratrol (3,5,4′-trihydroxystilbene), a naturally occurring phytoalexin, is found in several dietary items, e.g. red wine, grapes, peanuts, and berries (Fig. 1.1 and Table 1.1). Plants produce resveratrol as a result of natural physiological response to stress conditions, such as infection by the pathogen *Botrytis cinerea* (Delmas et al. 2006), vicissitudes in climate, exposure to ozone, sunlight and heavy metals (Bavaresco 2003). Resveratrol was first isolated from the roots of white hellebore (*Veratrum grandiflorum* O. Loes) and subsequently from the dried roots of *Polygonum cuspidatum*. It has traditionally been used as an anti-inflammatory agent. Other beneficial properties of resveratrol include antioxidant effects, cardioprotection and life span extension (Gupta et al. 2011; Iuga et al. 2012; Magyar et al. 2012). Resveratrol belongs to the stilbene class of polyphenolic compounds and exists in two isoforms; *trans*-resveratrol and *cis*-resveratrol (Baur

Table 1.1 Resveratrol content in various natural food products

Source	Amount	Reference
Fresh grape skin	>100 μg/g	Li et al. (2006)
Red wine	1–25 μmol/L	Gu et al. (1999)
Roasted peanuts	0.06 ppm	Sobolev and Cole (1999)
Boiled peanuts	5.1 ppm	Sobolev and Cole (1999)
Whole berry extract	7–5,800 ng/g	Rimando et al. (2004)

and Sinclair 2006; Gupta et al. 2011). Major form of resveratrol in plants is the glycosylated (3-*O*-β-*D*-glucosides) form (also known as piceid form). Upon glycosylation, resveratrol is protected from oxidative degradation, which is more stable and more soluble. The human gastrointestinal tract can readily absorb glycosylated resveratrol (Regev-Shoshani et al. 2003). Upon absorption, it is metabolized by liver phase 2 drug-metabolizing enzymes to water-soluble metabolites, which are primarily excreted in the urine (Walle et al. 2004). In the plasma, resveratrol has a half-life of 8–15 min whereas the metabolites have a half-life of 9.2 h (Walle et al. 2004).

Resveratrol modulates multiple cellular signaling pathways including cell death, cell cycle progression, and cell proliferation in tumor cells, thereby exerting its anticancer effects (Aggarwal et al. 2004; Harikumar and Aggarwal 2008; Shakibaei et al. 2009). This chapter will provide an overview of anticancer effects of resveratrol and its underlying molecular mechanisms.

1.2 Molecular Mechanisms of Resveratrol-induced Cancer Cell Death

1.2.1 Apoptosis Signaling

Resveratrol exerts anticancer effects either by inducing apoptosis or by inhibiting survival signaling in many different human cancers. Apoptosis is a multistep process culminating into the activation of caspases. Caspases are activated *via* death-receptor mediated signaling or mitochondrial signaling (Ashkenazi and Dixit 1998; Shi 2002, 2004). In either case, the cytochrome c released from the mitochondria interacts with cytosolic adapter protein, apoptotic protease activating factor 1 (Apaf-1) to form the apoptosome leading to the recruitment and activation of caspase-9, which activates effector caspases such as caspase-3 and caspase-7 to execute apoptosis (Li et al. 1997; Zou et al. 1999). The proteolytic activities of caspases are inhibited by the increased expression of inhibitors of apoptotic proteins (IAPs) in cancer cells (Schimmer 2004). For instance, active caspase-9 and caspase-3 are inhibited by XIAP and other IAPs (including cIAP1 and cIAP2) and survivin leading to the inhibition of the caspase cascade and apoptosis (Deveraux et al. 1998; Srinivasula et al. 2001; Shi 2002). However, Smac, another proapoptotic protein is released from the mitochondria and binds with IAPs to promote apoptotic cell death (Deveraux et al. 1998; Du et al. 2000;

Srinivasula et al. 2001). Release of proapoptotic proteins from mitochondria is tightly regulated by proapoptotic and antiapoptotic Bcl-2 family proteins (Danial 2007; Brunelle and Letai 2009). Proapoptotic BH3-only proteins (such as Bim and Bid) bind with antiapoptotic proteins (including Bcl-2 and Bcl-xL) to allow proapoptotic multidomain proteins Bax/Bak to form channels on the mitochondrial membrane (Willis et al. 2007; Merino et al. 2009). Bim and Bid can also directly activate Bax/Bak leading to the permeabilization of mitochondria (Merino et al. 2009; Gavathiotis et al. 2010).

1.2.2 Resveratrol and Death-receptor Signaling

Multiple evidences suggest the involvement of death-receptor signaling in resveratrol-mediated apoptosis. For example, resveratrol induces redistribution of FAS/CD95 in the rafts and triggers death receptor-mediated apoptosis (Clement et al. 1998; Delmas et al. 2003), which causes sensitization of colon cancer cells to death-receptor agonists (Delmas et al. 2004). Resveratrol triggers FAS-dependent apoptosis in HL-60 promyelocytic leukemia cells and T47D breast carcinoma cells (Clement et al. 1998). Resveratrol enhances expression of FAS/CD95, and thus causes induction of death receptor-mediated apoptosis (Ko et al. 2011). Inhibition of death-receptor signaling using dominant negative FADD leads to the inhibition of resveratrol-induced apoptosis (Reis-Sobreiro et al. 2009), thus suggesting the involvement of death-receptor signaling in resveratrol-induced apoptotic cell death in cancer. In contrast, multiple reports also indicate that death-receptor is not involved in resveratrol-induced apoptotic cell death (Bernhard et al. 2000; Dorrie et al. 2001; Wang et al. 2003). We have recently demonstrated that mitochondria-mediated apoptosis is an initiating event in resveratrol-induced apoptosis (Gogada et al. 2011), however, existence of death-receptor mediated apoptosis in some cancer cell types or additive effect of death-receptor pathway could not be ruled out.

1.2.3 Resveratrol Targets Mitochondria to Induce Apoptotic Cell Death

Resveratrol-induced mitochondrial apoptosis has been reported to be a p53-dependent mechanism in various types of cells. For example, resveratrol induces expression of p53 (Hsieh et al. 1999; She et al. 2001), which is known to activate Bax and Bak resulting into permeabilization of the outer mitochondrial membrane. Resveratrol also causes acetylation (Kai et al. 2010) and phosphorylation of p53 (Shih et al. 2004) leading to the expression of proapoptotic genes, and thus enhancing apoptosis. We have recently demonstrated that resveratrol-induced apoptosis does not require p53-dependent activation of Bax and Bak, suggesting

that p53-dependent apoptosis may not play critical role in response to resveratrol in some cancer cell types (Gogada et al. 2011). Multiple other reports also suggest that resveratrol induces p53-independent apoptosis in cancer cells (Mahyar-Roemer et al. 2001; Chow et al. 2010b; Pavet et al. 2010). In both p53-dependent and p53–independent scenarios, release of cytochrome c is critical for resveratrol-induced mitochondrial apoptosis. It has been reported that resveratrol induces Bax/Bak-dependent apoptosis. Although multiple mechanisms could be involved in the activation and oligomerization of Bax/Bak, our recent findings have identified that antiapoptotic protein, XIAP plays an important role in Bax-mediated cytochrome c release during resveratrol-induced apoptosis (Gogada et al. 2011).

1.2.4 Importance of Other Cellular Signaling in Resveratrol-induced Death of Cancer Cells

In addition to death-receptor and mitochondria-mediated apoptosis, resveratrol also activates multiple signaling pathways to induce cancer cell death. For example, resveratrol sensitizes cancer cells to apoptosis upon ceramide accumulation. Increase in growth inhibitory/proapoptotic ceramide has been associated with the anti-proliferative effects of resveratrol in colon cancer cells (Ulrich et al. 2007). Pretreatment of human prostate cancer cells with resveratrol inhibits growth and induces apoptosis *via* ceramide accumulation (Scarlatti et al. 2007). Similarly, in metastatic breast cancer cells (MDA-MB-231), resveratrol induces apoptosis with concomitant ceramide accumulation (Scarlatti et al. 2003).

Resveratrol treatment causes decreased expression of proteins critical for hedgehog signaling, and thus inhibits pancreatic cancer cell survival (Mo et al. 2012). Resveratrol and its dimers inhibit the activity of sphingosine kinase 1, which leads to reduced survival of breast cancer cells (Lim et al. 2012). Resveratrol inhibits AKT/PKB survival signaling and induces expression of DR5, which induces TRAIL-mediated apoptosis, suggesting that dual role (i.e. inhibition of AKT survival signaling and induction of DR5) may play a role in cancer therapy (Hussain et al. 2011). Proteome analysis of colon cancer cells upon resveratrol treatment demonstrates the suppression of pentose phosphate pathway, a metabolic pathway for cell cycle progression, and thus leading to the inhibition of cell growth and induction of apoptosis (Vanamala et al. 2011). Activation of caspase-2 has also been shown to activate both Bax/Bak-dependent and -independent apoptosis in response to resveratrol treatment (Mohan et al. 2006). Resveratrol impairs survival signaling in multiple myeloma by targeting the endoplasmic reticulum (ER) stress response component inositol-requiring enzyme 1α (IRE1α)/X-box binding protein 1 (XBP1) (Wang et al. 2011). Thus, not only mitochondria, but ER, calcium, and calpain are also involved in resveratrol-induced apoptotic cell death (Park et al. 2007; Sareen et al. 2007). Resveratrol also targets mitochondrial respiration to

induce apoptotic cell death in leukemic cancer cells (Low et al. 2010). Additionally, resveratrol also induces lysosomal cathepsin D-dependent apoptosis in colorectal cancer cells (Trincheri et al. 2007, 2008). These studies clearly demonstrate that resveratrol induces apoptotic cell death in cancer cells *via* death-receptor, mitochondrial, and multiple other apoptotic signaling pathways.

1.2.5 Resveratrol Induces Autophagy and Caspase-independent Death in Cancer Cells

In addition to apoptosis, resveratrol also induces autophagy, causing death of cancer cells. For example, resveratrol induces ROS-mediated autophagy causing toxicity in colon cancer cells (Miki et al. 2012). It seems that resveratrol-induced apoptosis and autophagy are sufficient to induce cell death in glioblastoma cancer stem cells as inhibition of apoptosis or autophagy alone does not inhibit resveratrol-induced cytotoxicity (Filippi-Chiela et al. 2011). Resveratrol induces caspase-independent and apoptosis inducing factor-mediated apoptosis in human lung adenocarcinoma cells (Zhang et al. 2011). Evidence supports that resveratrol induces, apoptosis, autophagy, and mitotic catastrophe in cancer cells (Delmas et al. 2011). Resveratrol also induces Beclin 1-independent autophagy in breast cancer cells (Scarlatti et al. 2008). Recently, we have reported that autophagy induction may act as an adaptive response during resveratrol-induced apoptosis (Prabhu et al. 2012). These findings suggest that resveratrol-induced autophagy could be considered as both cell death and survival mechanism. Therefore, existence of multiple cell death pathways provides various avenues to induce death in different cancer cell types.

1.2.6 Cancer Cell Survival Signaling During Resveratrol-induced Cell Death

Multiple genes involved in tumor cell survival, proliferation, metastasis, invasion, and angiogenesis are regulated by NF-κB (Sethi et al. 2008; Baud and Karin 2009). Although NF-κB activity is crucial for normal cellular proliferation, whereas, aberrant and/or constitutive activation of NF-κB is implicated in various cancers (Bharti and Aggarwal 2002b). Activation of NF-κB inhibits chemotherapy-induced apoptosis in several tumor types (Wang et al. 1996; Bharti and Aggarwal 2002a, b). Resveratrol inhibits NF-κB activation in mammary carcinogenesis induced by 7,12-dimethylbenz(a)anthracene *via* downregulation of cyclooxygenase-2 (COX-2) and matrix metalloproteinase (MMP)-9 (Banerjee et al. 2002). Resveratrol blocks NF-κB activation in various cancer cells induced by different activators of NF-κB, such as TNF, PMA, LPS, H_2O_2, okadaic acid, or ceramide (Manna et al. 2000).

Resveratrol downregulates the expression of NF-κB-regulated genes including interleukin-6, Bcl-2, Bcl-xL, XIAP, c-IAP, vascular endothelial growth factor (VEGF), and MMP-9 in multiple myeloma cells (Sun et al. 2006). Resveratrol inhibits NF-κB and MMP-9 activities to block migration of breast cancer cells (Pozo-Guisado et al. 2005). Thus resveratrol-induced NF-κB suppression may be essential for its anticancer activities.

In addition to NF-κB-mediated survival signaling, resveratrol also targets multiple biochemical signaling to exert anticancer activities. For instance, resveratrol induces p53-independent upregulation of p21, cell cycle arrest, and survivin depletion (Fulda and Debatin 2004). Downregulation of survivin expression during resveratrol-induced apoptosis was observed in T-cell leukemia (Hayashibara et al. 2002). In addition to cell culture model, resveratrol suppresses survivin in ultraviolet B radiation-induced skin carcinogenesis in SKH-1 hairless mice, thus providing protection from ultraviolet B radiation-mediated cutaneous damages (Aziz et al. 2005a). Resveratrol inhibits the expression of survivin in *BRCA1* mutant breast cancer cells, suggesting that inclusion of resveratrol for the treatment of breast cancer may serve as an excellent strategy (Wang et al. 2008a). Besides survivin, resveratrol also suppresses the expression of other antiapoptotic proteins, such as, Bcl-xL and Mcl-1. In some non-Hodgkin's lymphoma and multiple myeloma cell types, resveratrol downregulates expression of the antiapoptotic proteins Bcl-xL and Mcl-1 (Jazirehi and Bonavida 2004). Our recent findings demonstrated the accumulation of XIAP on mitochondria causes activation of Bax leading to Bax-channel formation and permeabilization of the outer mitochondrial membrane. Our findings suggest that resveratrol-induced expression of antiapoptotic proteins such as XIAP may also perform proapoptotic function to sensitize cancer cells (Gogada et al. 2011).

Hsp70 functions as a prosurvival protein by inhibiting: apoptosome function (Beere et al. 2000; Saleh et al. 2000), AIF-mediated apoptosis (Ravagnan et al. 2001), Bax translocation to mitochondria (Stankiewicz et al. 2005), and lysosomal membrane permeabilization (Nylandsted et al. 2004). Resveratrol-mediated downregulation of Hsp70 by targeting heat shock factor 1 transcriptional activity may lead to caspase activation and apoptosis in cancer cells (Cardile et al. 2003; Chakraborty et al. 2008). Resveratrol has also been shown to activate or enhance expression of sirtuin 1 (Wang et al. 2008a; Li et al. 2013). However, other studies suggest that resveratrol does not modulate the levels of sirtuins and sirtuin 1 inhibitor sirtinol does not affect apoptosis induction by resveratrol. These studies suggest that resveratrol also induces sirtuin 1-independent apoptosis in cancer cells (Wang et al. 2012b).

1.3 Resveratrol Target Genes Regulating Cell Cycle Progression and Proliferation

The alterations in regulation of different phases of cell cycle have been implicated in cancer development (Sherr 2000). Various reports indicate that resveratrol inhibits cell cycle progression, and cell proliferation. For instance, resveratrol is

known to arrest G1-phase, S-phase, S/G2-phase, and G2-phase of cell cycle (Hsieh et al. 1999; Ahmad et al. 2001; Wolter et al. 2002; Estrov et al. 2003; Liang et al. 2003).

Resveratrol causes G2-phase arrest through the inhibition of p34 (Cdc2) and Cdk7 protein kinase activity in colon carcinoma HT-29 cells (Liang et al. 2003). Resveratrol downregulates cyclin D1/Cdk4 complex in colon cancer cell lines (Wolter et al. 2001). In H22 tumor-bearing mice, the expression of cyclin B1 and Cdc2 protein was decreased in resveratrol-treated animals (Yu et al. 2003). In human SK-Mel-28 melanoma cells, resveratrol induced S-phase arrest and upregulation of cyclins A, E and B1 (Larrosa et al. 2003). Resveratrol induces p21WAF1 and p27KIP1 accumulation and downregulation of cyclins and cyclin-dependent kinases, thus implicating its anti-proliferative activity (Ahmad et al. 2001; Kim et al. 2006). Resveratrol reduces the expression of cyclins D1, E, and Cdk4, and a reduced cyclin D1/Cdk4 kinase activity is associated with the apoptosis induction by resveratrol in prostate cancer cells (e.g., LNCaP) (Benitez et al. 2007). Resveratrol decreases cyclin B and Cdk1 expression and cyclin B/Cdk1 kinase activity in both androgen-sensitive and androgen-insensitive prostate cancer cells (Benitez et al. 2007). In human malignant B cells, resveratrol induces S-phase cell cycle arrest *via* ATM/Chk pathway (Shimizu et al. 2006). Moreover resveratrol phosphorylates Cdc2 at tyr15 through the activation of ATM/ATR-Chk1/2-Cdc25C pathway in ovarian cancer cells (Tyagi et al. 2005). Overall, the anti-proliferative activity of resveratrol involves the regulation of multiple cell cycle targets by blocking cell cycle progression.

1.4 Resveratrol Potentiates the Anticancer Activities of Multiple Cancer Drugs

Resveratrol induces expression of perforin A and granzyme B leading to apoptosis induction in prostate cancer cells. In the presence of radiation, resveratrol further enhanced the levels of these proteins, suggesting the synergistic effect of resveratrol and radiation in cancer cells (Fang et al. 2011). Indeed, various phytochemicals including resveratrol in combination with irradiation have been suggested to enhance the therapeutic index of cancer treatment (Nambiar et al. 2011). Additionally, resveratrol inhibits cell proliferation and promotes apoptosis to enhance radiation sensitivity in prostate cancer (Fang et al. 2012).

Resveratrol reduced the associated toxicity without compromising the anticancer activities of cisplatin, suggesting resveratrol in combination with known anticancer agents such as cisplatin could be beneficial in cancer therapy to reduce toxicity and enhance cancer cell killing (Attia 2012). Similarly, pretreatment with resveratrol enhances apoptosis in response to platinum drug such as cisplatin and oxaliplatin in ovarian cancer cells (Nessa et al. 2012). Resveratrol enhances the antitumor activity of gemcitabine *in vitro* and in an orthotopic mouse model of

human pancreatic cancer *via* suppression of several proteins associated with proliferation, invasion, angiogenesis and metastasis (Harikumar et al. 2010). Resveratrol potentiates the effects of temozolomide against malignant glioma *in vitro* and *in vivo* by inhibiting autophagy (Lin et al. 2012). Resveratrol reverses temozolomide resistance in T98G glioblastoma cells *via* NF-κB-dependent mechanism (Huang et al. 2012).

Resveratrol in combination with glucan and vitamin C is more potent in inhibiting the growth of tumors *in vivo* (Vetvicka and Vetvickova 2012). Combined treatment of resveratrol and 5-fluorouracil (5-FU) enhanced expression of p-JNK and p38 causing increased apoptosis in colon cancer cells at lower doses of 5-FU (Mohapatra et al. 2011). Resveratrol sensitizes TRAIL-resistant LNCaP cells, suggesting the link between TRAIL and resveratrol for the improvement of prostate cancer therapy and various other cancers (Fulda and Debatin 2004, 2005; Shankar et al. 2007). Resveratrol also induces AMPK-dependent apoptosis in chemo-resistant cells (Hwang et al. 2007). Additionally, resveratrol modulates protein kinase C (PKC) signaling to exert anticancer activities (Shih et al. 2004). In another study, Majumdar et al. (2009) studied the synergistic effect of curcumin and resveratrol and found that the combination of resveratrol and curcumin is more effective in inhibiting growth of p53-positive or p53-negative colon cancer HCT-116 cells *in vitro* and *in vivo* in SCID xenografts than either agent alone. This suggests that the combination of curcumin and resveratrol could be an effective strategy for colon cancer treatment.

Interestingly, resveratrol can sensitize several cancer cells to apoptosis induced by death-receptor ligation or anticancer drugs. Resveratrol sensitizes tumor cells for TRAIL-induced apoptosis through p53-independent induction of p21 leading to cell cycle arrest and survivin depletion (Fulda and Debatin 2004). In another study Ganapathy et al. (2010) showed that resveratrol can enhance the apoptosis-inducing potential of TRAIL in a xenograft model of prostate cancer by activating FKHRL1 and its target genes.

It is also known that, resveratrol at low doses sensitizes drug-refractory non-Hodgkin's lymphoma and multiple myeloma cells to apoptosis induced by paclitaxel (Jazirehi and Bonavida 2004). Furthermore, resveratrol was shown to enhance the apoptotic effects of bortezomib and thalidomide in chemo-resistant human multiple myeloma cells (Bhardwaj et al. 2007). These findings suggest that resveratrol could function as a sensitizing agent for the improvement of cancer therapy. Indeed, resveratrol sensitizes multiple tumors including lung carcinoma, acute myeloid leukemia, promyelocytic leukemia, multiple myeloma, prostate cancer, oral epidermoid carcinoma, and pancreatic cancer to chemotherapeutic agents such as vincristine, adriamycin, paclitaxel, doxorubicin, cisplatin, gefitinib, 5-FU, velcade, and gemcitabine (Gupta et al. 2011). Together, available evidences suggest that resveratrol can sensitize cancer cells, by blocking survival and antiapoptotic pathways to render tumor cells more susceptible for death induction.

Resveratrol may have benefit in protection against side effects caused by cancer therapy. A study showed that resveratrol protects against irradiation-induced

hepatic and ileal damage through its antioxidative activity. Thus, cancer patients treated with adjuvant resveratrol therapy may have some benefit resulting in successful radiotherapy with reduced toxicity (Velioglu-Ogunc et al. 2009).

1.5 Anticancer Activities of Resveratrol in Mouse Tumor Models

Various animal studies provide evidence for the anticancer and chemopreventive potential of resveratrol in multiple cancer types (Bishayee 2009). Effect of resveratrol on cancer chemoprevention was first studied by Jang et al. (1997). They demonstrated that resveratrol inhibits preneoplastic lesions in carcinogen-treated mammary glands in culture and inhibits tumorigenesis in a mouse skin cancer model (Jang et al. 1997). Further studies in skin carcinogenesis demonstrated that, resveratrol prevents UVB skin tumorigenesis by upregulating survivin and downregulating proapoptotic Smac/DIABLO protein (Aziz et al. 2005b). Later, anticancer activities of resveratrol were observed in multiple cancers. For instance, resveratrol suppresses prostate cancer growth through downregulation of androgen receptor (Seeni et al. 2008). Suppression of prostate cancer growth was associated with downregulation of phospho-extracellular signal-regulated kinase (ERK)-1and ERK-2, and increase in the levels of estrogen receptor-β (Harper et al. 2007). A delay in spontaneous mammary tumor development was observed after resveratrol administration and was associated with the downregulation of HER2/neu (Provinciali et al. 2005). A decrease in tumor growth and inhibition in angiogenesis with an increased apoptosis was observed in breast cancer xenograft tumors upon resveratrol treatment (Garvin et al. 2006). Resveratrol reduces growth and development of tumors in 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis in Wistar rats (Sengottuvelan et al. 2006). Resveratrol suppresses growth of human ovarian cancer cells *in vitro* and in xenograft tumors by targeting eukaryotic elongation factor 1A2, which is known to cause transformation of fibroblasts and stimulates cell growth in nude mice (Lee et al. 2009). Resveratrol inhibits growth and metastasis of lung tumors (Kimura and Okuda 2001). Resveratrol inhibits tumor growth in liver by upregulating proapoptotic Bax and downregulating antiapoptotic Bcl-2 proteins (Bishayee and Dhir 2009). Nanoparticles containing resveratrol have demonstrated increased retardation of ovarian tumor growth without weight loss in animals (Guo et al. 2010). Since bioavailability of resveratrol in tumor is a problem, peritumor injection of resveratrol leads to increased apoptosis and rapid neuroblastoma tumor regression (up to 90 %) was observed (van Ginkel et al. 2007). Similarly, peritumor injection of resveratrol inhibits uveal melanoma tumor growth in animal models by inducing apoptosis *via* the intrinsic mitochondrial pathway (van Ginkel et al. 2008). However, some animal studies show that resveratrol is rapidly metabolized and does not inhibit human melanoma xenograft tumor growth (Niles et al. 2006). Resveratrol induces angiogenesis and inhibits

apoptosis in prostate tumor xenografts (Wang et al. 2008b). Similarly, resveratrol does not show anticancer effects in lung tumorigenesis as shown in multiple reports (Hecht et al. 1999; Berge et al. 2004). Based on these information it is possible that, the doses of resveratrol may not be sufficient to exert anticancer activities or resveratrol is metabolized very fast leading to the lack of its sufficient accumulation in tumors.

1.6 Current Updates on Clinical Studies with Resveratrol

Few studies have been conducted on the bioavailability of resveratrol in human subjects (Goldberg et al. 2003; Walle et al. 2004; Vitaglione et al. 2005; Boocock et al. 2007), and a limited number of clinical trials have evaluated the anticancer activities of resveratrol. For example, 10-year of epidemiological study shows 50 % reduction in breast cancer among women with resveratrol consumption from grapes (Levi et al. 2005). Clinical trials on colorectal cancer patients (age: 46–83 years, sex: 9 males and 11 females) demonstrate that daily doses of 0.5 or 1.0 g for 8 days produces sufficient levels of resveratrol to elicit anticarcinogenic effects (Patel et al. 2010). Resveratrol and resveratrol-3-*O*-glucuronide were detected from tissues at mean concentrations of 674 and 86 nmol/g, respectively. Resveratrol intake reduced tumor cell proliferation by 5 % (Patel et al. 2010). Resveratrol doses of up to 5 g/day are tolerated by humans without severe side effects (Brown et al. 2010; Patel et al. 2010). A phase 1 randomized and double-blinded pilot study on safety and anticancer activities of micronized resveratrol demonstrated that resveratrol up to 5 g/day for 14 days is tolerated by human. Significant apoptosis with an increase in caspase-3 activity was observed at these doses of resveratrol in malignant hepatic tissues (Howells et al. 2011). However, Chow et. al., demonstrated that in healthy volunteers resveratrol daily dose of 1 g for 4 weeks modulates cytochrome P450 and phase 2 detoxification enzymes, which may lead to adverse drug reactions or altered drug efficacy (Chow et al. 2010a). Additionally, mild to moderate gastrointestinal symptoms have been reported with 2.5 and 5 g doses of resveratrol given to healthy volunteers daily for 29 days (Brown et al. 2010). Such data should be taken into consideration for future clinical trials or therapy. Additional information on clinical trials are described in details by Brown's group (Patel et al. 2011), which are mentioned in Table 1.2. Further clinical trials are needed to conclusively demonstrate the utility of resveratrol in cancer therapy.

1.7 Concluding Remarks and Future Perspectives

Anticancer agents that target multiple pathways in tumor cells without toxicity to normal tissues will be highly significant for cancer therapy. Resveratrol targets multiple cellular signaling pathways involved in different stages of cancer

Table 1.2 Summary of published clinical trials involving resveratrol

Cohort	Form of resveratrol	Resveratrol dose	Dosing schedule	Study outcome
Healthy males (12)	Delivered in white wine, white grape juice or vegetable juice	25 mg/70 kg	Single	Resveratrol absorption was similar in all three matrices
Healthy males (3)	Dissolved in 5 mL whisky mixed with 50 mL water	0.03, 0.5, or 1 mg/kg	Single	Pharmacokinetic and metabolite profile
Healthy males (3)	Delivered in grape juice (200, 400, 600 or 1,200 mL)	0.32, 0.64, 0.96, or 1.92 mg	Single	Pharmacokinetic and metabolite profile
Healthy males (3) and females (3)	^{14}C-resveratrol taken orally and intravenously	25 mg	Single	Pharmacokinetic and metabolite profile
Healthy males (11)	250 mL red wine	5.38 mg	Single	Resveratrol and metabolites identified in low-density lipoprotein after moderate wine intake
Healthy males (14) and females (11)	300 or 600 mL red wine, consumed after fasting, or with meals of varying lipid content	246, 480 μg, or 1.92 mg	Single	Resveratrol bioavailability was not influenced by food, or lipid content
Healthy males (18) and females (22)	500 mg capsules	0.5, 1, 2.5, or 5.0 g	Single	Pharmacokinetic and metabolite profile. Resveratrol did not cause serious adverse events
Healthy males (9)	Dissolved in 100 mL of 15 % ethanol, made up in low-fat milk to a total volume of 500 mL	85.5 mg/ 70 kg	Single	Pharmacokinetic and metabolite profile. Resveratrol and its metabolites shown to have a high affinity for protein binding
Healthy males (11)	250 mL red wine, 1 L grape juice, or 10 tablets	14 μg/kg	Single	Bioavailability of resveratrol from wine and grape juice six fold higher than that from tablets
Healthy males (4) and females (20)	Capsules	250 or 500 mg	Single; once daily on 3 separate days	Single doses of resveratrol can modulate cerebral blood flow variables

Healthy males (12 young) and females (12 elderly)	Capsules	200 mg	Single followed by multiple doses thrice daily (2 days) and a final single dose	Pharmacokinetic and metabolite profile. Resveratrol was well-tolerated by young and elderly subjects
Healthy males (20) and females (20)	Capsules	25, 50, 100, or 150 mg	Multiple; six times/day, for 13 doses	Pharmacokinetic and metabolite profile. Resveratrol was well-tolerated, but with some mild adverse events reported
Healthy males (3) and females (5)	Capsules; taken with food, quercetin or 100 mL 5 % alcohol	2 g	Multiple; twice daily	Pharmacokinetic and metabolite profile. A high-fat meal reduced AUC and C_{max}. Resveratrol was well-tolerated, although diarrhea was frequently observed
Healthy males (22) and females (18)	500 mg caplets	0.5, 1, 2.5, or 5.0 g	Multiple; once daily for 29 days	Pharmacokinetic and metabolite profile. Resveratrol caused a reduction in IGF-1 and IGFBP-3 plasma levels. 2.5 and 5.0 g caused mild to moderate gastrointestinal symptoms
Male (9) and female (11) colorectal cancer patients	500 mg caplets	0.5 or 1 g	Multiple; once daily for 8 days	Pharmacokinetic and metabolite profile in colon/tumor tissue
Healthy males (11) and females (31)	500 mg caplets	1 g	Multiple; once daily for 28 days	Resveratrol shown to modulate enzyme systems involved in carcinogen activation and detoxification. Resveratrol was well-tolerated
Healthy males (10) and females (10)	300 mL sparkling wine or 200 mL either white wine or red wine	0.357, 0.398 or 2.56 mg/day	Multiple; once daily for 28 days	Resveratrol metabolites in urine may be useful biomarkers of wine intake in epidemiological and intervention studies

The figures in parentheses (column 1) refer to the number of participants in each study

development. Several studies have explored the benefits of resveratrol treatment in combination with other agents for cancer treatment. However, long-term epidemiological studies on humans will be needed to determine therapeutic efficiency of resveratrol in human cancers. Since bioavailability of resveratrol is a major drawback in cancer therapy, various attempts have been made to synthesize resveratrol analogs to enhance stability, bioavailability and apoptosis. For example, a resveratrol analogs, phoyunbene B (PYB, *trans*-3,4′-dihydroxy-2′,3′,5-trimethoxystilbene) has been shown to be more effective in inhibiting the growth of cancer cells than resveratrol itself (Wang et al. 2012a). This study suggests that resveratrol analogs showing increased bioavailability and/or anticancer activities may lead to cancer cure with minimal toxicity in cancer patients. Taken together, identification of new molecular targets and/or future clinical studies on resveratrol is required to harness its cancer therapeutic potential.

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Chapter 2
Effect of Flavonoids from Fruits and Vegetables in the Prevention and Treatment of Cancer

Min-Hsiung Pan, Ching-Shu Lai, Jia-Ching Wu, and Chi-Tang Ho

Abstract Cancer is the major health problem worldwide over a century. The development of cancer is a multi-step process, including initiation, promotion and progression. This process is driven by genetic changes, elicit transformation of initiated cell into cancerous cell with abnormal proliferative and invasive capabilities, and ultimately distant metastasis. Recent studies also exhibit that chronic inflammation is implicated in tumorigenesis by over-production of inflammatory mediators and creation of an inflammatory microenvironment. Prevention and treatment of cancer through dietary intervention has been considered as a rational approach. Convincing evidence shows regular consumption of fruit and vegetables is associated with reduced risks of cancer. Flavonoids are widely present in diet such as fruits and vegetables, they have been demonstrated a broad spectrum of biological activities for human health. They have been found to interfere with cancer development at different stage by targeting on various signaling molecules, genes, proteins and enzymes involved in tumorigenesis. Accumulating evidences report the potential of dietary flavonoids for both chemopreventive and chemotherapeutic effects which act on regulation of redox status, cellular proliferation, differentiation, programmed cell death, inflammation angiogenesis and metastasis. Some of them display synergy effect in combination of conventional therapies for drug-resistant cancer cells. In this chapter, we summarize recent knowledge and the underlying mechanism on chemopreventive and chemotherapeutic activities of dietary flavonoids that may offer effective approach for the control of cancer incidence.

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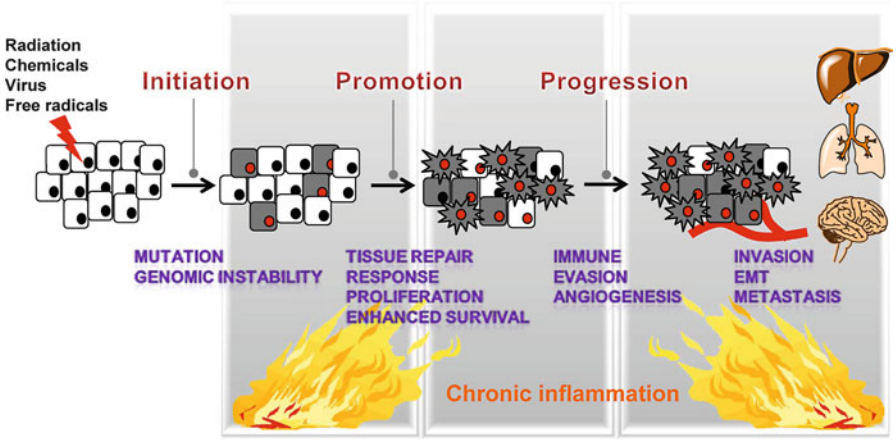


Fig. 2.1 Development of cancer is a multiple step, including initiation, promotion and progression process

2.1 Cancer Development

Cancer is the major leading cause of death worldwide. Numerous researches show that cancer development in humans is a multistep process. In 2000, Hanahan and Weinberg (2000) proposed that tumorigenesis acquires six biological capabilities including sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, and activating invasion and metastasis. Recently, several lines of evidence also reveal that chronic inflammation is the seventh hallmark of cancer (Hanahan and Weinberg 2011). Tumorigenesis has been characterized by three critical steps including initiation, promotion and progression in many types of human cancers (Fig. 2.1). This process can be activates by any one of the various environmental carcinogens, inflammatory agents, tumor promoters and reactive oxygen species (ROS) (Pan and Ho 2008; Shields and Harris 1991).

- Initiation stage: it begins with DNA damage in a cell population exposed to various environmental carcinogens, free radicals, inflammatory agents, and tumor promoters. The most relevant in this regard are mutations activating proto-oncogenes and inactivating tumor suppressor genes, such as *c-myc* and *p53*, or increase of genomic instability in damaged cells.
- Promotion stage: it could be defined as a process of escape programmed cell death with enhanced survival property of initiated cells (with mutated gene) that results in clonal expansion and further producing nodules, polyps or papillomas.
- Progression stage: this stage is characterized by the transformation of pre-neoplastic cells into malignant tumor invading surrounding tissues and forming metastases by enhanced angiogenesis, epithelial-mesenchymal transition (EMT) immune suppression/evasion capabilities.

In progress of clinical technology and medicine development, cancer diagnosis, surgical techniques, and adjuvant therapies are greatly improved, but still with limitation on reduction of cancer incidence and mortality. The major problem in current cancer therapy is the eventual development of recurrent or metastatic cancers caused by drug resistance in most patients (Alexander and Friedl 2012). Scientists have proposed and identified many critical molecules involved in tumorigenesis in past few decades. A number of genes with genetic mutation or loss of function, dysregulation of signaling molecules, proteins and enzymes are contributes to cancer development (Tonon 2008; William et al. 2009). However, despite understanding of the process and molecular mechanism in tumorigenesis, present therapies are still limited for advanced tumors. Due to the limitation of current cancer treatment and side effects of chemotherapy, researchers attempt to search for new approach to control cancer development through cancer chemoprevention or by specific/multi-targets approach to improve efficiency of conventional therapies.

2.2 Cancer Chemoprevention and Treatment by Natural Compounds

Chemoprevention is the use of a chemical substance of either natural or synthetic origin to prevent, hamper, arrest, or reverse a disease. The term chemoprevention was coined by Sporn et al. (1976) in the mid-1970s. His work on retinoids against chemical carcinogenesis showed the time that cancer takes to develop in humans through the initiation, promotion, and progression stages. Cancer chemoprevention has a dual goal, i.e. prevention of occurrence of the disease (primary prevention) and early detection and reversion of tumors at a premalignant stage (secondary prevention). At a later stage, attempts can be made to prevent local recurrences as well as invasion and metastasis of malignant cells (tertiary prevention) (De et al. 2001; De and Ferguson 2005).

- Primary prevention: includes inhibition of mutation and cancer initiation, in the extracellular environment or inside cells, followed by inhibition of tumor promotion, such as, modifying transmembrane transport, modulating metabolism, blocking reactive species, detoxification, inhibiting cell replication, maintaining DNA structure, DNA repair, and controlling gene expression.
- Secondary prevention: exploits a variety of mechanisms aimed at inhibiting progression of a timely diagnosed benign tumor towards malignancy. It is possible to inhibit tumor progression *via* the same mechanisms, and in addition by affecting the hormonal status and the immune system in various ways, and by inhibiting angiogenesis and disruption of inflammatory microenvironment.
- Tertiary prevention: has the goal of preventing local relapses of the disease and of inhibiting invasion and metastasis or induction of programmed cell death in cancer cells.

Accumulated genetic alterations and dysregulated intracellular signaling are critical characteristics in cancer cells. Targeting on modulation of signaling molecules involved in tumorigenesis or leading to programmed cell death of cancer cells are promising approach in cancer therapy (Cho 2012). Both apoptosis and autophagy are types of programmed cell death that with entirely different mechanism and biological function (Edinger and Thompson 2004). Apoptosis is involving the concerted action of a number of intracellular signaling pathways, including members of the caspases family of cysteine proteases stored in most cells as zymogens or procaspases (Martin and Green 1995). Proteolytic cleavage of procaspases is an important step leading to caspase activation, which in turn is amplified by the cleavage and activation of other downstream caspases in the apoptosis cascade (Earnshaw et al. 1999; Stennicke and Salvesen 2000). The maintenance of homeostasis in normal mammalian tissues reflects a critical balance between cell proliferation and cell death *via* apoptosis. Induction of tumor cell apoptosis can be induced to augment interventions designed to suppress or reverse the development of cancer (Debatin 2004). Autophagy is defined as type 2 programmed cell death and is crucial for maintaining cellular homeostasis that responded to various microenvironment stresses, including starvation, pathogen infestation and chemotherapy (Cecconi and Levine 2008). It also functions as a backup when apoptosis is disabled (Maycotte and Thorburn 2011). However, the role of autophagy in cancer treatment is still controversial. Several reports exhibit that autophagy is a possible mechanism for tumor cell survival after cancer treatment (Hippert et al. 2006; Ravikumar et al. 2006; Degenhardt et al. 2006). Other studies reveal that autophagy induction appears to facilitate successful therapy-induced killing of tumor cells that suggesting a novel therapeutic strategy (Yang et al. 2011).

Over the past few decades, growing interesting in identify agents from natural sources which provide with preventing the initiation of tumors, arresting the development or metastasis of overt tumors and others (Yang et al. 2001). An effective and acceptable chemopreventive or anticancer agent should possess certain properties, include low cost, no toxic effects in normal and healthy cells, capability of oral consumption, high efficacy against multiple cancers, known mechanism of action, and acceptance by the human population (Galati and O'Brien 2004). Because of their pharmacological safety, most chemopreventive agents can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy-induced toxicity (Ferguson et al. 2005).

Many dietary phytochemicals such as curcumin, resveratrol, gingerols, sulforaphane and β-carotene have been shown to have cancer-preventing and therapeutic activities in laboratory studies (Pan and Ho 2008). As an example, tea and tea preparations have been shown to inhibit tumorigenesis in a variety of animal models of carcinogenesis, involving organ sites such as the skin, lungs, oral cavity, esophagus, stomach, liver, pancreas, small intestine, colon, and prostate (De and Ferguson 2005; Ferguson et al. 2005). The chemopreventive and chemotherapeutic mechanisms of natural dietary compounds are acting on regulation of redox status

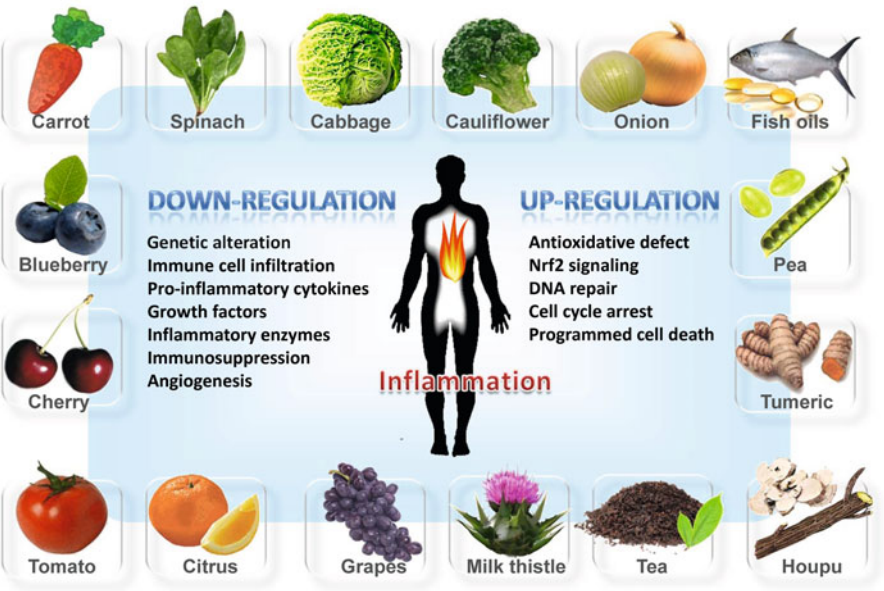


Fig. 2.2 Proposed mechanism of natural dietary compounds on cancer chemoprevention and chemotherapy

and signal transduction, modulation of gene expression involved in the suppression of inflammation, regulation of cell proliferation, differentiation, cell cycle and apoptosis and suppression of angiogenesis and metastasis, and thus inhibition of carcinogenesis (Pan and Ho 2008) (Fig. 2.2).

2.3 Chemopreventive and Chemotherapeutic Mechanisms of Flavonoids in Human Cancer

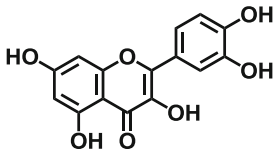
Numerous epidemiologic studies showed that the incidence of cancer is inversely correlated with the consumption of fruits and vegetables (Block et al. 1992; Vainio and Weiderpass 2006). Flavonoids are natural plant secondary metabolites in fruits, vegetables, nuts, seeds and plants and, with the burgeoning interest in alternative medicine, which increasingly been ingested by the general population. Chemically, flavonoids possess a basic 15-carbon skeleton and can be represented as C6-C3-C6 consisting of two benzene rings (C6) joined by a three carbon chain (C3). They can be classified by flavones, flavanones, flavonols, flavanonols, flavanols (catechins), anthocyanidins and isoflavones based on the differences in the structure of the aglycones C ring. The structural diversity of flavonoids is present in the pattern of basic structure such as hydroxylation and methoxylation, and the type of conjugation includes sulfonation, prenylation, or glycosylation, that display various biological activities (Beecher 2003).

Growing evidences exhibit a broad spectrum of pharmacological properties of flavonoids such as antioxidant, free radical-scavenging, anti-inflammatory, anti-carcinogenic, anti-viral, anti-bacterial, anti-thrombogenic and anti-atherogenic activities. It has been reported that human intake of all flavonoids is a few hundred milligrams to 650 mg/day in our diet (Liu 2004). Several researches indicate that dietary flavonoids may reduce cancer risk and display benefit for human health (Neuhouser 2004; Rossi et al. 2008; Graf et al. 2005). The chemopreventive and chemotherapeutic effects of flavonoids on various human cancers as well as their molecular mechanism are described below.

2.3.1 Flavonols (Table 2.1)

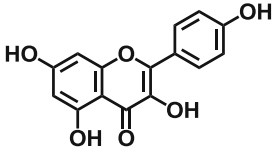
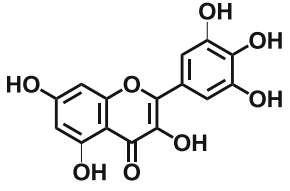
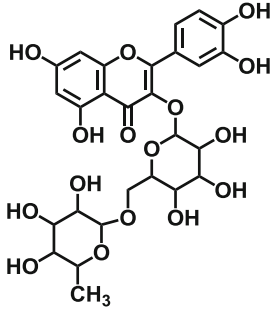
Quercetin is found typically in onions, broccoli, apples, grapes, wine, tea, and leafy green vegetables, and it is well known as a potent antioxidant and anti-inflammatory agent and effective in the prevention and modulation of different type of cancers. Dietary quercetin reduces azoxymethane (AOM)-induced aberrant crypt foci (ACF) formation by lowering crypt cell proliferation and increasing apoptosis in both F344 and SD rats (Warren et al. 2009; Turner et al. 2009). Decreased level of inflammatory enzyme cyclooxygenase-2 (COX-2) is also one of possible mechanisms for preventing colonic tumorigenesis in early stage (Turner et al. 2009). Quercetin is reported to inhibit the growth of colon cancer cells by induction of G2/M cell cycle arrest *via* downregulation of β-catenin/T cell factor (Tcf) transcriptional activity, thus decreasing gene expression of both *cyclin D1* and *survivin* that involved in cell cycle progression (Shan et al. 2009). Quercetin also induces apoptosis through targeting on epidermal growth factor receptor (EGFR) and AMP-activated protein kinase (AMPK) signaling in HT-29 colon cancer cells (Kim et al. 2005) and HT-29 xenograft tumor (Kim et al. 2010a), respectively. Moreover, quercetin enhances tumor radio-sensitivity in DLD-1 human colorectal cancer xenograft model through inhibiting Ataxia telangiectasia mutated (ATM) kinase that contributes to abate repair signaling in response to DNA damage (Lin et al. 2012a). Similar to the role of quercetin in colon cancer, it inhibits tumor growth by induction of apoptosis in human breast cancer MDA-MB-453 tumor growth *in vivo* (Dechsupa et al. 2007) and enhances radio-sensitivity in MCF-7 cancer cells (Lin et al. 2012a). In T98G and U87 glioblastoma cells, treatment with quercetin inhibits interleukin (IL)-6 triggered cell proliferation and migration through targeting on signal transducer and activator of transcription 3 (STAT3) and downstream target gene *cyclin D1* and matrix metalloproteinases (*MMP*)*-2* (Michaud-Levesque et al. 2012). In prostate cancer, quercetin causes endoplasmic reticulum (ER)-dependent apoptosis signaling in PC-3 cells and enhances eliminating prostate cancer stem cells (CSCs) characteristics by inhibition of self-renewal properties, and lowering vimentin, slug, snail levels involved in EMT, thus suppression of invasion and migration (Liu et al. 2012; Tang et al. 2010).

Table 2.1 Chemopreventive and chemotherapeutic effects of dietary flavonols on human cancers

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
Quercetin		Broccoli, onion, apples and grapes	Colon cancer	Inhibits AOM-induced colorectal carcinogenesis by suppression of COX-2 expression, crypt cell proliferation and induction of apoptosis	Warren et al. (2009); Turner et al. (2009)
				Reduces β-catenin/T cell factor transcriptional activity, induces G2/M cell cycle arrest through decreasing gene expression of *cyclin D1* and *survivin* in SW480 colon cancer cells	Shan et al. (2009)
				Induces apoptosis *in vitro* and *in vivo* through targeting epidermal growth factor receptor and AMP-activated protein kinase signaling	Kim et al. (2005, 2010a)
				Enhances tumor radio-sensitivity in xenograft model through targeting ATM kinase	Lin et al. (2012a)
			Breast cancer	Induces apoptosis in human breast cancer MDA-MB-435 cells xenograft model	Dechsupa et al. (2007)
				Enhances radio-sensitivity *in vitro*	Lin et al. (2012a)
			Glioblastoma	Inhibits glioblastoma cells proliferation and migration by reduction of IL-6/signal transducer and activator of transcription 3 mediated *cyclin D1* and *MMP-2*	Michaud-Levesque et al. (2012)
			Prostate cancer	Induces apoptosis *via* ER-stress and mitochondrial signaling	Liu et al. (2012)
				Synergizes with EGCG in eliminating prostate cancer stem cells characteristics by inhibition of self-renewal properties, invasion and migration	Tang et al. (2010)
Kaempferol		Broccoli and tea	Colon cancer	Prevents 1,2-dimethyl hydrazine induced colonic tumorigenesis by reducing lipid peroxidation and increasing anti-oxidative enzymes in rats	Nirmala and Ramanathan (2011a)

(continued)

Table 2.1 (continued)

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
			Ovarian cancer	Inhibits angiogenesis and tumor growth by decreasing vascular endothelial growth factor levels *via* hypoxia-inducible factor dependent and independent pathway	Luo et al. (2009)
				Induces apoptosis *via* activation of p53	Luo et al. (2011)
				Enhances chemotherapeutic effect by downregulation of c-myc in cisplatin-treated cells	Luo et al. (2010)
Myricetin		Grapes, berries and other fruits and vegetables	Pancreatic cancer	Induces apoptotic cell death *in vitro*, regresses tumor growth and reduces metastasis *in vivo*	Phillips et al. (2011)
			Bladder cancer	Induces G2/M cell cycle arrest and inhibits cell migration by targeting MMP-9 *in vitro*, reduces tumor growth in bladder cancer xenograft model	Sun et al. (2012)
			Colon cancer	Decreases 1,2-dimethylhydrazine-induced colonic tumorigenesis *via* upregulation of antioxidant levels and reduced lipid peroxidation	Nirmala and Ramanathan (2011b)
Rutin		Apple, orange, onion and citrus fruits	Leukemia	Synergistically inhibits vascular endothelial growth factor production with vitamin E by downregulating activator protein-1 and insulin receptor substrate 1 signaling	Chuang et al. (2010)
				Reduces tumor growth in leukemia xenograft model	Lin et al. (2012b)
				Reduces cell adhesion-mediated drug resistance by inducing apoptosis adherent leukemic progenitors *via* downregulation of active GSK3	Bourogaa et al. (2011)
			–	Protects benzo[*a*]pyrene-induced DNA damage in hepatoma tissue culture cells	Cristina et al. (2011)

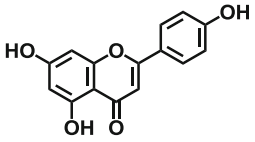
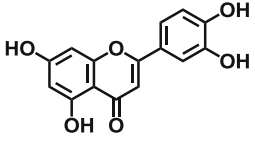
Kaempferol is another typical flavonol that commonly present in broccoli, tea and various vegetables. Study shows that dietary administration of 50–200 mg/kg kaempferol to rats causes significant upregulation of antioxidant enzymes including catalase, super oxide dismutase (SOD) and glutathione peroxidase (GPx) as well as decreasing lipid peroxidation that reduces 1,2-dimethyl hydrazine-induced colonic carcinoma in rats (Nirmala and Ramanathan 2011a). Several reports demonstrate the therapeutic effect of kaempferol in ovarian cancer. Kaempferol displays anti-angiogenic activity by inhibition of hypoxia-inducible factor (HIF)-dependent and independent vascular endothelial growth factor (VEGF) expression thus reduces tumor growth in human ovarian cancer cell lines and chorioallantoic membranes of chicken embryos model (Luo et al. 2009). Treatment with kaempferol induces apoptosis in ovarian cancer cells through p53-dependent mechanism (Luo et al. 2011). In addition, kaempferol sensitizes cisplatin-induced apoptosis by decreasing c-myc that contributes to overcome chemoresistance (Luo et al. 2010).

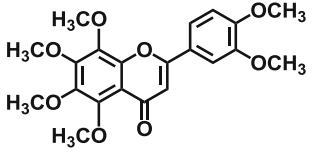
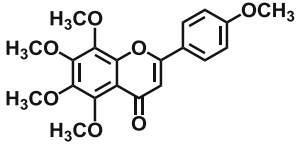
Myricetin, a naturally occurring flavonol in grapes, berries and other fruits and vegetables, has been found to possess anticancer property. In pancreatic cancer, myricetin induces apoptosis *in vitro*, regress tumor growth and decrease metastatic spreads in orthotopic pancreatic tumors through inhibition of phosphoinositide-3-kinase (PI3K) activity (Phillips et al. 2011). Myricetin also inhibits bladder cancer cells proliferation and migration by induction of G2/M phase cell cycle arrest and decreases of MMP-9 production that markedly reduces tumor growth in xenograft model (Sun et al. 2012). Consumption of myricetin at a dose of 50 and 100 mg/kg significantly suppress 1,2 dimethylhydrazine-induced colonic tumorigenesis through modulation of redox statue (Nirmala and Ramanathan 2011b). Rutin, also known as rutoside or sophorin, is a flavonol glycoside has a similar structure with quercetin that commonly present in apple, orange, onion and citrus fruits. Many studies have documented the potential of rutin on treatment of leukemia. In human promyelocytic leukemia (HL-60) cells, rutin and vitamin E synergistically inhibits VEGF secretion by suppression of activator protein-1 DNA-binding activity and interference with insulin receptor substrate-1 (IRS-1) signaling (Chuang et al. 2010). Treatment with rutin at a dose of 120 mg/kg inhibits tumor growth in a HL-60 xenograft animal model (Lin et al. 2012b). Rutin also displays a chemotherapeutic effect by induce apoptosis specifically in adherent leukemic cells thus contributes to abolish cell adhesion-mediated drug resistance (CAM-DR) (Bourogaa et al. 2011). Moreover, rutin is found to protect benzo[*a*]pyrene-induced DNA damage in hepatoma tissue culture (HTC) cells that may against carcinogen-induced toxic effect during metabolism (Cristina et al. 2011).

2.3.2 Flavones (Table 2.2)

Apigenin majorly presents in parsley and celery has been considered as an anticancer agent. Dietary apigenin (at a dose of 0.1 %) reduces AOM-induced ACF number by increasing colonocytes apoptosis in SD rats (Leonardi et al. 2010). In prostate

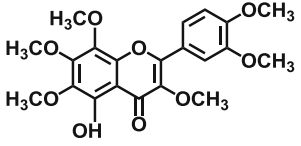
Table 2.2 Chemopreventive and chemotherapeutic effects of dietary flavones on human cancers

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
Apigenin		Parsley and celery	Colon cancer	Reduces number of high multiplicity ACF and increases apoptosis in AOM-treated rats	Leonardi et al. (2010)
			Prostate cancer	Reduces prostate cancer cell motility and invasion by interfering with actin cytoskeleton and focal adhesion kinase/Src signalings	Franzen et al. (2009)
				Induces cell cycle arrest and apoptosis in prostate cancer cells and reduces tumor growth in xenograft model by inhibition of histone deacetylase	Pandey et al. (2012)
			Ovarian cancer	Sensitizes paclitaxel-induced apoptosis through accumulation of reactive oxygen species and activation of caspase-2 in ovarian cancer cells	Xu et al. (2011)
Luteolin		Beets, brussels sprouts and cabbage	Colon cancer	Prevents AOM-induced ACF formation by decreasing malondialdehyde-DNA adduct and increasing activities of antioxidant enzymes in mouse colon	Ashokkumar and Sudhandiran (2008)
				Decreases tumor incidence and size through interference with Wnt/β-catenin signaling and cyclin D1 levels in AOM-treated mice	Ashokkumar and Sudhandiran (2011)
			Lung cancer	Induces G2/M phase cell cycle arrest and apoptosis through inhibiting translocation of NF-κB	Cai et al. (2011)
				Inhibits cell invasion and growth of tumor xenografts in nude mice by targeting HDAC	Attoub et al. (2011)
				Inhibits hypoxia-induced epithelial mesenchymal transition by downregulation of integrin β1 and FAK	Ruan et al. (2012)

				Enhances tumor necrosis factor-related apoptosis-inducing ligand-induced apoptosis in lung cancer xenograft model	Yan et al. (2012)
			Liver injury	Reduces CCl_4-induced liver fibrosis by increasing MMP-9 and metallothionein I/II expression	Domitrovic et al. (2009)
				Protects D-galactosamine/lipopolysaccharide-induced liver injury by decreasing apoptosis and tumor necrosis factor-α release	Lee et al. (2011a)
Nobiletin		Citrus peel	Colon cancer	Reduces AOM and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced ACF formation in mice	Suzuki et al. (2004); Tang et al. (2011)
				Reduces AOM and dextran sulfate sodium-induced tumor number partly through decreasing leptin in mice	Miyamoto et al. (2008)
			Prostate cancer	Inhibits PhIP-induced incidence and multiplicity of prostate adenocarcinomas by inhibiting cell proliferation	Tang et al. (2011)
			Gastric cancer	Suppresses cell invasion and migration through FAK/PI3K/Akt-mediated *MMP-2* and *MMP-9* gene expression and enzyme activity	Lee et al. (2011b)
Tangeretin			Lung cancer	Suppresses IL-1α-induced COX-2 expression *via* interfering with p38 mitogen-activated protein kinase, c-Jun N-terminal kinases and Akt signalings as well as downstream NF-κB activation	Chen et al. (2007)
			Ovarian cancer	Synergistically induces apoptosis and cell cycle arrest in cisplatin-resistant ovarian cancer cells through downregulation of PI3K/Akt signaling	Arafa et al. (2009)

(continued)

Table 2.2 (continued)

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
			Colon and breast cancer	Inhibits cancer cell proliferation by induction of G1 cell cycle arrest	Morley et al. (2007)
5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone		Citrus peel	Leukemia	Induces apoptosis through ROS production and oxidative DNA damage	Pan et al. (2007)
			Colon cancer	Inhibits colony formation in various colon cancer cells by decreasing β-catenin and NF-κB	Qiu et al. (2011)
			Skin cancer	Inhibits 12-*O*-tetradecanoylphorbol-13-acetate-induced skin inflammation and tumor promotion by downregulation of inducible NO synthase and COX-2 *via* multiple signaling in mouse skin	Lai et al. (2007)

cancer cells, treatment with apigenin inhibits cell motility and invasion by alteration of cytoskeleton and accumulates focal adhesion proteins *via* decreased focal adhesion kinase (FAK)/Src signaling, thus promotes cell adhesion (Franzen et al. 2009). Otherwise, apigenin inhibits prostate tumor growth through epigenetic mechanism that evidenced by decreasing histone deacetylases (HDACs) activity that results in cell cycle arrest and apoptosis in nude mice (Pandey et al. 2012). Ovarian cancer cells treated with apigenin shows a synergistic effects in paclitaxel-induced apoptosis through increases of oxidative stress, indicating apigenin may act as a sensitizer with cancer therapy drugs (Xu et al. 2011).

Luteolin exists abundantly in thyme and also presents in beets, Brussels sprouts, cabbage and cauliflower that has been shown to possess chemopreventive and chemotherapeutic effects on various human cancers corresponding to its anti-oxidative, anti-proliferative, anti-invasion and apoptosis-inducing activity. In AOM-induced colon tumorigenesis model, orally treatment of luteolin at a dose of 1.2 mg/kg to rats reduces AOM-induced ACF formation through decreasing lipid peroxidation and increasing anti-oxidative enzyme activity that contributes to preventing malondialdehyde (MDA)-DNA adduct formation in rat colon (Ashokkumar and Sudhandiran 2008). In addition, luteolin decreases colonic tumor size by downregulation of Wnt/β-catenin signaling and downstream target gene *cyclin D1* expression (Ashokkumar and Sudhandiran 2011). A large body of studies demonstrates the effect of luteolin on lung cancer. Luteolin induces lung cancer cell apoptosis, cell cycle arrest, inhibits invasion and tumor growth *in vitro* and *in vivo* through multiple mechanisms, including downregulation of nuclear factor-κB (NF-κB) (Cai et al. 2011), decreasing HDACs activity (Attoub et al. 2011) and suppression of EMT (Ruan et al. 2012). Luteolin also enhances therapeutic efficacy in lung cancer xenograft model when it combines with tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) (Yan et al. 2012). It has been reported that the hepatoprotective property of luteolin is attributed to its ability on anti-fibrosis and reduction of hepatotoxicity induced by carbon tetrachloride (CCl_4) and D-galactosamine/lipopolysaccharide *in vivo* (Domitrovic et al. 2009; Lee et al. 2011a).

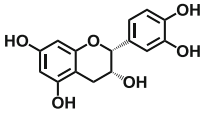
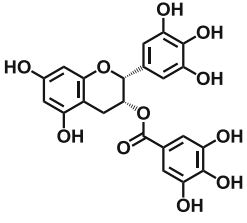
Nobiletin, tangeretin and 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone are polymethoxyflavones exist almost exclusively in citrus plants, particularly in citrus peel. Owning to the substituted methoxy groups, PMFs has a superior metabolic stability and membrane permeability over flavonoids (Wen and Walle 2006). Dietary feeding of nobiletin not only reduces ACF formation but also suppressed incidence and multiplicity of adenocarcinoma in AOM- and 2-amino-1-methyl-6-phenylimidazo [4,5-b]pyridine (PhIP)-treated animals (Suzuki et al. 2004; Tang et al. 2011). Nobiletin also completely abolished AOM/dextran sulfate sodium (DSS)-induced adenocarcinoma formation by reduction of leptin levels in ICR mice (Miyamoto et al. 2008). In PhIP-induced prostate cancer model, dietary feeding 0.05 % nobiletin significantly reduces number of prostate adenocarcinoma by suppression of cell proliferation (Tang et al. 2011). Nobiletin also displays anti-invasion activity in human gastric cancer cells by interfere with FAK and PI3K/AKT signaling and downstream target gene, *MMP-2* and *MMP-9* (Lee et al. 2011b).

Tangeretin exhibits anticancer property through modulation of intracellular signaling. Studies have supported the role of inflammation in the pathogenesis of lung cancer. Over-production of inflammatory mediators in lung cancer cells has been believed to implicate in tumor growth, invasion, migration and metastasis (Cho et al. 2011). Human lung carcinoma cells treated with tangeretin inhibits IL-1α-mediated NF-κB-dependent COX-2 expression *via* interference with multiple signaling molecules, such as p38 mitogen-activated protein kinase (MAPK), c-Jun N-terminal kinases (JNK) and Akt (Chen et al. 2007). In cisplatin-resistant human ovarian cancer cells, tangeretin synergistically induces apoptosis and cell cycle arrest with cisplatin by targeting PI3K/Akt signaling (Arafa et al. 2009). Tangeretin also inhibits human colon cancer and breast cancer cells proliferation through induction of G1 phase cell cycle arrest (Morley et al. 2007). 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone, a hydroxylated polymethoxyflavone, is particularly exists in the peels of sweet orange, has been reported to induce apoptosis in human leukemia cells and inhibit colony formation through downregulation of β-catenin and NF-κB in human colon cancer cells (Pan et al. 2007; Qiu et al. 2011). Topical application of 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone decreased expression levels of inducible NO synthase (iNOS) and COX-2, reduced nuclear translocation of NF-κB, and suppressed activation of extracellular regulated protein kinase (ERK) 1/2, p38 and AKT signaling in a 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced skin inflammation mouse model (Lai et al. 2007). Moreover, 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone inhibits 7,12-dimethylbenz[α]anthracene (DMBA)/TPA-induced skin tumor formation in mice. This study indicates that 5-hydroxy-3,6,7,8,3′,4′-hexamethoxyflavone is a potent chemopreventive agent.

2.3.3 Flavanols (Catechins) (Table 2.3)

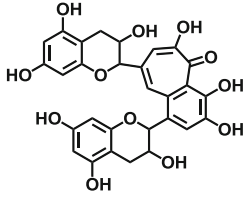
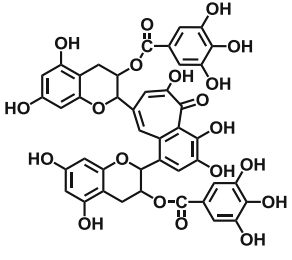
Tea is the most popular flavored and functional drink worldwide and possesses a broad spectrum of biological activities, including antioxidant, anti-carcinogenic, anti-inflammatory, anti-diabetic, anti-hyperlipidemia and anti-obesity. Green and black tea is account for about 20 and 78 % worldwide tea consumption, respectively. Nemours studies have demonstrated the potential of green tea and black tea on cancer chemoprevention, including colon cancer, prostate cancer, ovarian cancer and rectal cancer are attributing to their polyphenolic compounds, catechins and theaflavins (Yang et al. 2007; Yuan et al. 2011; Baker et al. 2007; Dora et al. 2003). In rats with acute myeloid leukemia, (−)-epicatechin (EC) is found to induce apoptosis of leukemia cells in the spleen but without cause toxic effect in splenocytes that may against acute myeloid leukemia (Papiez et al. 2010). When combine with curcumin, EC enhances growth inhibitory effect and induction of apoptosis in human lung cancer cell line (Saha et al. 2010). *In vivo* study also demonstrates that the combination of EC with vitamin E effectively protects nicotine-induced oxidative damage in rats (Al-Malki and Moselhy 2013). (−)-Epigallocatechin-3-gallate (EGCG), the most abundant catechin in green tea,

Table 2.3 Chemopreventive and chemotherapeutic effects of dietary flavanols (catechins) on human cancers

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
(−)-Epicatechin		Tea	Leukemia	Induces apoptosis of leukemia cells in the spleen in leukemic rats	Papiez et al. (2010)
			Lung cancer	Enhances growth inhibitory and apoptotic effect with curcumin by upregulation of growth arrest and DNA damage induced gene (*GADD*)*153* and *GADD45*	Saha et al. (2010)
				Enhances protective effect on against nicotine-induced oxidative stress with vitamin E by upregulation of antioxidants in rat	Al-Malki and Moselhy (2013)
(−)-Epigallocatechin-3-gallate		Tea	Melanoma	Inhibits cell invasion and migration through targeting PGE receptors/COX-2/ prostaglandin E_2 and EMT	Singh and Katiyar (2011)
			Colon cancer	Inhibits colorectal ACF formation by interfere with IGF/ IGF-IR and β-catenin signaling in AOM-induced colonic carcinogenesis model	Shimizu et al. (2008)
			Liver cancer	Protects CCl_4-induced liver fibrosis through reducing oxidative stress, collagen accumulation and inflammatory mediators production	Tipoe et al. (2010)
			Skin cancer	Reactivation of tumor suppressor genes through inhibiting DNA methyltransferases and decreasing HDAC activity	Nandakumar et al. (2011)
			Breast cancer	Inhibits nicotine and estrogen-induced cell proliferation through downregulation of 9-α nicotinic acetylcholine receptor	Tu et al. (2011)
			Lung cancer	Inhibits cell invasion and migration through downregulating JNK-dependent MMP-2 expression	Deng and Lin (2011)
			Gastric cancer		Wu et al. (2012)

(continued)

Table 2.3 (continued)

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
				Enhances docetaxel-induced gastric tumor growth inhibition by suppression of angiogenesis in xenograft model	
Theaflavin		Black tea	Breast cancer	Induces apoptosis through death receptor cascade and inhibits pAkt/pBad survival pathway in p53-mutated human breast cancer cells	Lahiry et al. (2010)
				Inhibits fatty acid synthase level in MCF-7 breast cancer cells	Yeh et al. (2003)
			Lung cancer	Against benzo[*a*]pyrene-induced lung carcinogenesis through induction of apoptosis and inhibition of COX-2 expression in mice	Banerjee et al. (2005, 2006)
				Inhibits bronchiolar cell proliferation and tumor formation in 4-(methyl nitrosamino)-1-(3-pyridyl)-1-butanone (NNK)-induced lung carcinogenesis in A/J mice	Yang et al. (1997)
			Melanoma	Induction of apoptosis through increasing ROS production and activation of JNK-p38 MAPK signaling	Bhattacharya et al. (2009)
				Suppression of *MMP-2* gene expression and activity by downregulation of EGFR and NF-κB signaling	Sil et al. (2010)
Theaflavin-3,3′-digallate (TF-3)		Black tea	Oral cancer	Induced apoptosis by increasing oxidative stress in human oral squamous cells	Schuck et al. (2008)
			Lung cancer	Induces cell cycle arrest combined with ascorbic acid in human lung adenocarcinoma cells	Li et al. (2010)

has been considered as a promising chemopreventive and anticancer agent. EGCG inhibits human melanoma cells invasion and migration which is associate with suppression of transition of mesenchymal stage to epithelial stage and endogenous expression of COX-2 and prostaglandin E_2 (PGE_2) (Singh and Katiyar 2011). Supplementation with 0.01 and 0.1 % EGCG in drinking water suppresses AOM-induced ACF formation by downregulation of insulin-like growth factor (IGF), β-catenin and downstream gene *cyclin D1* and *COX-2* in C57BL/KsJ-db/db mice (Shimizu et al. 2008). EGCG also displays hepatoprotective and anti-fibrosis effect evidenced by reduction of oxidative stress, collagen accumulation and inflammatory mediators production including tumor necrosis factor (TNF)-α, COX-2 and iNOS (Tipoe et al. 2010). In human skin cancer cells, EGCG function as an epigenetic regulator that reactivation of tumor suppressor genes, *Cip1*/*p21* and *p16INK4a*, *via* reduction of DNA methylation and increases of histone acetylation (Nandakumar et al. 2011). Human breast cancer cells treated with EGCG inhibits nicotine-induced proliferation by targeting on α9-α nicotinic acetylcholine receptor (nAChR) signaling pathway (Tu et al. 2011). Additionally, EGCG shows anti-invasion and anti-migration activity in invasive human lung cancer cells by induction of G2/M phase cell cycle arrest and JNK-dependent MMP-2 expression (Deng and Lin 2011). When combination with chemotherapeutic drug, docetaxel, EGCG demonstrates an enhanced effect on suppression of gastric xenograft tumor growth by decreasing tumor angiogenesis *in vivo* (Wu et al. 2012).

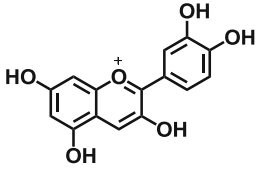
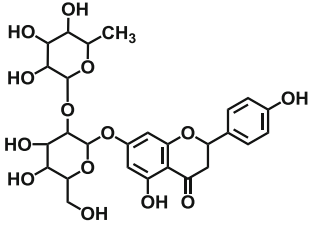
Theaflavins include theaflavin (TF1), theaflavin-3-*O*-gallate (TF2a), theaflavin-3′-*O*-gallate (TF2b) and theaflavin-3,3′-*O*,*O*-digallate (TF3) are major polyphenolic compounds in black tea. The anti-proliferative and apoptosis-inducing activity of TF1 has been documented in various cancer cell lines. Treatment with TF1 results in induction of death receptor/caspase-8 dependent apoptosis and inhibits pAkt/pBad survival signaling in p53-mutated human breast cancer cells that may reduce drug-resistance (Lahiry et al. 2010). TF1 also downregulates fatty acid synthase in human breast cancer MCF-7 cells that may contributes to reduce cell lipogenesis and proliferation (Yeh et al. 2003). Dietary TF-1 has been shown to against lung cancer in different animal model. Consumption of TF-1 prevents benzo [*a*]pyrene (BP)-induced lung carcinogenesis through induction of apoptosis and inhibition of COX-2 expression *in vivo* (Banerjee et al. 2005, 2006). Moreover, administration of TF-1 in drinking water reduces NNK-induced lung carcinogenesis caused in A/J mice (Yang et al. 1997). In human melanoma cell line, treatment with TF1 induces apoptosis *via* ROS generation and MAPKs signaling (Bhattacharya et al. 2009). Also, TF1 may against melanoma cell invasion by suppression of MMP-2 *via* downregulation of epidermal growth factor receptor (EGFR), ERK and NF-κB signaling (Sil et al. 2010). TF3, another theaflavin in black tea, is found to induce apoptosis *via* increased oxidative stress in human oral squamous cells (Schuck et al. 2008). TF3 also reveals synergistic effect on induction of cell cycle arrest when combined with ascorbic acid in human lung adenocarcinoma cells (Li et al. 2010).

2.3.4 *Flavonones (Table 2.4)*

Dietary consumption of 0.02 % naringenin, a naturally occurring citrus flavonone, has been found to suppress AOM-induced colonic ACF formation by decreasing cell proliferation and promoting apoptosis in colonocytes (Leonardi et al. 2010). Naringenin may be considered as immunomodulator supported by significantly reducing lung metastases in mice with pulmonary fibrosis through downregulation of transforming growth factor (TGF)-α1 and reducing regulatory T cells that involved in creation of immunosuppressive environment within tumor tissue (Du et al. 2009). Administration of naringenin reduces tumor growth by modulation of redox statue that against *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced gastric carcinogenesis in rats (Ekambaram et al. 2008). In cerebrally implanted C6 glioma cells rat model, naringenin is found to increase expression of connexin 43 (Cx43), a molecule involved in gap junction, thus promotes apoptosis of glioma cells (Sabarinathan et al. 2010). Naringenin also has been reported to protect against UVB-induced DNA damage by accelerating of cyclobutane pyrimidine dimers (CPD) removal and decreasing apoptosis in human keratinocytes HaCaT cells (El-Mahdy et al. 2008). Naringin is another flavonone naturally occurs in citrus. Administration of naringin at a dose of 25 mg/kg is effective on reducing tumor growth by downregulation of inflammatory cytokines TNF-α and IL-6 in rats with Walker 256 carcinosarcoma (Camargo et al. 2012). Naringin also demonstrates protective property against carcinogen-induced lung injury by increasing antioxidant, downregulation of inflammatory cytokine production and suppression of neutrophil infiltration in cigarette smoke (CS) and lipopolysaccharides (LPS)-treated animal models (Luo et al. 2012; Liu et al. 2011).

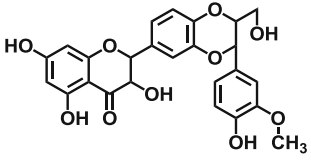
Silibinin is the major flavonolignan isolated from seed of milk thistle (*Silybum marianum*), has been believed to possess anticancer efficacy and liver protective effect with its mixture form, similarly. Dietary 0.5 % silibinin induces apoptosis and inhibits angiogenesis *via* downregulation of Bcl-2, survivin and VEGF expression, thus suppresses prostate tumor growth in nude mice (Singh et al. 2007). In metastatic prostate cancer cells, silibinin causes suppression of invasive property by reversing EMT *via* targeting on NF-κB, vimentin and MMP2 (Wu et al. 2010). Silibinin treatment induces apoptosis in human bladder cancer cells and bladder xenograft tumor growth through interfering with STAT3 signaling, and caspase- dependent and -independent pathway (Agarwal et al. 2007; Zeng et al. 2011). Orally feeding silibinin at a dose of 750 mg/kg suppressed colonic tumorigenesis through inhibition of cell proliferation (β-catenin, cyclin D1), inflammation (iNOS, COX-2), angiogenesis (VEGF) and induction of apoptosis in AOM-treated A/J mice and $APC^{min/+}$ mice (Ravichandran et al. 2010; Rajamanickam et al. 2010). In addition, silibinin displays anti-metastatic property evidenced by inhibition of migration and adhesion *via* decreasing cell division control protein 42 (Cdc42) and D4-GDI (a Rho GTPases regulator) that may prevent metastasis of human highly metastatic breast cancer cell to distant organs (Dastpeyman et al. 2011). In human cervical cancer cell line, silibinin treatment induces both apoptosis and autophagy by increasing of oxidative stress including ROS and reactive nitrogen species (RNS) (Fan et al. 2011).

Table 2.4 Chemopreventive and chemotherapeutic effects of dietary flavanones on human cancers

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
Naringenin		Orange peel	Colon cancer	Suppresses AOM-induced ACF formation by inhibiting colonocyte proliferation and increasing apoptosis	Leonardi et al. (2010)
			Lung cancer	Reduces lung metastases in mice with pulmonary fibrosis by downregulation of TGF-α1 and regulator T cells	Du et al. (2009)
			Gastric cancer	Reduces *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine-induced gastric carcinogenesis by upregulation of redox status	Ekambaram et al. (2008)
			Glioma	Promotes apoptosis through mitochondrial pathway and Cx43 in cerebrally implanted C6 glioma cells rat model	Sabarinathan et al. (2010)
			Skin cancer	Protects UVB-induced DNA damage by removal of cyclobutane pyrimidine dimers and apoptosis in human keratinocytes	El-Mahdy et al. (2008)
Naringin			Carcinosarcoma	Reduces tumor growth by decreasing TNF-α and IL-6 levels *in vivo*	Camargo et al. (2012)
			Lung cancer	Inhibits cigarette smoke exposure-induced chronic bronchitis by reducing inflammatory cytokines production and increasing SOD activity	Luo et al. (2012)
				Reduces lipopolysaccharides-induced lung injury and edema by decreasing neutrophil infiltration and TNF-α secretion *via*	Liu et al. (2011)

(continued)

Table 2.4 (continued)

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
				interference with NF-κB	
Silibinin		Milk thistle	Prostate cancer	Suppresses prostate xenographic tumor growth by induction of apoptosis and inhibition of angiogenesis	Singh et al. (2007)
				Reduces invasive property of metastatic prostate cancer cells by alteration of EMT *via* targeting NF-κB	Wu et al. (2010)
			Bladder cancer	Inhibits activation of STAT3 in DU145 cancer cells and inhibits bladder xenograft tumor growth through induction of caspase-dependent and -independent apoptosis	Agarwal et al. (2007); Zeng et al. (2011)
			Colon cancer	Inhibits colonic tumorigenesis through downregulation of inflammatory and angiogenic molecules and induction of apoptosis in AOM-treated and $APC^{min/+}$ mice	Ravichandran et al. (2010); Rajamanickam et al. (2010)
			Breast cancer	Inhibits cell migration and adhesion by decreasing cell division control protein 42 and D4-GDI levels	Dastpeyman et al. (2011)
			Cervical cancer	Induces apoptosis and autophagy by increasing ROS and reactive nitrogen species	Fan et al. (2011)

2.3.5 *Anthocyanidins (Table 2.5)*

Anthocyanidins are water-soluble glycosides and common plant pigments that give the red and blue colors in many cereal grains, and flowers, fruits and vegetables such as blueberries and grapes. Cyanidin is reported to synergistic against H_2O_2-induced cytotoxicity with peroxisome proliferator-activated receptors (PPAR) agonist through decreasing oxidative stress *via* activation of NF-E2-related factor 2 (Nrf2), an important transcription factor of antioxidant enzymes (Shih et al. 2012). Cyanidin also reduces UVB-induced COX-2 expression through targeting multiple signalings, such as MKK4, MAP kinase (MEK1) and Raf-1 in epidermal cells that contributes to suppression of UVB-induced inflammatory response in skin (Kim et al. 2010b). Cyanidin-3-glucoside, another natural colorant found in bilberries and other fruits, is shown to scavenge UVB-induced free radicals, block TPA-induced neoplastic transformation in epidermal cells, and decrease tumor number in DMBA/TPA skin tumorigenesis model *via* downregulation of COX-2 and TNF-α production (Ding et al. 2006). In *in vitro* and *in vivo* study, cyanidin-3-glucoside effectively suppresses lung cancer cell proliferation and metastasis by reduction of invasion and migration *via* decreasing MMP-2 level (Chen et al. 2006; Ding et al. 2006).

Delphinidin also shows chemoprotective and anticancer effects against prostate, breast and liver cancer. Studies show that delphinidin modulates NF-κB signaling that induces apoptosis, cell cycle arrest and inhibits prostate tumor growth *in vitro* and *in vivo* (Bin et al. 2008; Hafeez et al. 2008). In human breast cancer cells, treatment of delphinidin causes apoptosis through decreasing HER2 and ERK1/2 signalings (Ozbay and Nahta 2011). Administration with delphinidin inhibits CCl_4-indcued oxidative stress, reduces collagen accumulation, inactivates hepatic stellate cells (HSC) and restores hepatic injury that contribute to reduce liver fibrosis (Domitrovic and Jakovac 2010).

2.3.6 *Isoflavones (Table 2.6)*

Isoflavones are usually recognized as phytoestrogen compounds which rich in soybeans. Many studies have revealed the health benefits of soybeans are derived from isoflavones, such as anti-atherosclerotic and anticancer. Genistein and daidzein are major isoflavones that abundantly present in soybeans, possess chemopreventive and anticancer activity evidenced by numerous *in vitro* and *in vivo* studies. In human ovarian cancer cells, treatment of genistein induces both apoptosis and autophagy as well as decreases glucose uptake *via* downregulation of Akt that may contribute towards a mechanism to limit glucose utilization (Gossner et al. 2007). Genistein is effective on drug-resistant ovarian cancer by sensitizing cisplatin-induced apoptosis *via* targeting on NF-κB (Solomon et al. 2008). Dietary genistein displays protective effect against 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced chronic colitis *via*

Table 2.5 Chemopreventive and chemotherapeutic effects of dietary anthocyanidins on human cancers

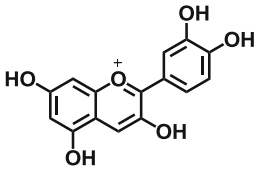
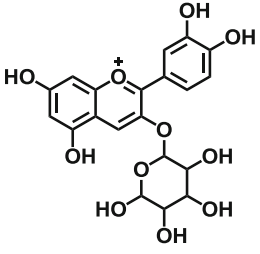
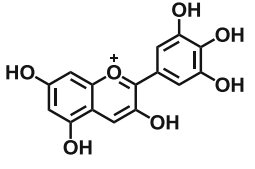
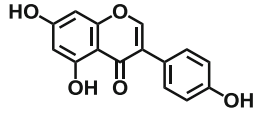
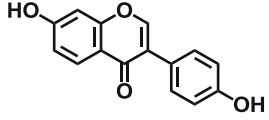
Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
Cyanidin		Cherries and strawberries	Liver cancer	Synergistic against nonalcoholic steatohepatitis-induced oxidative stress and cytotoxicity with peroxisome proliferator-activated receptors agonist through activation of NRF-2	Shih et al. (2012)
			Skin cancer	Suppresses UVB-induced COX-2 expression through interfere with MKK4, MAP kinase and Raf-1 activity	Kim et al. (2010b)
Cyanidin-3-glucoside		Blackberry	Skin cancer	Scavenges UVB-induced free radicals, inhibits TPA triggered neoplastic transformation and suppresses DMBA/TPA-induced skin tumor by decreasing inflammatory mediators production	Ding et al. (2006)
			Lung cancer	Inhibits lung cancer cell proliferation and metastasis in xenograft model by suppression of migration and invasion	Ding et al. (2006)
				Inhibits lung cancer cell migration and invasion by downregulation of MMP-2 and urokinase-plasminogen activator	Chen et al. (2006)
Delphinidin		Dark fruits and vegetables	Prostate cancer	Induces apoptosis, cell cycle arrest and inhibits tumor growth through interfere with NF-κB signaling *in vitro* and *in vivo*	Hafeez et al. (2008); Bin et al. (2008)
			Breast cancer	Induces apoptosis through inhibition of HER2 and ERK1/2 signaling	Ozbay and Nahta (2011)
			Liver cancer	Against CCl_4-induced liver fibrosis through decreasing oxidative stress, promoting extracellular matrix degradation and inactivation of hepatic stellate cells	Domitrovic and Jakovac (2010)

Table 2.6 Chemopreventive and chemotherapeutic effects of dietary isoflavones on human cancers

Compound	Structure	Dietary source	Target cancer	Molecular mechanism	References
Genistein		Soybean	Ovarian cancer	Induces autophagy and inhibits glucose uptake through downregulation of Akt	Gossner et al. (2007)
				Sensitizes cisplatin-induced cytotoxicity through induction of apoptosis and inhibition of NF-κB	Solomon et al. (2008)
			Colon cancer	Reduces 2,4,6-trinitrobenzenesulfonic acid-induced chronic colitis by decreasing COX-2 levels and myeloperoxidase activity	Seibel et al. (2009)
				Synergistic induces apoptosis with indol-3-carbinol through inhibition of Akt	Nakamura et al. (2009)
			Prostate cancer	Inhibits lung micrometastasis by increasing adhesion *via* upregulation of promotility proteins in nude mice	Lakshman et al. (2008)
				Reduces *N*-methylnitrosourea-induced advanced prostate cancer by increasing apoptosis and inhibiting proliferation *via* Akt/ phosphatase and tensin homolog signaling	Wang et al. (2009)
				Reduces COX-2 and PGE_2 levels in prostate cancer patients	Swami et al. (2009)
Daidzein		Soybean	Glioma	Enhances therapeutic effect with TRAIL by increasing apoptosis *via* downregulation of Bcl2 in chemoresistance glioma cells	Siegelin et al. (2009)
			Breast cancer	Inhibits proliferation by inducing G1 and G2/M cell cycle arrest	Choi and Kim (2008)
			Skin cancer	Synergistically protects UVB-induced DNA damage and reducing COX-2 levels with genistein	Iovine et al. (2011)
			Liver cancer	Against DMBA-induced oxidative stress *via* increasing antioxidant status and reduction of hepatocyte apoptosis in liver	Choi and Kim (2009)

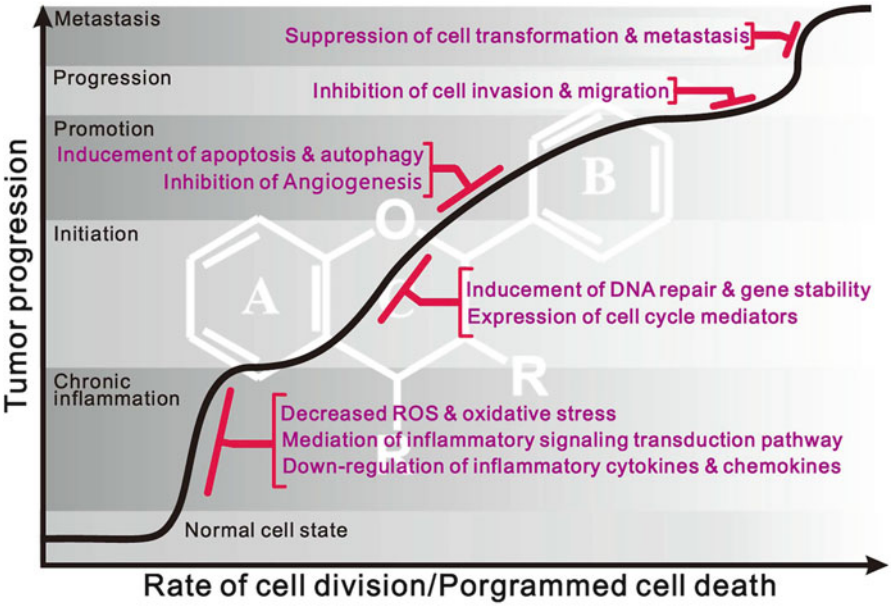


Fig. 2.3 Proposed mechanism of flavonoids from fruits and vegetables on cancer chemoprevention and chemotherapy

anti-inflammatory mechanism, including reduction of COX-2 expression and myeloperoxidase (MPO) activity (Seibel et al. 2009). In combination with indole-3-carbinol, a breakdown product of the glucobrassicin which can be found at relatively high levels in cruciferous vegetables, synergistically induces apoptosis through interfere with Akt in human colon cancer cells (Nakamura et al. 2009). Several studies demonstrate the potential of genistein against prostate cancer. Dietary genistein effectively inhibits lung micrometastasis of orthotopically implanted human prostate cancer cells by increasing promotility proteins that contributes to increase adhesion property of cancer cells (Lakshman et al. 2008). Genistein also reduces *N*-methylnitrosourea (NMU)-induced advanced prostate cancer *in vivo* through targeting on phosphatase and tensin homolog, known as a tumor suppressor, and Akt signaling, thus suppression of cell proliferation and increase of apoptosis (Wang et al. 2009). In prostate cancer patients, supplement with genistein significant decreases prostate COX-2/PGE_2 levels that may beneficial to the treatment prostate cancer (Swami et al. 2009).

Daidzein, another isoflavone exists in soybeans, is found as chemotherapy sensitizer supported by enhancement of TRAIL-induced apoptosis in chemoresistance glioma cells (Siegelin et al. 2009). Treatment of daidzein causes cell proliferation inhibition through induction of G1 and G2/M phase cell cycle arrest in different human breast cancer cells (Choi and Kim 2008). Additionally, combination of daidzein and genistein shows a synergistically protective effect against UVB-induced DNA damage and decreasing COX-2 levels in human fibroblasts, indicating a

protective role for UVB-induced skin damage and inflammation (Iovine et al. 2011). Orally feeding daidzein (5 and 25 mg/kg) displays hepatoprotective efficacy against DMBA-induced oxidative stress, increasing antioxidant statue (glutathione peroxidase and reductase) and reducing hepatocytes apoptosis in mouse liver (Choi and Kim 2009).

2.4 Conclusions and Perspectives

Cancer is the major challenge to human health. In concept of overcome this challenge, there is needed new approach to control cancer development through cancer chemoprevention or chemotherapy by specific/multi-targets to improve efficiency of conventional therapies. Natural compounds from diet are now considered to offer great potential in the prevention and management of cancer. Flavonoids are widely present in vegetables, fruits and edible plants that display great cancer chemopreventive and chemotherapeutic effects on various human cancers. Their possible mechanism includes interference in several of the steps that lead to the development of malignant tumors, such as protecting DNA from oxidative damage, inhibiting carcinogen activation, and activating carcinogen detoxifying systems (Fig. 2.3). They also inhibit the promotion stage of carcinogenesis by inhibiting oxygen radical-forming enzymes or enzymes that contribute to DNA synthesis or acts on inhibition of signaling molecules that contribute to proliferation, inflammation, EMT, angiogenesis, invasion and migration. Finally, they may prevent tumor development by inducing programmed cell death of tumor cell including apoptosis and autophagy as well as trigger cell cycle arrest.

Despite a number of studies have addressed the anticancer effect of flavonoids from fruits and vegetables, little is known about the mechanism of action of most compounds. Flavonoids can directly and indirectly influence cancer development likely to be an integrated effect of several distinct mechanisms. Therefore, identify specific targets and understanding of the critical events associated with tumorigenesis would provide the better investigation of their underlying mechanism. In addition, many *in vitro* and *in vivo* researches have reported the effects of flavonoids on various human cancers, but not final conclusion. In cell culture system and animal study, the dosage of flavonoids may not be attained in our regular diet. Although several epidemiological research report the efficacy of dietary flavonoids on against human cancer, but some are still contradictory. Before application of general public, well-designed and carefully clinical studies should be evaluated in intervention trials for potential of flavonoids as cancer chemopreventive agents. Moreover, the absorption, bioavailability, metabolism and pharmacokinetic properties are important issues for dietary intervention of flavonoids on human cancers. In fact, the structure and functional group of some flavonoids might limit their oral bioavailability. Poor absorption and extensive conjugative metabolisms in the intestine and liver greatly limit bioavailability of dietary flavonoids. Constructing an appropriate vehicle and the desired efficient formulation possess a challenge to dietary supplement

researchers. It is also possible that these dietary flavonoids can be used as sensitizers to enhance the efficacy of other known chemotherapeutic agent that offer effective approach on malignant or chemoresistance cancers. Further mechanistic insights are needed to elucidate for dietary flavonoids on cancer chemoprevention and treatment that should provide innovative approaches for control of cancers.

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Chapter 3
Beneficial Influence of Diets Enriched with Flaxseed and Flaxseed Oil on Cancer

Ashleigh K. Wiggins, Julie K. Mason, and Lilian U. Thompson

Abstract Dietary flaxseed and flaxseed oil are commonly consumed for their suggested anticancer effects. Flaxseed oil has an exceptionally high level of the omega-3 fatty acid α-linolenic acid and flaxseed is also the richest dietary source of phytoestrogens called lignans. This chapter provides information on flaxseed and flaxseed oil, including their composition and effects on the prevention and treatment of cancer. The major focus is on the effects in breast, colorectal and prostate cancer as observed in preclinical studies in cell culture and animal models, epidemiological and clinical studies. Limited studies on the effects in other forms of cancer are also discussed. Recent evidence supporting a potential anticancer role of flaxseed and flaxseed oil is for breast cancer. Extensive studies in rodent models suggest that flaxseed and its oil can reduce the various stages of carcinogenesis and there is increasing support from epidemiological and clinical studies. Studies in rodent models also suggest that flaxseed and its oil do not interfere with and may rather enhance the action of breast cancer drugs, including tamoxifen and trastuzumab. Regarding colorectal and prostate cancer, there are fewer studies with less consistent results. However, a protective effect is shown in general studies. The research in other forms of cancer is limited, inconsistent and warrants further investigation. Potential mechanisms of the action of flaxseed oil including effects on the properties of the cell membrane, the regulation of transcription, lipid peroxidation and others are discussed. Safety of diets enriched in flaxseed and flaxseed oil and flaxseed's regulatory status are outlined. Current limitations in the research and future directions are provided.

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3.1 Introduction

Cancer is one of the leading causes of death in the Western world (World Cancer Research Fund/American Institute for Cancer Research 2007). In North America, colorectal, prostate and breast are three of the most common and deadliest forms of cancer (Canadian Cancer Society 2010; American Cancer Society 2011). Although improvements in screening, detection and treatment have been made, strategies for prevention and further improvement of treatment outcomes are being sought. The role of bioactive dietary components in preventing and treating cancer has been a research focus for many years. Many cancer patients look to complementary and alternative medicine (CAM) to prevent cancer and assist traditional cancer therapy. A number of dietary compounds have been recommended for cancer treatment or prevention and are readily available to the general public through published books and websites. Studies have shown that flaxseed and flaxseed oil are among the most commonly used dietary supplements among cancer patients in North America (Boon et al. 2007; Greenlee et al. 2009; Rausch et al. 2010; Anderson and Taylor 2012; Boucher et al. 2012). Thus, there is a need to understand the scientific support for these agents.

This chapter will review the evidence for the role of flaxseed oil and its source, flaxseed, in the prevention and treatment of breast, colorectal and prostate cancer. Limited studies on the effect of flaxseed and its oil in other cancer types will also be discussed. Results from preclinical studies in rodent models and cell culture experiments, as well as observational and experimental human studies will be described and summarized. Potential mechanisms of effect and safety aspects of flaxseed and flaxseed oil rich diets will be discussed. Finally, comments on the limitations, current research gaps and future directions in the use of flaxseed oil-rich diets will be provided.

3.2 Flaxseed and Flaxseed Oil Composition

Of interest and the main reason for the use of flaxseed as a dietary supplement for health benefits is its high amount of oil, rich in the omega-3 (n-3) polyunsaturated fatty acid (PUFA) α-linolenic acid (ALA), the high amount of dietary fiber, high quality protein, and phytoestrogens called lignans. While flaxseed's exact composition varies by growth location, cultivar, and environment, it typically contains approximately 30 % dietary fiber, 20 % protein, 40 % oil and 820–1,050 μmol lignan per 100 g of flaxseed (Daun et al. 2003; Liu et al. 2006; Thompson et al. 2006).

Flaxseed oil's effect in reducing cancer growth has been of growing interest in recent years. The approximate composition of flaxseed oil is shown in Table 3.1 (Daun et al. 2003). Flaxseed oil is comprised primarily of neutral lipids (acylglycerols and fatty acids) and some polar lipids (glycolipids and phospholipids). About 57 % of flaxseed oil is ALA, an essential fatty acid, which

Table 3.1 Approximate fatty acid composition of flaxseed oil

Fatty acid class	% of total fat
Saturates	9.0
Monounsaturates	18.0
Polyunsaturates	73.0
Linoleic acid	16.0
α-linolenic acid	57.0

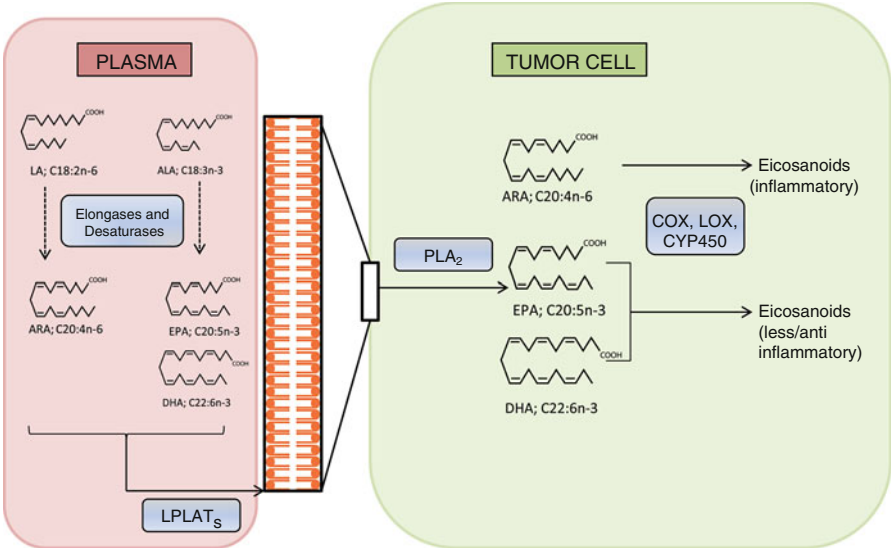

Fig. 3.1 n-3 and n-6 fatty acid metabolism. ALA and LA, the parent n-3 and n-6 fatty acids are converted by elongase and desaturase enzymes into the long chain fatty acids ARA (n-6), EPA (n-3) and DHA (n-3). Free PUFA are esterified into the membrane phospholipids by LPLATs and can then be released to the intracellular pool by PLA2. Free ARA, EPA and DHA are converted into eicosanoids through the actions of COX, LOX and CYP450 enzymes. n-3 and n-6 derived eicosanoids have different biological effects. *ALA* α-linolenic acid, *ARA* arachidonic acid, *COX* cyclooxygenases, *CYP450* cytochrome P450, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid, *LA* linoleic acid, *LOX* lipoxygenases, *LPLATS* lysophospholipid acyltransferases, *PLA2* phospholipase A2

can be metabolized to a limited extent to the longer chain n-3 PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), through a series of elongation (addition of 2 carbon) and desaturation (double bond insertion) steps (Cunnane 2003; Hall et al. 2006) (Fig. 3.1). Flaxseed is the richest plant source of ALA. Other sources rich in ALA include the chia seed, walnuts, perilla, canola, soybean and their oils.

This chapter focuses primarily on the specific effects of flaxseed oil as well as its source, flaxseed, on breast, colorectal and prostate carcinogenesis. It is important to note, however, that effects observed in intervention studies using flaxseed may not necessarily be solely due to the oil but also to other components including its lignan, fiber and protein.

3.3 Flaxseed and Cancer

The majority of the research focusing on the effect of flaxseed and flaxseed oil in cancer has been in hormone-related cancers, especially breast cancer. This came about because initial interest on the effect of flaxseed in cancer relates to its high amount of lignan. The predominant lignan in flaxseed is secoisolariciresinol diglucoside (SDG), which can be metabolized by colonic microbiota to the mammalian lignans enterodiol and enterolactone (Thompson et al. 1991, 2006). These mammalian lignans have chemical structural similarity to 17β-estradiol (E2), thus they are thought to have antiestrogenic/estrogenic properties that may influence hormone-related diseases, such as breast cancer (Adlercreutz 2007).

Elevated exposure to E2 is known to modulate breast cancer risk and factors that increase lifetime exposure to E2 such as early menarche, late menopause, hormone therapy use and adiposity are independent risk factors for breast cancer. As the research in the field of flaxseed and breast cancer progressed, the focus on the effect of the whole seed moved on to the study of the effect of its individual components including flaxseed oil and lignans. Through this research, support for the potential anticancer role of ALA-rich flaxseed oil developed. Unlike lignans, the proposed mechanisms of flaxseed oil effect do not relate directly to hormonal effects. Therefore, while ALA-rich flaxseed oil has mainly been studied in hormone-related cancers, effects have been observed in other types of cancer as well.

A number of preclinical models outlined in Fig. 3.2 have been useful in elucidating the effects of dietary components in carcinogenesis. These include rodent and cell culture models which have been used to study the effect of dietary agents such as flaxseed and flaxseed oil from a prevention or treatment perspective.

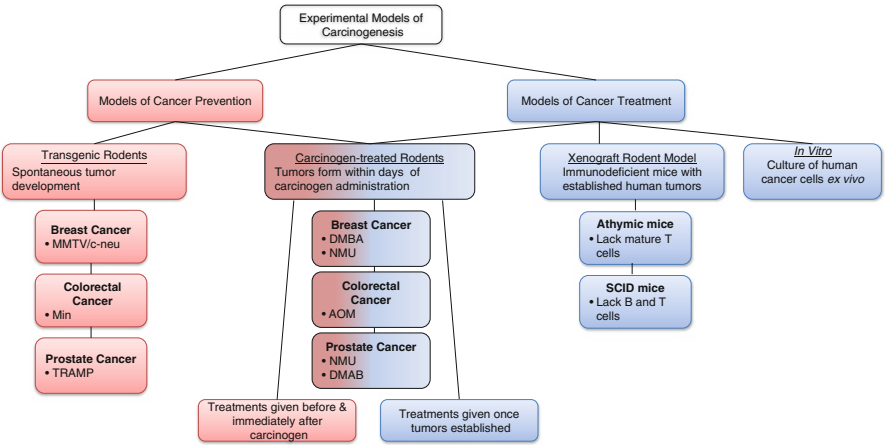

Fig. 3.2 Experimental models of carcinogenesis. *AOM* azoxymethane, *DMAB* 3,2′-dimethyl-4-aminobiphenyl, *DMBA* dimethylbenz(α)anthracene, *MMTV* mouse mammary tumor virus, *NMU* *N*-nitrosomethyl-urea, *SCID* severe combined immunodeficiency, *TRAMP* transgenic adenocarcinoma of the mouse prostate

3.4 Studies of Flaxseed and Flaxseed Oil in Breast Cancer

Breast cancer is a heterogeneous disease in terms of invasiveness, initiation site (duct or lobule), as well as cell receptor expression. Important protein receptors that can modify growth, prognosis and treatment are the estrogen receptor (ER) and the human epidermal growth factor receptor-2 (HER2). Based on these receptors, breast cancers are divided into molecular subtypes which have different prognosis and treatment approaches (Carey et al. 2006; Yang et al. 2007). When investigating potential chemopreventative or treatment options for breast cancer such as flaxseed, the specific subtypes and receptor status should be considered.

3.4.1 Breast Cancer Prevention

3.4.1.1 Preclinical Studies

The carcinogen-induced rodent model has been a useful tool in elucidating the effect of flaxseed and its components in the prevention of breast cancer (Table 3.2). The effect of flaxseed in breast cancer was first evaluated using rat models of the various stages of carcinogenesis (pre-initiation, initiation and promotion). The first study looked at the effects of diets rich in flaxseed on early markers of mammary carcinogenesis. Sprague-Dawley rats were fed high fat diets containing either no flaxseed (control), 5 or 10 % whole ground flaxseed (FF) or 5 or 10 % defatted flaxseed meal (FM) all matched for macronutrient and caloric content. The FF diets contributed 1.9–3.8 % flaxseed oil whereas the FM diets contributed 0.14–0.28 % flaxseed oil. Diets were fed for 4 weeks and measures of mitotic index, cell proliferation and nuclear aberrations after administration of the carcinogen dimethylbenz(α)anthracene (DMBA) in various structures of the mammary gland were conducted. The mitotic index in the terminal end buds (TEB), structures thought to be most highly implicated in carcinogenesis, was significantly lower in rats fed the 5 and 10 % FF diets with no significant reduction in those fed the FM diet. Similarly, cell proliferation and nuclear aberrations were significantly lower only in the TEB of rats fed the 5 % FF diet. The greater effect observed in the FF group which contains flaxseed oil suggests that the oil is likely playing an important role in flaxseed's anticancer effect at the pre-initiation stages (Serraino and Thompson 1991). In a follow up study by Serraino and Thompson (1992a), the effect of a 5 % FF diet on DMBA-induced carcinogenesis was shown to be complex. Rats were treated with DMBA at 50 days of age and followed for 21 weeks. The FF diet was fed either (i) throughout the study period (initiation and promotion), (ii) for 4 weeks prior to DMBA and followed by feeding with the control diet (initiation only) or (iii) starting 1 week after DMBA administration (promotion). Interestingly, when looking at the effect of flaxseed feeding throughout the study period (initiation and promotion) there was no difference in tumor

Table 3.2 Summary of studies investigating effect of flaxseed, flaxseed oil and ALA on breast cancer

Model	Treatments/measures	Results	References
In vitro studies			
MCF-7 cells	50 μM ALA + 1nM E2 for 5 days	↓ cell proliferation by 33 %	Truan et al. (2010)
MCF-7 cells	Up to 100 μM ALA for 24, 48, 72 h	↓ cell growth dose and time dependently ↑ apoptosis dose dependently	Kim et al. (2009)
MCF-7, MDA MB 231 cells	71.83 μM ALA, 5 days	↓ cell growth in MDA MB 231 but not MCF-7 No effect on cell viability	Chajes et al. (1995)
MDA MB 231	10–200 μM ALA, 24 h	↓ cell number	Horia and Watkins (2005)
SKBr3, BT 474 cells	10–20 μM ALA ± trastuzumab, 48 h	↓ HER2 expression dose dependently ↓ cell proliferation when ALA combined with trastuzumab	Menendez et al. (2006)
In vivo animal studies			
OVX athymic mice with MCF-7 xenografts	BD, FSO (38.5 g/kg), SDG (1 g/kg) and FSO + SDG Low E2	↑ tumor regression rate in all groups *vs*. control ↓ cell proliferation in all groups compared to control No effect on apoptosis	Saggar et al. (2010b)
OVX athymic mice with MCF-7 xenografts	BD, 10 % FS Low E2	↓ tumor growth, cell proliferation and ↑ apoptosis in FS *vs*. control	Chen et al. (2009)
OVX athymic mice with MCF-7 xenografts	BD, 10 % FS diet Low E2	No difference in tumor area, cell proliferation or apoptosis in FS *vs*. control	Power et al. (2008); Saarinen et al. (2006)
OVX athymic mice with MCF-7 xenografts	BD, 4 % FSO High E2	↓ tumor growth, cell proliferation and ↑ apoptosis in FSO *vs*. control	Truan et al. (2010)
OVX athymic mice with MCF-7 xenografts	BD, ED (15 mg/kg), EL (15 mg/kg) or 10 % FS High E2	↓ tumor growth and angiogenesis in all treatments *vs*. control	Bergman Jungestrom et al. (2007)

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
Athymic mice with MDA-MB-435 xenografts	BD, 10 % FS, SDG and FSO at levels present in 10 % FS or SDG + FSO High E2	↓ tumor growth, cell proliferation and ↑ apoptosis in all treatments except SDG *vs.* control	Wang et al. (2005)
Athymic mice with MDA-MB-435 xenografts	BD, 10 % FS High E2	↓ tumor growth and cell proliferation in FS compared to control	Chen et al. (2002)
Sprague-Dawley rats with DMBA-induced tumors (progression and tumor development stages)	BD, 2.5 or 5 % FS diet or FSO or SDG at levels present in 5 % FS	↓ established tumor growth in 2.5 and 5 % FS and FO compared to control; no effect of SDG	Thompson et al. (1996)
	Diet treatment started 13 weeks post DMBA	↓ new tumor volume in SDG *vs.* control; no effect of 2.5 or 5 % FS or FO No difference in tumor incidence and number between groups	
Sprague-Dawley rats with DMBA-induced tumors (initiation and early promotion stages)	BD, 5 % FS diet	↓ tumor size in rats fed FS at promotional stage; no effect of FS fed at initiation	Serraino and Thompson (1992a)
	FS fed at (i) initiation, (ii) early promotion or (iii) initiation and promotion	↑ tumor burden in promotion only *vs.* initiation and promotion FS groups	
Sprague-Dawley rats with DMBA-induced (initiation stage)	BD, 5 or 10 % FS flour (FF; 1.9–3.8 % FO) or defatted FS meal (FM; 0.14–0.28 % FO)	↓ mitotic index in terminal end buds of 5 % and 10 % FF groups	Serraino and Thompson (1991)
	Diets fed for 4 weeks pre DMBA exposure and rats sacrificed 24 h post DMBA	↓ cell proliferation in terminal end buds of 5 % FF groups ↓ nuclear aberrations in terminal end buds of 5 % FF, in terminal duct of 5 and 10 % FM, in alveolar buds of 10 % FF and 10 % FM	
Sprague-Dawley rats with NMU-induced tumors (early promotion stage)	BD, 2.5 or 5 % FS	↓ tumor invasiveness and grade in 2.5 and 5 % FS *vs.* control	Rickard et al. (1999)
	Diet treatment started 2 days post NMU	No effects on final tumor weight, volume, multiplicity and incidence	

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
Sprague-Dawley rats with NMU-induced (initiation stage)	Diets contained either 15 % FSO or 15 % palm oil/sunflower oil	↑ tumor growth FSO + vit E compared to FSO – vit E; no difference in tumor area and multiplicity, latency or incidence	Cognault et al. (2000)
	FSO ± vit E and + vit E + oxidant	↓ tumor area, multiplicity, incidence and number in FSO + vit E + oxidant compared to FSO + vit E	
Tg.NK (MMTV-c-neu) model	BD, FS diets (0.006, 0.018, 0.054 %) starting at day 25	↓ tumor incidence, number of tumors per mouse and number of large tumors in 0.054 % FS group *vs.* control	Birkved et al. (2011)
		No effect on the number of tumor bearing mice and tumor multiplicity	
Tg.NK (MMTV-c-neu) model	Gavage of FSO or melatonin in corn oil starting at 4 weeks of age	No significant effect of FSO on tumor incidence, multiplicity	Rao et al. (2000)
	Varying dose of FSO	Trend toward ↓ number of tumors/mouse in high dose FSO	
		↓ weight of tumors/mouse and mean tumor weight in high dose FSO group	
Athymic mice with 410 and 410.4 xenografts	BD, FSO or 4:1 fish oil (FO):corn oil (CO) fed (i) before implantation, (ii) before implantation with removal of primary tumor, (iii) after implantation	(i) No difference in tumor incidence or tumor size	Fritsche and Johnson (1990)
		(ii) Primary tumors grew faster and were larger in the FSO group *vs.* CO	
		(iii) Primary tumors were smallest in the FSO group *vs.* FO and lowest metastasis in FSO	

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
In vivo animal studies: drug-diet interaction			
OVX athymic mice with BT-474 xenografts	TRAS ± FSO (80 g/kg)	↓ tumor area, cell proliferation and ↑ apoptosis in FSO + TRAS2.5 *vs.* TRAS2.5	Mason et al. (2010)
OVX athymic mice with MCF-7 xenografts	BD, FSO (38.5 g/kg), SDG (1 g/kg) and FO + SDG ± TAM Low E2	↓ tumor growth, cell proliferation and ↑ apoptosis in all treatment groups *vs.* control FSO and FSO + SDG had the greatest effects	Saggar et al. (2010a)
OVX athymic mice with MCF-7 xenografts	BD ± TAM, ± 5, 10 % FS Low E2	↓ tumor regrowth, cell proliferation and ↑ apoptosis in TAM + 10 % FS *vs.* TAM alone	Chen et al. (2007b)
OVX athymic mice with MCF-7 xenografts	BD ± TAM, ± 5, 10 % FS High E2	↓ tumor growth, cell proliferation and ↑ apoptosis in all groups *vs.* control 10 % FS as effective as TAM alone; TAM + 5 % FS more effective than TAM or 5 % alone in ↓ tumor growth	Chen et al. (2007a)
OVX athymic mice with MCF-7 xenografts	BD ± TAM, ± 10 % FS Low and high E2	Low E2: ↓ tumor growth, cell proliferation and ↑ apoptosis in FS and FS + TAM *vs.* TAM and control High E2: ↓ tumor growth, cell proliferation and ↑ apoptosis in all treatments *vs.* control; ↓ cell proliferation in FS + TAM *vs.* TAM alone	Chen et al. (2004)
Clinical and epidemiological studies			
Case control; 123 breast cancer patients, 59 controls	Fatty acid composition of breast adipose tissue	↓ breast cancer risk with increasing ALA levels in breast adipose tissue (p trend = 0.026)	Klein et al. (2000)

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
Case control ; 365 breast cancer patients, 397 controls	Questionnaire and FFQ	↑ breast cancer risk with ALA intake, OR = 3.8 (1.5–9.4)	De Stefani et al. (1998)
Case control; 241 patients and 88 controls	Fatty acid composition of breast adipose tissue	↓ breast cancer risk with ALA breast adipose levels, adjusted OR = 0.39 (0.19–0.78), *p* trend = 0.01	Maillard et al. (2002)
Case control; 414 cases, 429 controls	FFQ for ALA intake	No association with breast cancer risk and ALA intake, OR = 1.27 (0.85–1.89), *p* trend = 0.284	Nkondjock et al. (2003a, b)
Case control; 196 cases, 388 controls	Fatty acid composition of serum phospholipids	No association with breast cancer risk and ALA levels in serum phospholipids OR = 1.36 (0.63–2.96), *p* trend = 0.424	Chajes et al. (1999)
Case Control; 322 cases, 1,030 controls	Erythrocyte fatty acid concentrations	No association with breast cancer risk and ALA levels of erythrocytes, OR = 0.99 (0.54–1.82), *p* trend = 0.59	Shannon et al. (2007)
Case Control; 103 cases, 309 controls	Erythrocyte fatty acid concentrations Dietary record	No association with breast cancer risk and ALA intake or erythrocyte composition	Kuriki et al. (2007)
Prospective Cohort; 121 breast cancer patients	Fatty acid methyl esters of breast adipose tissue	↓ breast cancer metastases when breast adipose ALA above 0.38 % of total fatty acids	Bougnoux et al. (1994)
Prospective cohort in 56,007 French women	Diet history questionnaires Followed for 8 years	↓ breast cancer hazard ratio with ALA intake from fruits and vegetables, and vegetable oils (*p* trend <0.0001, 0.017) ↑ with ALA intake from nut mixes (*p* trend 0.004) and processed foods (*p* trend 0.068)	Thiebaut et al. (2009)

(continued)

Table 3.2 (continued)

Model	Treatments/measures	Results	References
Cohort study; 62,573 women	FFQ for ALA intake	↓ breast cancer risk with ALA intake RR = 0.70 (0.51–0.97), p trend = 0.006	Voorrips et al. (2002)
Meta-analysis; fatty acid composition of adipose tissue/serum	Three cohort and seven case-control studies	Case control studies: high ALA content ↓ risk of breast cancer Cohort studies: no association between ALA content and breast cancer risk; in postmenopausal women ALA content ↑ breast cancer risk, RR = 1.14 (1.03–1.26)	Saadatian-Elahi et al. (2004)
RCT; 32 postmenopausal breast cancer patients	25 g FS muffin/day or control placebo muffin Biopsy tissue at diagnosis and surgery	↓ cell proliferation 34.2 % (p = 0.001) in FS group ↑ apoptosis 30.7 % (p = 0.007) in FS group ↓ HER2 expression 71 % (p = 0.003) in FS group	Thompson et al. (2005)

ALA α-linolenic acid, *BD* basal diet, *CO* corn oil, *DMBA* dimethylbenz(α)anthracene, *E2* estrogen, *ED* enterodiol, *EL* enterolactone, *FFQ* food frequency questionnaire, *FO* fish oil, *FS* flaxseed, *FSO* flaxseed oil, *HER2* human epidermal growth factor receptor 2, *NMU* *N*-nitrosomethyl-urea, *OR* odds ratio, *OVX* ovariectomized, *RCT* randomized controlled trial, *RR* relative risk, *SDG* secoisolariciresinol diglucoside, *TAM* tamoxifen, *TRAS* trastuzumab

burden (# of tumors/tumor bearing rat) or tumor volume compared to control. Dietary flaxseed fed only at the initiation stage tended to reduce the tumor burden, however, did not affect the final tumor size. On the other hand, flaxseed fed at the early promotion stage resulted in significantly smaller tumors compared to control despite greater tumor burden compared to the group fed the flaxseed diet throughout (Serraino and Thompson 1992a). Using the *N*-nitrosomethyl-urea (NMU)-induced rat model, Rickard et al. (1999), showed that dietary flaxseed fed 2 days post carcinogen administration had no effect on final tumor weight, volume, multiplicity or incidence although it did reduce the invasiveness and grade suggesting a flaxseed effect at the more advanced stages of carcinogenesis. The authors noted that the discrepancy in the results between their study and the previous DMBA-induced models described above may be due to a number of differences in experimental design including the use of soybean oil as the fat source in the basal diet (BD) which contributes ALA as opposed to the previously used corn oil-based BD which has very low ALA. Although the results of these studies were not straightforward, they stimulated a great interest in the role of dietary flaxseed in carcinogenesis.

Fewer studies have looked specifically at the role of flaxseed oil in the prevention of mammary carcinogenesis (Table 3.2). An early study compared the effect of various oils on mammary tumor growth in the C3H/Heston mouse model with DMBA administration and found flaxseed oil and fish oil fed mice had the lowest tumor incidence while corn oil and safflower oil had the greatest tumor incidence (Cameron et al. 1989). In a series of experiments, Cognault et al. (2000) demonstrated that flaxseed oil's effect on NMU-induced mammary tumor growth varies based on the presence of anti- or pro-oxidants in the diet, as (i) a flaxseed oil diet combined with vitamin E increased tumor growth in mice compared to a vitamin E-free diet, and (ii) a flaxseed oil diet with vitamin E plus prooxidant decreased tumor growth compared to the flaxseed oil with vitamin E diet alone. This study provides insight into how flaxseed oil may affect tumor growth (i.e. oxidation), but other dietary oils were not used so comparisons between flaxseed oil and other sources cannot be made (Cognault et al. 2000).

Flaxseed's effect in the prevention of HER2 overexpressing breast cancer has been studied using the MMTV/c-neu transgenic mouse model which spontaneously develops HER2+ tumors. When increasing levels of flaxseed were fed for 23 weeks, only the highest level of flaxseed (0.054 %) reduced tumor incidence, burden and number of large tumors compared to control levels. None of the flaxseed diets affected tumor multiplicity and number of tumor-bearing mice compared to control (Birkved et al. 2011). The levels used in this study were very low, almost 100 fold lower than the previously outlined studies, and therefore it is possible that a greater effect would have been achieved with higher levels of flaxseed in the diet. The effect of flaxseed oil has also been studied using this model. Mice were gavaged with 0.2 ml of oil containing increased proportion of flaxseed oil mixed into corn oil (0.05, 0.1 and 0.2 ml of flaxseed oil) for 30 weeks. The effect of flaxseed oil on mammary tumor development was complex; low dose of flaxseed oil resulted in a non significant increase in tumor incidence and number of tumors per mouse while there was a trend toward reduced tumor incidence with higher dose of flaxseed oil. The high dose flaxseed oil treated mice had lower overall weight of tumors per mouse and mean tumor weight compared to control (Rao et al. 2000). These results suggest that the n-6:n-3 ratio plays an important role in mediating the effect of flaxseed oil on HER2+ mammary tumorigenesis.

3.4.1.2 Clinical and Epidemiological Studies

Epidemiological and limited clinical studies that investigated flaxseed, flaxseed oil, ALA and breast cancer risk have produced inconsistent results (Table 3.2). A recent case-control study found that flaxseed consumption measured through Food Frequency Questionnaires (FFQ) significantly reduced breast cancer risk [OR = 0.82 (0.69–0.97)] (Lowcock et al. 2013). Two case control studies showed that ALA content in breast adipose tissue was inversely associated with breast cancer risk (Klein et al. 2000; Maillard et al. 2002). As well, a meta-analysis of five case-control studies found that there was a significant decrease in breast cancer risk with increasing levels of biomarkers of ALA intake (Saadatian-Elahi et al. 2004).

Contrary to these studies, a case control study in Uruguay found that ALA consumption measured by FFQs increased breast cancer risk (De Stefani et al. 1998), which may be partially explained by the high intake of red meat in Uruguay accounting for a large proportion of the ALA intake rather than vegetable sources (Bougnoux and Chajes 2003). Other case control studies measuring ALA intake with FFQs and erythrocyte ALA content all found that there was no association between ALA and breast cancer risk (Chajes et al. 1999; Nkondjock et al. 2003a; Kuriki et al. 2007; Shannon et al. 2007).

Cohort studies show more promise in terms of the potential role of ALA in breast cancer prevention. One study found that the ALA content in the breast adipose tissue was inversely associated with risk of subsequent metastasis in 121 non-metastatic breast carcinoma patients. When ALA levels were above 0.38 % of breast fat, there was a five-fold reduction in risk (Bougnoux et al. 1994). Similarly, a cohort study in the Netherlands measured ALA intake with a validated FFQ and found that intake was inversely associated with breast cancer risk (Voorrips et al. 2002). The food source of ALA may play an integral part in its effectiveness as a breast cancer preventative compound as highlighted in a French cohort study (Thiebaut et al. 2009). ALA intake from fruits and vegetables as well as vegetable oils as measured by a FFQ was inversely related to breast cancer risk whereas ALA from nuts and processed foods increased risk. Menopausal status may also alter the effects of ALA as a meta-analysis of three cohort studies found that in postmenopausal women only, ALA as measured by biomarkers increased breast cancer risk (Saadatian-Elahi et al. 2004). There are several limitations associated with the studies above which may explain inconsistent findings including variation in biomarkers used for ALA intake, poor FFQs, population characteristics (menopausal status, cancer subtype, BMI), quartiles of intake used, and the food source. To our knowledge there have been no completed clinical trials that specifically studied flaxseed or its components on breast cancer prevention. One ongoing study that will hopefully provide a clearer picture of flaxseed's role in preventing breast cancer is investigating the effect of a flaxseed enriched diet for 6 months on biomarkers of breast cancer (proliferation, apoptosis, and estrogen receptor genes) in premenopausal women at high risk of developing breast cancer (NCT00794989).

3.4.2 Breast Cancer Treatment

3.4.2.1 Preclinical Studies

In vitro studies are useful for investigating the role of potential anticancer agents as they provide information on specific effects and mechanisms of action to build upon in future *in vivo* and clinical studies. To date, *in vitro* studies investigating the role of ALA on breast cancer cell lines have produced inconsistent results which seem dependent on the receptor expression of the cells and ALA dose, as well as environmental factors such as estrogen levels (Table 3.2). Some studies in the ER+, low HER2 MCF-7 breast cancer cell line showed promise for ALA to inhibit

growth as (i) cell proliferation was reduced by 33 % when cells were treated with 50 μM ALA in a high estrogen environment (1 nM) for 5 days (Truan et al. 2010), and (ii) ALA concentrations varying from 0 to 100 μM for 24–72 h dose and time dependently inhibited MCF-7 cell growth and induced apoptosis (Kim et al. 2009). However, one study did find that 71.83 μM ALA for 5 days did not reduce MCF-7 cell growth (Chajes et al. 1995). In the HER2 overexpressing cell lines BT-474 and SKBr3, 10–20 μM ALA for 48 h dose dependently decreased HER2 expression at the transcriptional level, which would lead to a decrease in cancer cell growth through a reduction in growth factor signaling (Menendez et al. 2006). Finally, two studies of ALA effect in the basal MDA MB 231 cell lines (ER−, low HER2) found that 10–200 μM ALA treatment for time ranging from 24 h to 5 days decreased cell proliferation and growth (Chajes et al. 1995; Horia and Watkins 2005). Overall *in vitro* studies indicate ALA likely decreases breast cancer cell growth, but further exploration into the specific cell lines affected, adequate doses and mechanism is needed.

Rodent models with established tumors are often used to investigate the effect of dietary components in the treatment of breast cancer, and early studies with these models suggested a reduction in growth of established tumors with flaxseed and flaxseed oil supplementation (Table 3.2). For example, Sprague-Dawley rats were fed a BD or a 1.82 % flaxseed oil diet, BD with SDG treatment or 5 or 10 % flaxseed diets starting 13 weeks post DMBA administration when mammary tumors were established (Thompson et al. 1996). At the end of the study, tumors that were established at the start of treatment regressed in all treatments. All treatments except flaxseed oil reduced total tumor volume (established and new) compared to control, but only SDG lowered new tumor volume suggesting that the lignans are more effective at inhibiting new tumor development whereas flaxseed oil is more effective at reducing the growth of established tumors. An early study compared the effect of corn oil, flaxseed oil and fish oil diets on the growth of implanted tumors derived from mouse mammary tumors (410 and 410.1) (Fritsche and Johnston 1990). There was no difference between the diets on the growth of 410 tumors; however, flaxseed oil had the greatest effect at reducing the growth and metastasis of 410.1 tumors. These results further support the potential of flaxseed oil in reducing the growth of established mammary tumors.

Flaxseed, flaxseed oil and lignan effects have been studied in the xenograft model with various human breast cancer cell lines and at low and high circulating levels of E2. The effects vary depending on experimental conditions suggesting that the effect may differ based on cancer subtype and menopausal stage. Three studies observed the effect of dietary flaxseed and its components on established ER negative (ER−) MDA-MB-435 tumor growth (Chen et al. 2002; Dabrosin et al. 2002; Wang et al. 2005). A 10 % flaxseed diet fed for 7 weeks reduced the palpable tumor growth, lymph node metastasis, tumor cell proliferation and the expression of the marker of angiogenesis, VEGF (Chen et al. 2002; Dabrosin et al. 2002). Established MDA-MB-435 tumors were shown to have significantly lower growth rate, cell proliferation and increased apoptosis compared to control in athymic mice fed either 10 % flaxseed or 4 % flaxseed oil diets both alone and when combined with SDG treatment while SDG alone did not affect the palpable tumor growth rate (Wang et al. 2005). These results suggest that the oil may be the most effective component at reducing ER− tumor growth.

The role of flaxseed and its components in modulating the growth of MCF-7 (ER+, low HER2) breast tumors was the focus of several studies. At high circulating E2 levels, 5 and 10 % flaxseed diets were both shown to reduce the growth of established MCF-7 human breast tumors in the athymic mouse model which was related to a reduction in cell proliferation and increase in apoptosis (Chen et al. 2007b). Established MCF-7 tumors regressed upon removal of the E2 pellet (negative control) to lower circulating E2 levels but the regression caused by flaxseed did not differ from that of the control (Saarinen et al. 2006; Chen et al. 2007a). At low circulating E2 levels, both flaxseed oil and SDG reduced the growth of MCF-7 xenografts in athymic mice although SDG had the greatest effect (Saggar et al. 2010a). The effect on tumor growth was related to changes in cell proliferation rather than apoptosis. Flaxseed oil's anti-tumorigenic effect was supported by a study which showed that the flaxseed cotyledon, rich in flaxseed oil but low in lignan, similarly reduces the growth of MCF-7 tumors (Chen et al. 2011). At high circulating levels of E2, dietary flaxseed oil significantly reduced MCF-7 tumor growth rate in athymic mice compared to control (Truan et al. 2010). In contrast, in a similarly designed study, dietary SDG did not affect MCF-7 tumor growth rate compared to control (Truan et al. 2012). Evidently, the effect of flaxseed and its components on the growth of ER+ xenografts depends on the estrogen environment; however, flaxseed oil was shown to reduce tumor growth at both low and high circulating levels of E2.

3.4.2.2 Diet-Drug Interactions

Studies have investigated the potential interaction of flaxseed and its components with tamoxifen (TAM), a primary adjuvant therapy for the treatment of ER+ breast cancer (Table 3.2). At high circulating levels of E2, flaxseed enhanced the tumor-suppressing effect of TAM in the athymic mouse model with MCF-7 xenografts (Chen et al. 2004, 2007a, b). Additionally, at low circulating levels of E2, 10 % flaxseed prevented the tumor regrowth seen with TAM treatment alone through a decrease in cell proliferation and an increase in apoptosis (Chen et al. 2004, 2007a). Saggar et al. (2010a, b) investigated the effect of flaxseed oil, SDG and flaxseed oil + SDG in combination with TAM treatment in athymic mice with established MCF-7 human breast tumors. An E2 pellet was implanted into the mice to stimulate tumor growth. On removal, circulating E2 levels fall to within the range seen in postmenopausal women and MCF-7 tumors (E2 dependent) regressed. The tumors regressed to a smaller size in all of the treatment groups compared to control with the greatest effect seen in mice fed the flaxseed oil diet. Similarly, all treatments reduced cell proliferation and increased apoptosis with the greatest effect seen in mice fed the flaxseed oil diet (Saggar et al. 2010b). The effect of flaxseed oil in enhancing TAM action is supported by the results of a study which showed that a diet enriched with the flaxseed cotyledon fraction, rich in flaxseed oil and low in lignans, similarly showed a reduction in tumor growth rate alone and when combined with TAM while TAM alone did not reduce tumor growth (Chen et al. 2011).

Flaxseed oil has also been studied for its interaction with trastuzumab (TRAS, Herceptin), a primary therapy used in the treatment of HER2 overexpressing breast cancer (Menendez et al. 2006; Mason et al. 2010). In the athymic mouse model, 8 % dietary flaxseed oil was shown to enhance the effectiveness of TRAS (2.5 mg/kg) in reducing the growth of BT-474 xenografts (HER2+, ER+). This effect was related to both increased apoptosis and reduced cell proliferation (Mason et al. 2010). *In vitro* work has also demonstrated a significant synergism between ALA and TRAS. Treating BT-474 cells with both ALA (2.5–40 μM) and TRAS (5 μg/ml) resulted in greater cytotoxicity compared to TRAS alone (Menendez et al. 2006).

Although there are promising preclinical results supporting dietary flaxseed and flaxseed oil as complementary agents along with TAM and TRAS treatment, the effect must be confirmed in humans before any recommendations can be made regarding their therapeutic applications.

3.4.2.3 Clinical Studies

Very few studies have investigated flaxseed, flaxseed oil or ALA as a complementary breast cancer treatment in humans (Table 3.2). One randomized placebo controlled double blind study determined the effect of 25 g/day flaxseed incorporated into a muffin on postmenopausal women with newly diagnosed breast cancer (Thompson et al. 2005). In the flaxseed group, cell proliferation and HER2 expression decreased by 34 and 71 % respectively, and apoptosis increased by 31 %, compared to baseline while there were no changes in the placebo control group. An ongoing double blind, placebo controlled randomized control trial is investigating the effect of a 25 g/day flaxseed supplement with and without the aromatase inhibitor drug anastrozole in postmenopausal, ER + breast cancer patients with primary analysis on changes in proliferation, apoptosis and receptor expression (NCT00612560).

3.5 Studies of Flaxseed and Flaxseed Oil on Colorectal Cancer

Fewer studies have investigated the role of flaxseed and its components on colorectal carcinogenesis compared to breast/mammary carcinogenesis. Therefore, prevention and treatment effects are discussed together.

3.5.1 Preclinical Studies

The role of flaxseed and its components in colorectal cancer cells have been investigated *in vitro* and *in vivo* but the results are conflicting (Table 3.3). Habermann et al. (2009) showed in both highly transformed HT29 colorectal cancer

Table 3.3 Summary of studies investigating effect of flaxseed, flaxseed oil and ALA on colorectal cancer

Model	Treatments/measures	Results	References
In vitro studies			
LT97 (adenoma) and HT29 (carcinoma)	100 μM ALA 2–72 h treatment	↓ cell growth in both cell lines, LT97 to a greater extent	Habermann et al. (2009)
Colorectal cancer cell line HCT116	Cells treated with 10 μM ALA	↑ cell number by 30 %	Seti et al. (2009)
In vivo animal studies			
Sprague-Dawley male rats with AOM-induced tumors	BD, 5 or 10 % FS flour (FF; 1.9–3.8 % FO) or defatted FS meal (FM; 0.14–0.28 % FO)	↓ Aberrant crypts (AC) and aberrant crypt foci (ACF) in the descending colon in all groups *vs*. control	Serraino and Thompson (1992b)
	Diets fed starting in early promotion stage	↓ in AC and ACF in the ascending colon in the 10 % FF group *vs*. control	
		↓ cell proliferation in descending colon in 5 % FF group compared to control; ↓ in 5 % FF, 10 % FM and 10 % FF *vs*. 5 % FM	
Male Fischer rats with AOM induced tumors (initiation and promotion)	15 % corn oil *vs*. 15 % FSO diets Fed diets during initiation and promotion stages	↓ tumor incidence, size and number per rat in FSO group *vs*. corn oil	Dwivedi et al. (2005)
Male Fischer rats with AOM induced tumors (initiation and promotion)	15 % corn meal *vs*. 15 % FS meal diets Fed diets during initiation and promotion stages	↓ tumor incidence, size and number per rat in FS group *vs*. corn meal	Bommareddy et al. (2006)
Male Fischer rats with AOM induced carcinogenesis (initiation and promotion)	Control, 7 and 14 % soybean oil (SBO), 7 and 14 % FSO, 10 and 20 % FSM diets Fed diets during initiation and promotion stages	↓ ACF in 7 and 14 % FSO group and FSM groups compared to 7 and 14 % SBO groups ↓ ACF in 20 % FSM *vs*. 10 % FSM ↑ ACF in 14 % FSO *vs*. 7 % FSO	Williams et al. (2007)
Min mice	BD, 15 % FS diet (defatted FSM + FSO) and FSO diet Started at 5 weeks age	↓ # adenomas in FS compared to control; non-significant ↓ in FSO ↓ adenoma size in FS and FSO *vs*. control	Oikarinen et al. (2005)

(continued)

Table 3.3 (continued)

Model	Treatments/measures	Results	References
Min mice	BD, 15 % corn meal *vs.* 15 % FS meal diets and 15 % corn oil *vs.* 15 % FSO	↓ intestinal tumor multiplicity and size in FSM compared to corn meal and FSO compared to corn oil No effect on apoptosis	Bommareddy et al. (2009)
Clinical and epidemiological studies			
Normal and tumor tissue samples from nine colorectal cancer patients	Free fatty acids of cell membranes	↓ ALA % content in tumor tissue *vs.* control	Szachowicz et al. (2007)
Case-control; 3,166 controls, 1,597 adenoma polyp cases, 544 hyperplastic polyp cases	Dietary PUFA intake measured by FFQ	↑ polyp occurrence with ALA intake in men, OR = 1.51 (1.03, 2.21), *p* trend = 0.03	Murff et al. (2012)
Case control; 74 cases, 221 controls	Erythrocyte fatty acid content	No association with colorectal cancer risk and erythrocyte ALA content, OR = 1.18 (0.63–2.21), *p* trend = 0.51	Kuriki et al. (2006)
Prospective cohort; 99,080 subjects	PUFA intake and colorectal cancer risk	↑ trend in colorectal cancer risk in women with ↑ ALA intake (*p* trend = 0.13)	Daniel et al. (2009)
RCT; 523 patients with colorectal adenomas	2 g/day calcium, 3.5 g/day fibre, or placebo Questionnaires for dietary fat intake	No association between colorectal adenoma reoccurrence and ALA intake, OR = 0.87 (0.52–1.46), *p* trend = 0.43	Methy et al. (2008)
RCT; 2,079 (372 fully completed) participants with colon polyps	Low fat, high fiber, diet or control Four day food records, FFQ and 24 h recalls	No association with colorectal adenoma recurrence and ALA intake OR = 0.92 (0.48–1.78), *p* trend = 0.87	Cantwell et al. (2005)

AC aberrant crypt, *ACF* aberrant crypt foci, *ALA* α-linolenic acid, *AOM* azoxymethane, *BD* basal diet, *FF* flaxseed flour, *FFQ* food frequency questionnaire, *FM* defatted flaxseed meal, *FS* flaxseed, *FSO* flaxseed oil, *OR* odds ratio, *PUFA* polyunsaturated fatty acid, *RCT* randomized controlled trial, *SBO* soybean oil

cells and preneoplastic LT97 adenoma cells that ALA was taken up by the cells and growth was inhibited when treated with 100 μM ALA for 72 h. Contrary to these findings, Seti et al. (2009) observed that 10 μM ALA increased HCT1116 colorectal cancer cell growth. Limitations in the current *in vitro* studies include variation in cell lines and doses used, and different stages of cancer progression.

To date rodent studies have focused specifically on the role of flaxseed and its oil in the prevention rather than treatment of colorectal cancer (Table 3.3). Serraino and Thompson (1992b) were the first to investigate the role of dietary flaxseed in the prevention of colon carcinogenesis. Using an azoxymethane (AOM)-induced rat model, they showed that feeding diets rich in flaxseed, either 5 or 10 % FF (ground whole flaxseed) or 5 or 10 % FM (defatted flaxseed), for 4 weeks during the promotion stage of colon carcinogenesis significantly reduced the incidence of aberrant crypts (AC) and aberrant crypt foci (ACF) in the descending colon compared to rats fed a control diet. Only the 10 % FF diet decreased AC and ACF incidence in the ascending colon compared to control indicating a greater effect of oil-containing flaxseed than defatted flaxseed. In a similarly designed study, Jenab and Thompson (1996) showed that feeding AOM-induced rats diets containing 2.5 or 5 % FF (ground whole flaxseed) or 2.5 or 10 % FM (defatted flaxseed) for approximately 14 weeks in the promotion stage reduced the number of AC per ACF in the distal colon. In contrast to the Serraino and Thompson study, only the 2.5 % defatted flaxseed diet reduced the number of ACF in the proximal colon compared to control. Other studies have also shown that both ground whole flaxseed (Bommareddy et al. 2006) and flaxseed oil (Dwivedi et al. 2005) fed to rats 1 week before and for 35 weeks following AOM administration significantly reduced tumor incidence, multiplicity and size when compared to corn meal and corn oil respectively. Similarly, ground whole flaxseed and flaxseed oil fed for 4 weeks before and for 10 weeks following AOM administration reduced the number of ACF in the proximal, distal and total colon compared to the soybean oil control (Williams et al. 2007). Together these data suggest that flaxseed has components such as the oil capable of inhibiting the initiation and promotion stages of colon carcinogenesis in the rat model.

The Min mouse model which has a mutation in the Adenomatous polyposis coli (*APC*) gene spontaneously develops tumors and has been useful in further understanding the role of flaxseed and its oil in colon carcinogenesis. Compared to BD, feeding the BD enriched with a flaxseed mixture (2.6 % defatted flaxseed + 4.7 % flaxseed oil) or flaxseed oil (4.7 %) to Min mice for 10 weeks resulted in a significant decrease in adenoma size and number, although the reduction in adenoma multiplicity was only significant in the flaxseed mixture group (Oikarinen et al. 2005). Similarly, it was shown that 15 % ground whole flaxseed and 15 % flaxseed oil diets fed for 12 weeks to Min mice suppressed intestinal multiplicity and size compared to feeding 15 % corn meal and 15 % corn oil diets respectively (Bommareddy et al. 2009). These results further support the role of dietary flaxseed and flaxseed oil in the prevention of colon cancer.

3.5.2 Clinical and Epidemiological Studies

Data collected from both clinical and epidemiological studies on flaxseed, flaxseed oil and ALA and colorectal cancer have raised concern as several studies show no effect or protection, while others show an increase in colorectal cancer risk (Table 3.3). One prospective cohort study showed that ALA intake measured by a

FFQ was associated with an increase in colorectal cancer incidence in women only, however flaxseed oil supplements were omitted from the FFQ and ALA intake was primarily from non-plant sources (Daniel et al. 2009). Similarly in a case-control study, ALA intake measured *via* FFQ showed that increasing ALA intake was associated with an increase in hyperplastic polyps in men (Murff et al. 2012).

Contrary to the above studies, ALA content in cell membranes of normal colorectal tissue has been shown to be higher than that of tumor tissue (Szachowicz-Petelska et al. 2007). In a systematic review of fatty acids and colorectal cancer, two case control studies showed that ALA content was lower in cancer patients compared to controls indicating a protective effect, however one study did find an increase in ALA intake in subjects at high risk of colorectal cancer (Nkondjock et al. 2003b). Other case control and cohort studies all found no significant association of ALA and colorectal cancer incidence (Nkondjock et al. 2003b). Clinical trials would be useful to help resolve the controversy surrounding flaxseed and its components in colorectal cancer; however, this area of research is currently lacking. Two randomized control trials which focused on the effect of either a calcium or fiber supplement, or a low fat, high fiber, fruit and vegetable diet on colorectal adenoma recurrence indirectly found that ALA intake did not have a significant association with colorectal cancer incidence as measured by questionnaires and food records (Cantwell et al. 2005; Methy et al. 2008). Limitations in the current epidemiological and clinical studies which may explain the inconsistent results are the use of unreliable FFQs for intake measurements, variation in biomarkers measured, not stating specific colon/rectal subsite location, and variation in the food source of ALA.

3.6 Studies of Flaxseed and Flaxseed Oil on Prostate Cancer

Similar to colorectal cancer, fewer studies have investigated the role of flaxseed and its components on prostate carcinogenesis compared to breast/mammary carcinogenesis, therefore, this section will discuss prevention and treatment studies together.

3.6.1 Preclinical Studies

The few *in vitro* studies that have investigated the effect of ALA on prostate cancer cells have produced contradictory results (Table 3.4). Two studies using DU 145 human prostate tumor cells found that physiological levels of ALA decreased cell proliferation and increased the number of dead cells (du Toit et al. 1996; Motaung et al. 1999). However, one study using human metastatic prostate cell lines PC-3, LNCaP and TSU found that ALA actually promoted cell growth (Pandalai et al. 1996). Future studies should continue to investigate the role of ALA on prostate cell

Table 3.4 Summary of studies investigating effect of flaxseed, flaxseed oil and ALA on prostate cancer

Model	Treatments/measures	Results	References
In vitro studies			
Metastatic PC-3, LNCaP, TSU cells	0.003–0.359 μM	↑ cell growth in all cell lines	Pandalai et al. (1996)
DU-145 prostate tumor cells	4, 40, 200 μM ALA Six days	↓ cell growth with 40 and 200 μM ALA for 6 days	Toit et al. (1996)
DU-145 prostate tumor cells	4, 20, 40 μM ALA	↑ cell death with 20 μM and 40 μM ALA	Motaung et al. (1999)
In vivo animal studies			
TRAMP transgenic model	BD, 5 % FS Started at 5–6 weeks age Followed for 20 or 30 weeks	↓ urogenital/tumor weight, incidence of aggressive tumors at 30 weeks ↓ cell proliferation and ↑ apoptosis at both 20 and 30 weeks	Lin et al. (2002)
Male athymic nude mice with DU145 human prostate cancer xenografts	18 % corn oil/5 % FSO; 18 % FSO/5 % corn oil; 18 % fish oil/5 % corn oil Fed diets starting before implantation	No difference in tumor growth between the 18 % CO/5 % FSO; 18 % FSO/5 % CO diets ↓ tumor growth in the fish oil group compared to both other groups	Connolly et al. (1997)
Wistar rats (no carcinogen administration)	7 % soybean oil (SBO), 7 % SBO/FSO (1:1), 7 % FSO, 7 % SBO/pork fat (1:1), 7 % pork fat Fed for 10 weeks	↓ relative prostate weight in 7 % FSO group *vs*. SBO control and SBO/FSO ↑ cell proliferation in prostate tissue in lard group *vs*. SBO and FSO groups	Escobar et al. (2009)
Clinical and epidemiological studies			
Nested case control; 962 prostate cancer patients, 1,061 controls	Fatty acid composition of plasma phospholipids	No association with prostate cancer risk and % ALA; ↑ high grade cancer risk with ↑ % ALA (p trend 0.014)	Crowe et al. (2008)
Systematic review; eight case control, eight prospective	ALA intake or blood concentrations	↑ prostate cancer risk with ALA blood concentrations, RR = 1.20 (1.01–1.43), no association with dietary intake	Simon et al. (2009)
Meta analysis; five prospective studies	FFQ for ALA intake	No association with prostate cancer risk and ALA intake, pooled RR = 0.97 (0.86–1.10) If consumed >1.5 g/day ALA ↓ risk, RR = 0.95 (0.91–0.99)	Carayol et al. (2010)

(continued)

Table 3.4 (continued)

Model	Treatments/measures	Results	References
Meta analysis; nine case control and cohort	ALA intake and blood level	↑ prostate cancer risk with ALA intake and blood levels, RR = 1.70 (1.12–2.58)	Brouwer et al. (2004)
Pilot Study, 15 healthy men	Low fat, FS (30 g/day) diet for 6 months	↓ cell proliferation with low fat FS diet (0.022 ± 0.027 baseline to 0.007 ± 0.014 at 6 months, $p = 0.0168$)	Demark-Wahnefried et al. (2004)
Pilot clinical study, 25 prostate cancer patients	Low fat, FS (30 g/day) diet for 34 days	↓ proliferation in treatment group (5.0 ± 4.9 treatment, 7.4 ± 7.8 for control, $p = 0.05$) ↑ apoptosis in treatment group $p = 0.01$	Demark-Wahnefried et al. (2001)
RCT; 161 prostate cancer patients	Control, FS diet (30 g/day), low fat (<20 % energy), combination ; 30 days average	↓ proliferation in FS group ($p < 0.002$) No effect on apoptosis	Demark-Wahnefried et al. (2008)

ALA α-linolenic acid, *FS* flaxseed, *FSO* flaxseed oil, *RCT* randomized controlled trial, *RR* relative risk, *SBO* soybean oil, *TRAMP* transgenic adenocarcinoma of mouse prostate

growth, and take into account differences between cell lines, doses used and other components in the media environment that may alter ALA effects.

Animal models have shown varying effects of flaxseed and flaxseed oil rich diets on the growth of prostate tissue and tumor growth (Table 3.4). Current studies have focused on prostate cancer prevention. Healthy male Wistar rats fed a 7 % flaxseed oil diet had lower relative prostate weight compared to a 7 % soybean oil diet and lower relative prostate weight and prostate cell proliferation compared to a 7 % rendered pork fat diet rich in saturated fat (Escobar et al. 2009). Using the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, a 5 % flaxseed diet was shown to reduce the urogenital/tumor weight, number of aggressive tumors and prostate tissue cell proliferation and increase apoptosis after 30 weeks (Lin et al. 2002). On the other hand, an early study compared the effects of diets rich in corn oil (18 % corn oil/5 % flaxseed oil), flaxseed oil (18 % flaxseed oil/5 % corn oil) and fish oil (18 % menhaden oil/5 % corn oil). When fed 1 week before injection of DU145 human prostate cancer cells in athymic mice the diet with greater flaxseed oil did not affect tumor growth while the fish oil rich diet reduced tumor growth (Connolly et al. 1997). The major differences in design of these animal studies make it difficult to compare them, however, based on results in the healthy rats and transgenic mice, there is some indication that flaxseed and flaxseed oil may prevent the development of prostate cancer.

3.6.2 Clinical and Epidemiological Studies

Several reviews and meta-analyses have been conducted on the role of ALA in prostate cancer development with conflicting results (Brouwer et al. 2004; Simon et al. 2009; Carayol et al. 2010) (Table 3.4). Brouwer et al. (2004) found that increasing ALA dietary intake and/or blood level significantly increased prostate cancer risk in five case control and four cohort studies. Simon et al. (2009) pooled eight case control and nine prospective studies and found a weak association between ALA (dietary intake or concentration in tissues) and increased prostate cancer risk. Carayol et al. (2010) looked specifically at five prospective cohort studies and the pooled relative risk showed no significant association between ALA intake and prostate cancer; however, it was found that those who consumed more than 1.5 g/day of ALA were actually at a decreased risk of prostate cancer compared to those below 1.5 g/day. The European Prospective Investigation into Cancer and Nutrition (EPIC) also investigated the role of plasma phospholipid fatty acid composition on prostate cancer in a nested case-control study and found ALA composition of the plasma phospholipids was not significantly associated with prostate cancer risk, with the exception of high-grade prostate cancer in which ALA increased risk (Crowe et al. 2008). Limitations of these studies overall include the use of unreliable FFQs to measure ALA intake, the food source of ALA and significant heterogeneity across some studies and highlight the need for controlled clinical trials to resolve the controversy.

In a clinical pilot intervention study, 15 men scheduled for a repeat prostate biopsy adopted a low fat (<20 % of energy), flaxseed supplemented (30 g/day) diet for 6 months prior to second biopsy and showed that cell proliferation of the benign prostate epithelium slightly decreased with the low fat, flaxseed diet from baseline to 6 months, indicating flaxseed may prevent prostate cancer (Demark-Wahnefried et al. 2004). Two clinical trials have assessed the ability of a flaxseed supplemented diet prior to prostatectomy to act as a potential treatment through measurement of cell proliferation and apoptosis of prostate cancer cells from the excised prostate tumors of prostate cancer patients (Demark-Wahnefried et al. 2001, 2008). The 25 patient pilot study found that a low fat (<20 % of energy), flaxseed supplemented (30 g/day) diet for ~30 days prior to prostatectomy decreased cell proliferation and increased apoptosis compared to matched historic cases, indicating a decrease in cancer cell growth. The follow-up randomized control study in 161 prostate cancer patients found that a 30 g/day flaxseed diet for 30 days significantly decreased cell proliferation compared to control ($p < 0.002$), although no significant difference was seen on apoptosis, suggesting that flaxseed may provide a benefit in reducing cancer cell growth in prostate cancer patients (Demark-Wahnefried et al. 2008). Further studies are required to provide a better understanding of the effect of flaxseed, flaxseed oil and ALA on prostate cancer and potential mechanism of effect.

3.7 Studies of Flaxseed and Flaxseed Oil in Other Cancers

The effect of flaxseed and its components have also been determined in other cancers but the studies are limited and results are inconsistent. A study using laying hens as a model for spontaneous ovarian surface epithelial cancer found that a 10 % flaxseed diet fed for 1 year decreased late stage tumors and increased survival compared to the control diet, suggesting protective effect against ovarian cancer (Ansenberger et al. 2010). In pancreatic cancer cell lines (MIS PaCa-2, PANC-1 and CFPAC), ALA in doses of 10–20 μM significantly decreased cell number across the three cell lines indicating a beneficial effect (Falconer et al. 1994). In contrast, in Syrian golden hamster models of BOP-induced pancreatic ductular carcinoma, liver metastases occurred when 2.5–10 % ALA was incorporated into the diet (Wenger et al. 1999, 2000). However, the diets in these studies varied greatly in fat content (3 % fat control diets *vs*. 25 % fat treatment diets), carbohydrate to fat ratio, and protein and fibre contents so conclusions made regarding increased liver metastases may not necessarily be due to dietary ALA. Two other studies also looked at the effect of flaxseed and flaxseed oil on metastasis to the liver and lungs in animal models. In C57B1/6 mice with murine melanoma cells injections, a 2.5–10 % flaxseed diet 2 weeks pre and post melanoma cell injection dose dependently decreased the number, area, and volume of secondary lung tumors (Yan et al. 1998). In the same C57B1/6 mouse model using H59 lung carcinoma cells; however, an 8 % flaxseed oil diet 4 weeks pre injection and roughly 5 weeks post injection increased metastasis to the liver compared to a control no fat diet, a saturated fat diet, and an n-6 PUFA diet (Coulombe et al. 1997). These limited and contradictory studies highlight the need for further work in the area of flaxseed supplementation for a variety of cancer types.

3.8 Proposed Mechanisms of Anticancer Effect

The anticancer effect of flaxseed oil is thought to be due to ALA which is the predominant fatty acid. The mechanisms by which ALA modulates carcinogenesis are not yet understood although several have been proposed (Fig. 3.3). ALA's effect was thought to be due to both direct effects and indirect effects through conversion to EPA and DHA (Fig. 3.1). However, the conversion of ALA to EPA and DHA is quite low although humans have functional enzymes for the metabolic pathway for the conversion (Cunnane 2003; Brenna et al. 2009). Nevertheless, flaxseed, flaxseed oil and ALA have all been shown to have anticancer properties and hypothesized mechanisms of effect include: (1) incorporation into the cancer cell membrane thus (1a) increasing the synthesis of n-3-derived eicosanoids and (1b) disrupting the localization, expression and signalling of growth factor receptors; (2) alteration of the regulation of transcription; (3) increased lipid peroxidation; and (4) other emerging potential mechanisms (Fig. 3.3).

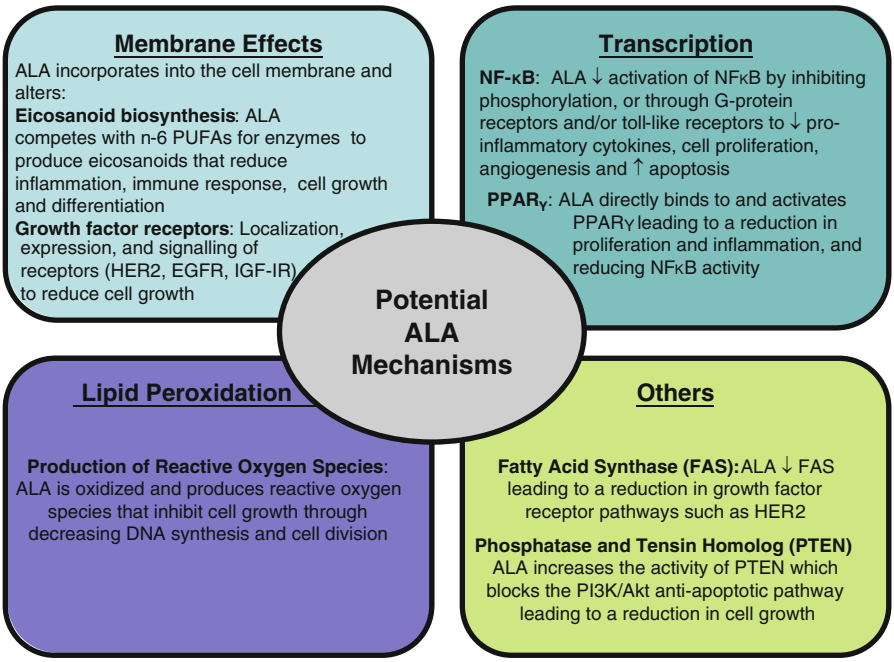

Fig. 3.3 Overview of the potential mechanisms of ALA to reduce cancer promotion and progression. *ALA* α-linolenic acid, *EGFR* epidermal growth factor receptor, *FAS* fatty acid synthase, *HER2* human epidermal growth factor receptor 2, *IGF-IR* insulin-like growth factor-I receptor, *NF-κB* nuclear factor-kappa B, *PTEN* phosphatase and tensin homolog, *PUFA* polyunsaturated fatty acid

3.8.1 Cell Membrane Effects

Increased exposure of cancer cells to ALA, EPA and DHA results in an increase in the n-3 PUFA and a decrease in the n-6 PUFA content of membrane phospholipids (Connolly et al. 1997; Dwivedi et al. 2005; Dabadie et al. 2006; Schley et al. 2007; Truan et al. 2010). This modulation of the tumor fatty acid profile may result in reduced tumor growth. One potential mechanism for this effect is through the modulation of the biosynthesis of eicosanoids, mediators of cellular processes including inflammation, immune response, cell growth and differentiation (Wang and Dubois 2010). n-3 and n-6 PUFA compete for the same enzymes for metabolic conversion, esterification into membrane phospholipids, release into the intracellular free fatty acid pool and conversion to eicosanoids and n-3 PUFA are the preferred substrate for several of these enzymes (Cunnane 2003; Larsson et al. 2004). Therefore, increased exposure to n-3 PUFA increases their level in the membrane phospholipids thereby increasing n-3 substrate availability for enzymatic cleavage to release free n-3 PUFA and for the subsequent conversion to eicosanoids (Rose and Connolly 1999). n-6 and n-3-derived eicosanoids have

different biological effects: n-6-derived eicosanoids are generally pro-inflammatory whereas those derived from n-3 PUFA are anti-inflammatory or less potent inflammatory agents (Larsson et al. 2004; Wang and Dubois 2010). Few studies have measured flaxseed oil's modulation of eicosanoid biosynthesis. Feeding rats a flaxseed oil rich diet suppressed the production of the n-6-derived prostaglandin E2 (Marshall and Johnston 1982). More studies are required to confirm this effect and to see whether other eicosanoids are affected by dietary flaxseed oil.

Another mechanism through which membrane fatty acid changes may affect tumor growth is through the alteration of the localization, expression and signalling of growth factor receptors. Several studies have demonstrated that flaxseed and flaxseed oil rich diets decrease the expression of Akt and MAPK which are biomarkers of growth factor receptor signalling (Saggar et al. 2010a, b; Truan et al. 2010). This may be related to decreased expression of receptors including HER2 (Thompson et al. 2005; Menendez et al. 2006; Saggar et al. 2010b; Truan et al. 2010), IGF-IR (Chen et al. 2007a; Saggar et al. 2010b) and EGFR (Chen et al. 2002; Truan et al. 2010). An emerging potential mechanism for the altered growth factor receptor signaling is the translocation of receptor from the lipid raft microdomain to the non-raft domain (Staubach and Hanisch 2011). Treatment of breast cancer cells with EPA and DHA has been shown to cause translocation of EGFR out of the lipid rafts (Schley et al. 2007).

3.8.2 Regulation of Transcription

Fatty acids, including ALA, have been shown to alter the activity for a variety of transcription factors, including peroxisome proliferator-activated receptor (PPAR), nuclear factor-kappa B (NF-κB) and sterol regulatory element-binding protein (SREBP) (Jump 2004). PPARs have cancer implications due to their regulation of cell differentiation, proliferation and inflammation, and ALA binding and activating PPAR may decrease cancer cell growth through these pathways (Larsson et al. 2004). Evidence for ALA effectiveness at activating PPARγ was shown in an *in vitro* leukemia cell line (Zhao et al. 2005). Treatment of cells with 100 μM ALA was shown to increase PPARγ expression and decrease cytokines that induce inflammation (TNF-α, IL-6). The transcription factor NF-κB has pro-cancer effects through alteration of inflammation, apoptosis, cell proliferation and angiogenesis (Ghosh and Karin 2002; Karin and Lin 2002; Shibata et al. 2002). Fatty acids have been shown to decrease NF-κB activity through decreasing phosphorylation as well as indirectly through regulating activity of G coupled protein receptors and toll-like receptors which regulate NF-κB activity (Lee et al. 2003; Oh et al. 2010). One study highlighting the ability for ALA to alter NF-κB activity used a rat model of colitis and showed that ALA treatment decreased NF-κB expression, as well as pro-inflammatory TNF-α and COX-2 expression (Hassan et al. 2010). To date, few studies have investigated specifically flaxseed, flaxseed oil and ALA as regulators of transcription factors and more research into this area is warranted.

3.8.3 Lipid Peroxidation

n-3 PUFA are highly unsaturated and therefore are susceptible to oxidation resulting in the production of free radicals and reactive oxygen species (Larsson et al. 2004). Several studies suggest that the observed reduction in tumor and cancer cell growth by n-3 PUFA is related to the extent of lipid peroxidation (Gonzalez et al. 1991; Chajes et al. 1995; Cognault et al. 2000). Further support for the role of lipid peroxidation stems from the fact that the flaxseed oil's tumor-reducing effect was decreased with the addition of antioxidants and was enhanced with the addition of pro-oxidants (Cognault et al. 2000).

3.8.4 Other Potential Mechanisms

Other potential mechanisms of ALA in reducing carcinogenesis have been proposed. These include modulation of the tumor suppressor phosphatase and tensin homologue (Ghosh-Choudhury et al. 2009), fatty acid synthase (FAS) (Menendez et al. 2004) and angiogenesis (Dabrosin et al. 2002; Bergman Jungestrom et al. 2007). Further studies are required to more clearly understand the effect of ALA on these mechanisms and how these effects relate to tumor growth.

3.9 Safety and Regulatory Status

Flaxseed is an ancient crop which has been consumed in populations around the world since around 1000 BC (Thompson and Mason 2010). There is currently no regulation regarding the level of flaxseed that can be added to foods in North America. The United States Food and Drug Administration (FDA) recently issued a no objection decision to the request of Flax 2015 and Flax Council of Canada to consider whole and milled flaxseed for Generally Recognized as Safe (GRAS) status (Cheeseman 2009). As noted above it contains many health beneficial components, however, it does contain anti-nutritional factors which have raised concerns when consumed in excess. Examples include cadmium, cyanogenic glucosides, phytic acid and the vitamin B6 antagonist linatine. However, very high levels would need to be consumed to see adverse reactions. For example, while cyanogenic glucosides produce toxic hydrogen cyanide, it is estimated that adults can detoxify between 30 and 100 mg of cyanide per day (Roseling 1994; Daun et al. 2003). Therefore very large amounts of flaxseed would need to be consumed to show cyanide toxicity considering that studies have estimated the cyanide equivalent content of flaxseed to vary from 190 to 1,000 mg HCN/kg (Daun et al. 2003). A clinical study in healthy females shows that consumption of 50 g of ground raw flaxseed per day for 4 weeks did not cause a significant increase in

urinary thiocyanate excretion (Cunnane et al. 1993). The majority of the studies on flaxseed effect in cancer use levels around 25 g/day. Furthermore, heat treatment destroys cyanogens in flaxseed thus amounts present in baked products or cereals may not pose problems (Cunnane et al. 1993). Adverse reactions to flaxseed are rare although gastrointestinal discomfort has been documented with high consumption which is likely related to the high fiber intake (Demark-Wahnefried et al. 2001; Thompson et al. 2005; Thompson and Mason 2010). Hence, moderate consumption of flaxseed appears to be safe.

3.10 Conclusion and Future Directions

Flaxseed and its oil and lignan components have been most extensively studied for breast cancer treatment and prevention compared to other cancer types. Preclinical animal studies have overwhelmingly shown that they decrease breast cancer growth and may work beneficially with common breast cancer drugs such as tamoxifen and trastuzumab. Although epidemiological and clinical studies are limited, they generally indicate a beneficial role of flaxseed and its components in breast cancer and overall agree with the preclinical studies that flaxseed and its components are safe and likely beneficial. However, more clinical trials still need to be conducted before a definitive general recommendation can be made regarding their use for breast cancer prevention and treatment. Clinical trials on the effect of flaxseed on breast cancer patients have been done but not on the effect of flaxseed oil alone, which thus should be conducted in the future. Promising beneficial interaction with drugs such as tamoxifen and trastuzumab have been studied in rodent models but have yet to be demonstrated clinically. While preclinical and clinical studies have been conducted with ER+ and ER− breast cancer, very little has been done with HER2+ breast cancer. As the effect could differ with different breast cancer types, there is a need to further clarify the role of flaxseed and its oil components on different breast cancer types. More studies on their interaction with other breast cancer drugs should also be conducted.

The majority of preclinical studies focusing on colorectal cancer have also shown a benefit from flaxseed, flaxseed oil and ALA supplementation, however, current epidemiological evidence is weak and clinical trials have yet to be conducted. Therefore, conclusions cannot yet be made regarding their potential use in colorectal cancer prevention and treatment. Similarly, preclinical prostate cancer models have generally shown a benefit from flaxseed, flaxseed oil and ALA treatment and the few completed clinical trials have also shown a decrease in prostate cancer proliferation with no adverse outcomes. However, the epidemiological evidence on ALA association with prostate cancer is conflicting with some studies showing an increase in prostate cancer risk. Therefore, further studies are also needed to understand the effects of flaxseed and flaxseed oil and ensure their safety in prostate cancer patients before recommendations can be made. Studies assessing the effect of flaxseed and its components on other cancer types such as

ovarian and pancreatic are very limited and inconsistent and further preclinical, epidemiological and clinical investigations are needed to further understand their role in these cancer types.

Several mechanisms of flaxseed oil/ALA effect on cancer have been suggested but not all have been systematically studied in relation to specific cancer type; they should be further addressed in future studies. Inconsistencies commonly seen in epidemiological studies maybe reduced by improvements in or methods standardization of future experimental design considering the following which contribute to variability: (i) differences in type, specific subtypes (e.g. with different receptor expressions) and subsites (e.g. proximal or distal colon, rectal) of the cancers, (ii) methods of measuring ALA exposure, (iii) use of appropriate biomarkers, (iv) ALA level/dose and exposure time, and (iv) food source of ALA. Accurate measurement of ALA intake can pose a challenge and FFQs and dietary recalls have the potential to obscure results. The food source providing the ALA is also of importance as effects likely vary between flaxseed, vegetable, fruit, nut, processed and supplemental sources.

Overall, current evidence is encouraging that flaxseed and its oil component are safe and may have a beneficial effect in both prevention and treatment of several types of cancer, but further studies are still needed before making definitive claims and recommendations for their prevention and treatment of cancer.

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Chapter 4
Cancer Prevention with Green Tea Polyphenols

Hong Wang, Hong Zhou, and Chung S. Yang

Abstract Consumption of green tea (*Camellia sinensis*) has been suggested to have beneficial health effect, including cancer prevention. Extensive studies have established that the active cancer preventive constituents in green tea are a group of polyphenols. Green tea polyphenols display anticancer activity in many organ sites in different experimental models in rodents and in cultured cell lines *in vitro*. Treatment with green tea polyphenols leads to the inhibition of cancer cell proliferation, cancer-associated angiogenesis, and metastasis, as well as the induction of cancer cell apoptosis. Experimental studies demonstrate that these activities are likely resulted from the antioxidant activity and the direct binding of green tea polyphenols to proteins, resulting in the modulation of multiple cellular signaling pathways. The findings of polyphenol binding proteins reveal the mechanisms of the effectiveness and specificity of the anticancer actions. However, the inverse association between of green tea consumption and cancer risk is supported by epidemiological studies, but not all. This inconsistence may due to the lower blood and tissue levels of polyphenols from green tea drink, and may also depend on various etiology factors. Using much higher doses, results from some interventional studies support the safety and effectiveness of green tea polyphenols in cancer prevention. Well-designed clinical studies are required to fully evaluate the usefulness of green tea polyphenols in cancer prevention.

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4.1 Introduction

The consumption of green tea, a beverage derived from the dried leaves of the *Camellia sinensis* plant, has a long history in Asia and is believed to have beneficial health effects such as preventing cancer, diabetes, neurodegeneration, eliminating toxic chemicals, anti-aging, improving cardiac function (Weisburger 1999, 2003; Yang et al. 2002; Higdon and Frei 2003; Lambert et al. 2005a; Yang et al. 2009a, b). Recent years, its cancer preventive activity has drawn the most attentions. Since 2000, there are over 2,000 research publications associated to tea and cancer. Extensive studies have established that there are two types of chemicals in green tea constituents, polyphenols and caffeine, responsible for cancer preventive activities (Conney et al. 2007; Yang et al. 2009a, b). The cancer preventive activity of polyphenols is the focus of this chapter.

Green tea catechin are a group of polyphenols, including (−)-epigallocatechin-3-gallate (EGCG), (−)-epigallocatechin (EGC), (−)-epicatechin-3-gallate (ECG), (−)-epicatechin (EC) and other minor catechins (Lambert et al. 2007; Yang et al. 2009a, b, 2011; Sang et al. 2011). Among them, EGCG is the most abundant and active constituent in green tea. The anticancer activity of green tea polyphenols has been demonstrated in many types of cancers in experimental modeling systems range from different cancer cells cultured *in vitro* to various animal models (Yang et al. 2002, 2011). Treatment with green tea polyphenols leads to the direct effects on cancer cells such as the inhibition of tumor cell growth and the induction of tumor cell apoptosis (Yang et al. 2002, 2009b, 2011; Lambert et al. 2005a). Such effects have been reported to involve not only the regulation of specific genes, but also the modulations of multiple cellular signaling pathways (Yang et al. 2002, 2009b, 2011; Lambert et al. 2005a). The cancer preventive activity may be a result of combinatory effects on multiple targets, although the relative importance of the different pathways may depend upon the cellular context. Despite a large body of experimental evidences supporting the anticancer activity of green tea polyphenols, epidemiology studies conducted to determine the potential inverse association between the consumption of green tea and human cancer risk has shown inconsistent results. Some epidemiological studies conclude that green tea consumption is associated with reduced cancer risk, whereas some studies suggest that green tea is not associated with the reduced cancer risk (Boehm et al. 2009; Yang et al. 2009b). Albeit there are only a few clinical studies on the application of green tea polyphenols in cancer prevention, it is suggested that higher doses of green tea polyphenols can be tolerated and effective in cancer prevention. Here, we review the experimental evidences of the biological activity of green tea polyphenols and discuss the existing data from epidemiology and clinical studies regarding whether green tea polyphenol is effective in reducing human cancer risk in order to understand the potential application of green tea polyphenols for cancer prevention.

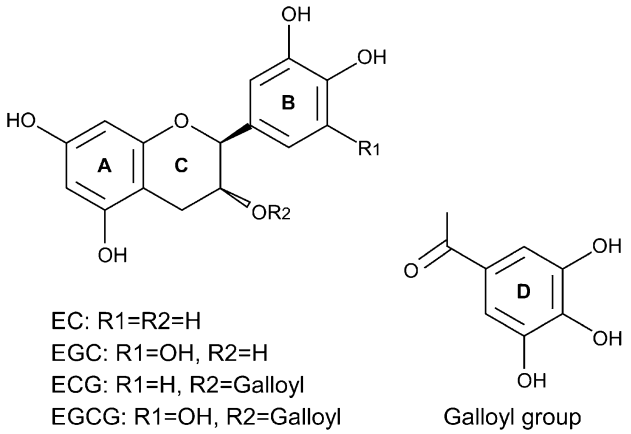

Fig. 4.1 The structures of the major green tea polyphenols

4.1.1 Tea Constituents and Their Biochemical Properties

A typical cup of green tea, brewed with 2.5 g of dry tea leaves in 250 mL hot water, contains 620–880 mg of water extractable chemicals among which tea polyphenols account for 30–42 % (Balentine et al. 1997). The major polyphenols are four catechins, EGCG, EGC, ECG, and EC, and their structures are shown in Fig. 4.1. The water extractable fraction is the green tea extract (GTE) and has often been used for numerous experimental studies in earlier years. Because EGCG is accounted for 50–80 % of the total catechins in tea, it is considered as the major tea catechin and purified EGCG has been used in substantial amount of studies. Thus the bioactivities of tea polyphenols are often represented by the activity of EGCG in many studies.

A well-defined activity of green tea polyphenols is the antioxidant activity, which is associated with the multiple phenolic groups on each ring: dihydroxyl or trihydroxyl substitutions on the B ring and the *m*-5,7-dihydroxyl substitutions on the A ring (Fig. 4.1) (Balentine et al. 1997). The B ring is the principle site of antioxidant reactions (Valcic et al. 2000; Meng et al. 2002), while the trihydroxyl D ring (gallate) of EGCG or ECG provides the extra potentials for antioxidant reactions. The polyphenolic structures allow electron delocalization and give green tea polyphenols ability to quench free radicals. Indeed, it is demonstrated that tea polyphenols are able to trap reactive oxygen and nitrogen species (RONS) including superoxide radical, singlet oxygen, hydroxyl radical, peroxyl radical, nitric oxide, nitrogen dioxide, and peroxynitrite (Balentine et al. 1997; Valcic et al. 2000; Meng et al. 2002). EGCG has the most hydroxyl groups (a total of eight hydroxyl groups) and is the most potent in reacting with RONS. Besides the direct mechanism to quench free radicals, green tea polyphenols are strong chelators of metal ions. Chelation of free metal ions by green tea polyphenols

prevents the formation of reactive oxygen species (ROS) from the auto-oxidation of many compounds that requires metal ions.

However, the vicinal dihydroxy or trihydroxy structure of tea polyphenols not only contributes to the anti-oxidative activity, but also increases the susceptibility of these compounds to air oxidation under alkaline or neutral pH. This is a particularly important feature of EGCG as the auto-oxidation of EGCG in solution generates superoxide anion and hydrogen peroxide and leads to the formation of several unstable intermediates including EGCG quinone, quinone-dimer, and theasinensins (Yang et al. 2009b; Lambert and Elias 2010). It is worth noting that, in the cell culture experiment, the auto-oxidation of EGCG is enhanced in cell culture medium containing metal ions. For example, a half-life of EGCG less than 30 min was observed in McCoy's 5A medium, commonly used for colon cancer cell line culture (Sang et al. 2007). By a real-time mass analysis, the kinetic of EGCG auto-oxidation at the concentrations of 50 and 200 μM in a Tris–HCl buffer (pH 7.2) has been elucidated (Sang et al. 2007). It has been proposed that oxygen reacts with EGCG to produce EGCG radical ($EGCG^{\cdot}$) and superoxide radical ($O_2^{-}\cdot$), both of which are unstable and active. This reaction is probably catalyzed by metal ions such as Cu^{2+} or Fe^{2+}. Then $O_2^{-}\cdot$ reacts with another EGCG molecule to produce EGCG· and hydrogen peroxide (H_2O_2). EGCG· reacts with oxygen to produce EGCG quinone and generate $O_2\cdot^{-}$. $O_2\cdot^{-}$ can then react with another molecule of EGCG for the propagation of a chain reaction of EGCG auto-oxidation (Fig. 4.2), which produces significant amount of ROS.

ROS generated by the auto-oxidation of EGCG is given more attentions in our experimental system using cultured cells. We have found that EGCG is very effective in killing cultured cells as analyzed by cytotoxic or cell proliferation assays with effective concentration as low as 5 μM. This is due to the cytotoxicity of quinone and ROS. However, when we add superoxide dismutase (SOD) into the medium prior to the addition of EGCG to block $O_2^{\cdot-}$, the cell killing effect is significantly reduced. The half maximal inhibitory concentration (IC_{50}) of EGCG required for inhibiting cell proliferation at 48 h is determined to be around 30–50 μM. This issue is particularly important for evaluating the results of the studies on cell signaling mechanism using cultured cells since ROS is able to cause changes on variety of cell signaling pathways. For example, ROS contributes to the inactivation of epidermal growth factor receptor (EGFR) (Naasani et al. 2003; Hou et al. 2005). Thus, we strongly suggest the addition of SOD to remove $O_2\cdot^{-}$ and catalase to remove H_2O_2 for the prevention of EGCG auto-oxidation when EGCG is studied in cultured cells *in vitro*. This addition reduces the cytotoxic effect but does not change the biological action of EGCG. In our recent study identifying *miR-210* up regulated by EGCG, we found no difference in the upregulation of *miR-210* by EGCG in lung cancer cells in the presence or absence of SOD and catalase (Wang et al. 2011). On the other hand, the effect associated with the ROS produced by the EGCG auto-oxidation, such as its inhibition on TGF (Vittal et al. 2004) and EGF (Hou et al. 2005), is prevented by the addition of SOD. Thus, the addition of SOD and catalase to remove ROS in the medium can distinct whether the action is mediated by ROS produced by the auto-oxidation of EGCG.

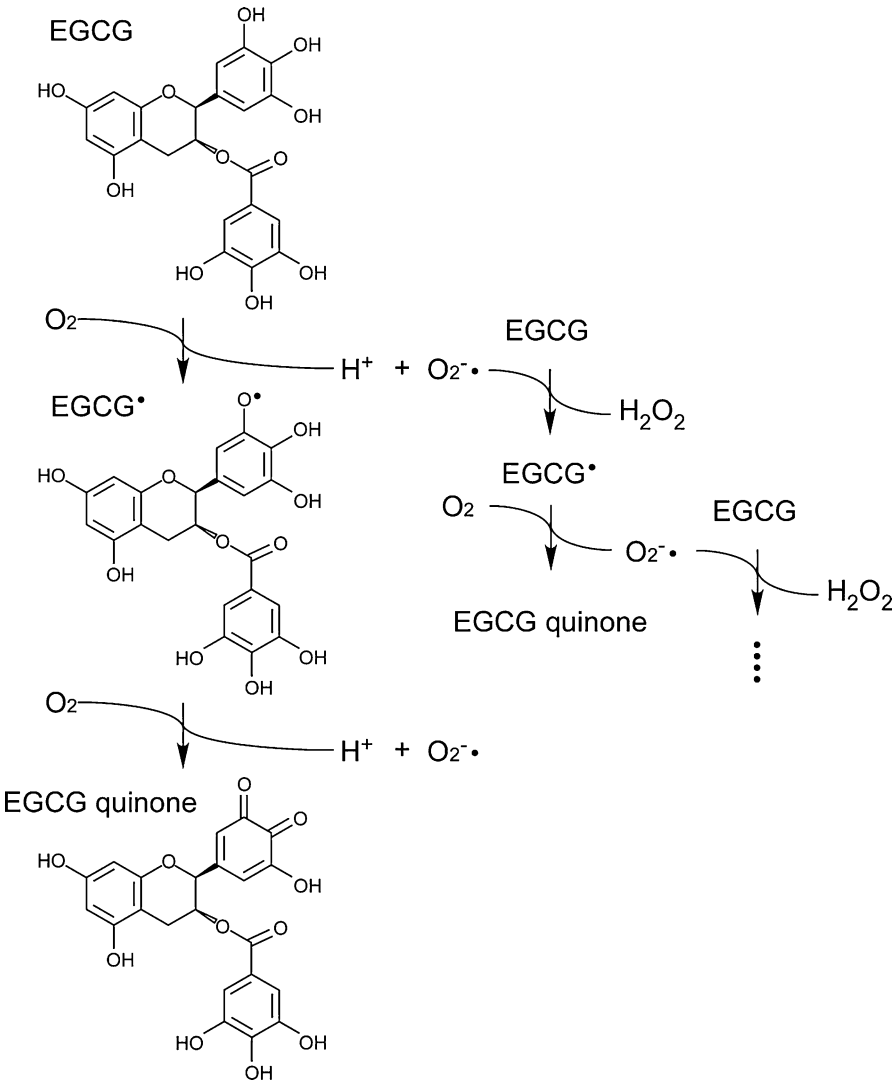

Fig 4.2 The auto-oxidation reaction of EGCG

4.1.2 Green Tea Polyphenol Biotransformation and the Activities of Metabolites

After drinking green tea, it takes about 1–1.5 h for green tea polyphenols to reach peak levels in the blood. When drinking an equivalent of two cups of green tea, the peak values of EGCG, EGC, and EC were 0.26, 0.48 and 0.19 μM, respectively (Lee et al. 2002). Considering the application of EGCG as the cancer prevention

agent, pharmacological doses have been studied and the highest blood peak value reported were as high as 7 μM (Lee et al. 2002). In most animal and clinical studies, the peak blood levels were usually below 1 μM and the half life of EGCG was approximately 2–3.5 h (Lee et al. 2002; Lambert et al. 2008). These observed peak blood values of EGCG were lower than the concentrations of EGCG that were used in most mechanistic studies, which often are about 10–100 μM, in cell culture. Although local concentration of EGCG in cancer tissue may be different, these data about the peak blood levels should be used as references for designing experiments and evaluating the results from *in vitro* studies.

After ingestion, green tea polyphenols have been found to undergo extensive biotransformation, including methylation, glucuronidation, and sulfation, as well as the microbial metabolism (i.e. ring fission) in the digestion tracts (Yang et al. 2002; Feng 2006; Sang et al. 2011). In the plasma, most EGCG is unconjugated (Chow et al. 2001), whereas most of ECG, EGC and EC are in the glucuronidated or sulfated (Lee et al. 1995; Zhu et al. 2000; Yang et al. 2002). However, it is less clear how these processes affect the anticancer activity of green tea polyphenols. Some *in vitro* experimental results suggest that the inhibitory activities of EGCG metabolites on cancer cell growth are less effective than EGCG (Lambert et al. 2005b, 2006; Nakagawa et al. 2007). Thus, it is reasonable to assume that the anticancer activities of green tea polyphenols are not due to their metabolites. EGCG is mainly excreted through bile, whereas EGC is excreted in urine. Since the bioavailability of polyphenols is a key parameter for understanding this biological effect, to measure tea polyphenols and their metabolites may provide useful information.

4.2 Application of Green Tea Polyphenols in Cancer Prevention

Cancer prevention by green tea polyphenols has been extensively studied for many years from *in vitro* and *in vivo* models as well as epidemiology and clinical studies. The overall conclusions are that green tea polyphenols are effective in inhibiting or preventing cancer progression in the majority of animal models. However, not all epidemiology studies support the inverse association between green tea consumption and cancer risk.

4.2.1 Inhibition of Tumorigenesis in Animal Studies

Green tea polyphenols display inhibitory activity against carcinogenesis in animal models at many organ sites, including lung, oral cavity, esophagus, stomach, small intestine, colon, skin, prostate, breast, liver, bladder, pancreas and thyroid (Yang et al. 2009b, 2011; Yang and Wang 2011). Among the models, lung, colon, prostate, breast, and skin cancers have been investigated extensively and will be discussed in details as examples of our current understanding of this subject.

4.2.1.1 Prevention of Lung Carcinogenesis by Green Tea Polyphenols

Administration of green tea polyphenols has been demonstrated to be effective in inhibiting lung carcinogenesis in 19 out of 21 studies using mice, rats, and hamsters (Ju et al. 2007; Yang et al. 2009b). Among these animal models, the inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) or benzo[*a*]pyrene (B[*a*]P)-induced lung carcinogenesis draws the most attentions. NNK and B[*a*]P are the major carcinogens found in cigarette smoke and used to mimic cigarette smoke to induce lung cancer. In A/J mice, NNK treatment induces lung carcinogenesis with the development of adenoma within 20 weeks and the progression of adenoma to adenocarcinoma between 20 and 50 weeks (Hoffmann et al. 1996). When 0.5 % green tea polyphenol extract was given to A/J mice bearing NNK-induced lung tumors as drink fluid for 32 weeks, adenoma progression to adenocarcinoma was inhibited (Lu et al. 2006a). Further, EGCG has been demonstrated to inhibit the xenograft tumors of human lung cancer cell lines H1299 and H460 in nude mice (Li et al. 2010). Apoptosis specific in tumors and not in normal lung tissues was induced by EGCG treatment while pro-proliferation signaling (i.e. c-Jun and phospho-ERK1/2) in tumors were reduced (Lu et al. 2006a; Li et al. 2010). Differential gene expression had also been profiled in tumors from the mice treated with green tea polyphenol (Lu et al. 2006b). Together with other studies on lung cancer, green tea polyphenols display multiple activities in inhibiting different aspects of lung carcinogenesis in this experimental model.

4.2.1.2 Prevention of Colon Carcinogenesis by Green Tea Polyphenols

The cancer preventive activity of green tea polyphenols is also demonstrated in different colon cancer animal models (Ju et al. 2007; Yang et al. 2009b). First, EGCG significantly inhibits colon tumorigenesis in $Apc^{min/+}$ transgenic mouse model. Administration of $Apc^{min/+}$ transgenic mouse with 0.02–0.32 % EGCG as the drink fluid shows a dose-dependent inhibition on the tumorigenesis in the small intestine (Ju et al. 2005; Hao et al. 2007). EGCG treatment leads to reduced Wnt signaling activity as indicated by the increased level of E-cadherin, decreased level of nuclear β-catenin, and reduced level of Wnt target such as c-myc, and pro-proliferation signaling such as phospho-Akt and phospho-ERK1/2 (Ju et al. 2005). Second, EGCG inhibits the chemical carcinogen induced colon cancer in rodent models. The incidence of aberrant crypt foci (ACF), representing colonic premalignant lesion, in azoxymethane (AOM)-treated F334 rats is reduced significantly by 0.01 % EGCG in drinking water (Ohishi et al. 2002). Besides, 0.1 % EGCG in drinking water further inhibits the high-fat diet enhanced incidence of ACF in the AOM-treated CF-1 mice (Ju et al. 2003). However, the involved mechanism remains unclear.

4.2.1.3 Prevention of Prostate Carcinogenesis by Green Tea Polyphenols

Green tea polyphenols display inhibitory activity in mouse prostate cancer models in several studies (Ju et al. 2007; Yang et al. 2009b). In study using the transgenic adenocarcinoma of the mouse prostate (TRAMP) model, 0.1 % green tea polyphenols in drinking water is reported to be effective in inhibiting tumor incidence, burden, and metastasis (Gupta et al. 2001; Caporali et al. 2004). Similar to other cancer models, the pro-proliferation signalings (i.e. phospho-Akt and phospho-ERK1/2) are reduced in the prostate cancer of TRAMP model (Adhami et al. 2004). In this model, IGF-1 is reduced and IGFBP3 is increased (Adhami et al. 2004), which suggest that green tea polyphenols block IGF-1 signaling. However, these studies may not be sufficient to support the inhibitory activity of green tea polyphenols in prostate carcinogenesis. Prostate cancer developed in TRAMP model is androgen-independent and most of them are endocrine origin, whereas human prostate cancer is epithelial cell origin and hormone dependence in the earlier stage (Shen and Abate-Shen 2010). Thus, a proper model is necessary for addressing these issues for determining the effectiveness of the inhibition on prostate carcinogenesis.

4.2.1.4 Prevention of Breast Cancer by Green Tea Polyphenols

The studies of the anticancer activity of green tea polyphenols in mammary cancer in animal models are found to be somewhat inconsistent (Ju et al. 2007; Yang et al. 2011). Some studies show the anticancer activity of green tea polyphenols while others suggest no effect. The overall results can be seen in three different categories: potent inhibitory activity, partial inhibitory effect on certain aspect of mammary tumor, and no effect. It has been suggested that the poor bioavailability of green tea polyphenols in mammary gland tissues might be the reason behind these differences (Yang et al. 2011). Therefore, green tea polyphenols may not be able to target mammary cancer directly. The observed anticancer activity could be an indirect effect resulted from the inhibition of green tea polyphenols on other aspects such as inflammation or oxidative stress.

4.2.1.5 Prevention of Skin Cancer by Green Tea Polyphenols

Skin cancer can be treated by topical application, which overcomes the possible poor bioavailability as proposed in mammary gland tissue described above. Green tea polyphenols can be used more frequently and at higher concentrations. As matter of fact, this approach is very effective in treating mouse skin cancer by EGCG. For instance, topical application of EGCG to the skin in SKH-1 mice treated by UVB results in the reduction of tumor incidence, multiplicity, and size (Lu et al. 2002, 2005). Interestingly, the treatment of green tea polyphenols is found

to also decrease adipose tissue in skin and the inhibition of UVB-induced skin cancer appears to be associated to the reduction of adipose tissue (Lu et al. 2001). It remains to be determined whether this phenomenon is a coincidence or an indirect inhibition of skin cancer.

4.2.2 Epidemiology Studies on the Association Between Consumption of Green Tea and Cancer Risk

Many epidemiology studies, including both cohort and case–control studies, have investigated the cancer preventive activity of green tea against different types of cancers. Most of the studies on possible inverse association between green tea consumption and cancer risk have been conducted in Asian countries such as Japan and China, where green tea is widely consumed. Based on the quality of these studies as assessed by several systematic analyses (Liu et al. 2008; Zhou et al. 2008; Boehm et al. 2009; Myung et al. 2009; Sasazuki et al. 2012), we selected 18 cohort and 28 case control studies and compiled a summary in Table 4.1. About half of these studies were focused on cancers in digestive tract, especially gastric cancer.

Among the studies on gastric cancer, the conclusion from the cohort studies except one found no association between gastric cancer risk and green tea consumption. The meta-analyses on the available data conclude that there is no sufficient evidence to support the inverse association (Liu et al. 2008; Zhou et al. 2008; Sasazuki et al. 2012). However, a recent meta-analyses on selected six cohort studies including more than 218,000 Japanese aged 40 or older and over 3,500 incident stomach cancer cases found the statistically significant, inverse association between green tea consumption and stomach cancer risk in nonsmoker women but not in men. A significantly decreased risk was observed for nonsmoker women with consumption of $\geq$5 cups/day (Inoue et al. 2009).

There are about half of the case–control studies listed in Table 4.1 supporting the inverse association between green tea consumption and gastric cancer risk. Apparently, the studies supporting the inverse association are conducted in China, while the similar studies conducted in Japan do not support the inverse association. Possible error resulted from random events can be ruled out because the sample numbers in all the related studies were sufficient. Inconsistence resulted from the tea composition can also be ruled out because it is generally accepted that the green tea consumed in Japan and China contains the same tea constituents. Perhaps, more details involving the life styles are necessary in order to understand the inconsistence between Chinese and Japanese studies.

The discrepancy can be also found from the studies on the inverse association between green tea consumption and the risk of breast, colorectal, lung, pancreatic, and prostate cancers in Table 4.1. We cannot speculate whether other factors are involved. However, the results from the studies on oral/esophageal and ovarian cancers appear to be consistent. In oral and esophageal cancers, the inverse

Table 4.1 Epidemiology studies on the association between green tea consumption and reduced risk of cancer

Cancer type	Study type	Country	Participants	Association			References
				Women	Men	All	
Breast	Cohort	Japan	488,989	No			Key et al. (1999)
		Japan	35,004	No			Suzuki et al. (2004)
		Japan	63,257	No			Inoue et al. (2008)
	Case control	USA	1,095	Yes			Wu et al. (2003)
		China	2,018	Yes			Zhang et al. (2007)
Colorectal	Cohort	Japan	65,915			No	Suzuki et al. (2005)
		China	69,710	Yes			Yang et al. (2007)
		Singapore	61,320	No	Inverse	Inverse	Sun et al. (2007)
	Case control	Japan	1,324			Yes/no[c]	Kato et al. (1990)
Gastric	Cohort	USA[a]	11,907	No	Inverse		Galanis et al. (1998)
		Japan	26,311			No	Tsubono et al. (2001)
		Japan	44,930	No	No		Fujino et al. (2002)
		Japan	72,851	No	No		Hoshiyama et al. (2002)
		Japan	65,915			No	Koizumi et al. (2003)
		Japan	72,273	Yes	No		Sasazuki et al. (2004, 2008)
	Case control	Japan	376			No	Tajima and Tominaga (1985)
		Japan	4,855			No	Kato et al. (1992)
		Japan	1,336			No	Inoue et al. (1994)
		China	1,422			Yes	Yu et al. (1995)
		China	2,575	Yes	Yes		Ji et al. (1996)
		Japan	2,991			Yes	Kono et al. (1988)
		China	816			Yes	Ye et al. (1998)
		Japan	22,834			No	Inoue et al. (1998)
		Japan	29,506			No	Huang et al. (1999)
		Japan	732			Yes	Setiawan et al. (2001)
		China	1,043			Yes	Mu et al. (2003)

Leukemia/lymphoma	Case control	China	217			Yes	Zhang et al. (2008a, b)
		China[b]	889			Yes	Kuo et al. (2009)
Lung	Case control	China	1,320	Yes			Zhong et al. (2001)
		China	244			No	Bonner et al. (2005)
Oral and esophageal	Cohort	Japan	78,950			Inverse	Ishikawa et al. (2006)
		Japan	50,221	Yes	No	Yes	Ide et al. (2007)
	Case control	China	2,454	Yes	No		Gao et al. (1994)
		China	418			Yes	Wang et al. (1999)
		China	703	Yes	No		Wang et al. (2007)
Ovarian	Case control	China	706	Yes			Zhang et al. (2002)
		USA	2,017	Yes			Song et al. (2008)
Pancreatic	Cohort	Japan	102,137			No	Luo et al. (2007)
		Japan	77,850	No	No	No	Lin et al. (2008)
	Case control	Japan	213			Yes	Goto et al. (1990)
		Japan	248			Inverse	Mizuno et al. (1992)
		China	3,818	Yes	Yes	Yes	Ji et al. (1997)
Prostate	Cohort	Japan	19,561		No		Kikuchi et al. (2006)
		Japan	49,920		Yes		Kurahashi et al. (2008)
	Case control	Japan	280		No		Sonoda et al. (2004)
		China	404		Yes		Jian et al. (2004)

[a]Only including Japanese residents in Hawaii, USA
[b]Only in Taiwan, China
[c]Yes in colon cancer but no in rectal cancer

association has been found in women, but not men in both Japan and China. Results from the two case control studies on leukemia in China are also consistent. Therefore, based on the current data, it is safe to propose that the consumption of green tea can be cancer preventive, but whether it is effective may be related to the etiology of certain cancer.

It should be pointed out again that green tea polyphenols in the subjects of these studies are from the daily consumption and can only reach ~0.1 μM in the blood. The dose of green tea polyphenols found to be effective in animal studies are at a much higher level (>1 μM in the blood). Considering that the bioavailability is a key issue, the intake level of green tea polyphenols should be documented in the future case–control and cohort studies. This can be done by indirectly monitoring the metabolites in urine based on our knowledge about polyphenol metabolism. Such data would be helpful to classify the subjects according to bioavailability levels and to rule out the possible difference in the composition of green tea when similar studies from different area or time are applied for comparison. Given that etiology factors are often related to life-style which could be very different depending on geography or culture, to collect different data for further study such as meta-analysis should involve these records or the related etiology study result. In one example described above, although several meta-analyses found no association between gastric cancer risk and green tea consumption when all studied were combined, the statistically significant inverse association is clear in nonsmoker women (Inoue et al. 2009).

4.2.3 Clinic and Intervention Studies

Limited clinical and intervention studies have been conducted to further explore the application of green tea polyphenols against cancer. In these studies, patients or healthy personals are given the higher doses of green tea polyphenols resulting in the blood levels higher than levels obtained from usual tea consumption. While most results are positive, a clear conclusion in support of anticancer effect cannot be reached. This might due to the fact that the numbers of people in these studies are often very small and the duration of treatment is short compared to that in animal studies. Here, we discuss a few studies in order to understand the opportunity for the application of green tea polyphenols in cancer prevention.

Healthy person can be benefited by the antioxidant activity of green tea polyphenols. Supplementation of green tea polyphenols (500 mg/day) in the diet of healthy persons for 4 weeks reduced oxidized low-density lipoproteins in blood by 18 %, compared to the placebo (Inami et al. 2007). When the similar dose (455 mg/day) was given to patients on haemodialysis for 3 months, plasma hydrogen peroxide, hypochlorous acid, C-reactive protein, and pro-inflammatory cytokines were significantly reduced (Hsu et al. 2007). These results support the concept that green tea polyphenols can improve the antioxidant activity in our body and prevent the damage due oxidative stress.

Some intervention studies provide suggestive evidences for the application of green tea polyphenols against cancer in high risk population. In a double-blind, placebo-controlled study, 60 volunteers with high-grade prostatic intraepithelial neoplasia (HG-PIN) were randomized to receive three capsules (200 mg of green tea polyphenols each; a total of 600 mg/day) or placebo for 1 year. One subject was diagnosed with prostate cancer among 30 men receiving green tea polyphenols (incidence = ~3 %), whereas nine cancers were found among 30 men receiving placebo (incidence = 30 %) (Bettuzzi et al. 2006). The 30 % incident rate in the placebo group was consistent with the clinical data that about 30 % HG-PIN patients develop advanced cancer. This result strongly supports that green tea polyphenols are effective in treating premalignant lesions and preventing its development to advanced tumor. A 2-year follow-up study on a subset of these 60 patients showed a promising protective effect against prostate cancer development (Brausi et al. 2008). In another study on 26 patients receiving green tea polyphenols (1.3 g/day containing 800 mg EGCG) for an average of 35 days during the interval between positive biopsies and radical prostatectomy, the application of green tea polyphenols reduced the levels of cancer-associated biomarkers such as PSA, HGF, VEGF, IGF-1 and IGF-1:IGF binding protein 3 ratio (McLarty et al. 2009). Similarly, in a phase 2 randomized trial consist of 41 patients with high-risk oral premalignant lesions (11 receiving placebo, 11 receiving 500 mg GTE/m^2, 9 receiving 750 mg GTE/m^2, and 10 receiving 1 g GTE/m^2 for 12 weeks), biomarkers such as VEGF and cyclin D1 were significantly reduced in the lesion biopsies (Tsao et al. 2009). This result is consistent with the finding in another randomized, placebo-controlled phase 2 trial that 3 g/day of green tea extract reduced the size of oral mucosa leukoplakia, a precancerous lesion, in 37.9 % patients (Li et al. 1999). In a Japan trial comprised of 136 patients with colorectal adenomas first removed by endoscopic polypectomy and confirmed the clean colon 1 year later (71 receiving with 1.5 g GTE/day for 12 months and 65 as control), the incidence of adenomas at the end-point colonoscopy was 31 % in the control group and 15 % in the GTE group (Shimizu et al. 2008a).

However, there are also studies showing negative results of green tea polyphenols against cancer. An earlier phase 2 trial on 42 patients with hormone-independent prostate cancer showed that receiving green tea polyphenols (a total 6 g/day) for a month increased PSA levels by 43 % (Jatoi et al. 2003). In a recent intervention study randomizing 50 prostate cancer patients scheduled to undergo radical prostatectomy, patients receiving 800 mg EGCG for 3–6 weeks before surgery show favorable when compared to placebo but not statistically significant changes in PSA, IGF, and oxidative DNA damage (Nguyen et al. 2012).

Taken together, the available clinical studies support the cancer preventive activity of green tea polyphenols, but these studies are rather preliminary since the size of the trials is small and the long-term effect is unknown. Albeit, green tea supplement already became the most commonly used product for self-treating breast cancer survivors in Canada (Boon et al. 2007). Now, various green tea

products are available over counter and self-treatment may run ahead of our knowledge. The opportunity is on the horizon, but the challenge is how to better design and conduct further clinical study to clearly address whether green tea polyphenols are applicable against cancer. A larger-scale study such as phase 3 trial is necessary to determine the efficacy. Given the fact that we know more about the metabolism of green tea polyphenols, the intake level should be monitored by examining the urine samples frequently. More, future clinical study should integrate the experimental study to apply biomarkers to determine the short-term responsiveness in addition to long-term effect. Furthermore, we suggest to preserve blood, urine, and tissue samples for "-omic" studies. The genomic and proteomic studies using these materials will provide data for understanding the molecular mechanism in-depth. These materials will also be useful to better categorize cancers by the genetic or epigenetic features, the so-called molecular pathological characteristics. Although personalized medicine has not been established in this field, the responsiveness could be possibly associated to specific subtype of cancer if the "-omic" data is available. Based on our knowledge from experimental studies, it is anticipated that a well-designed phase 3 clinical study could determine the efficacy of green tea polyphenols in cancer prevention and clarify whether its application depends on the types or stages of cancer.

4.3 Mechanisms of Tea Polyphenols in Cancer Prevention

To better understand the cancer preventive activity of green tea polyphenols found in animal studies and to promote them for human cancer prevention, substantial studies have been conducted to uncover the mechanism at the cellular and molecular levels. Experimental results collectively show that the treatment of animal cancer models or cancer cells *in vitro* with green tea polyphenols leads to wide range of responses. It has been reported that green tea polyphenols enhance detoxification to prevent the carcinogen-induced genetic and epigenetic damage (Na and Surh 2006, 2008; Chow et al. 2007), alter epigenetic modification on chromosome such as reducing DNA hypermethylation-associated tumor suppressor silence (Fang et al. 2003; Navarro-Peran et al. 2005; Gao et al. 2009; Choudhury et al. 2011; Nandakumar et al. 2011; Wong et al. 2011), inhibit tumor cell growth by inducing cell cycle arrest and apoptosis (Yang et al. 2009b; Singh et al. 2011), decrease inflammation (Hong et al. 2001; Na and Surh 2006; Pan et al. 2011), and inhibit tumor-associated angiogenesis (Noonan et al. 2007; Yang et al. 2009b; Singh et al. 2011). These activities are consequences of the direct scavenge of ROS and the physical interactions on proteins with various functions. The cancer preventive activity could be resulted from a combinatory effects on multiple targets. The actions of green tea polyphenols mediated through different mechanisms directly lead to the

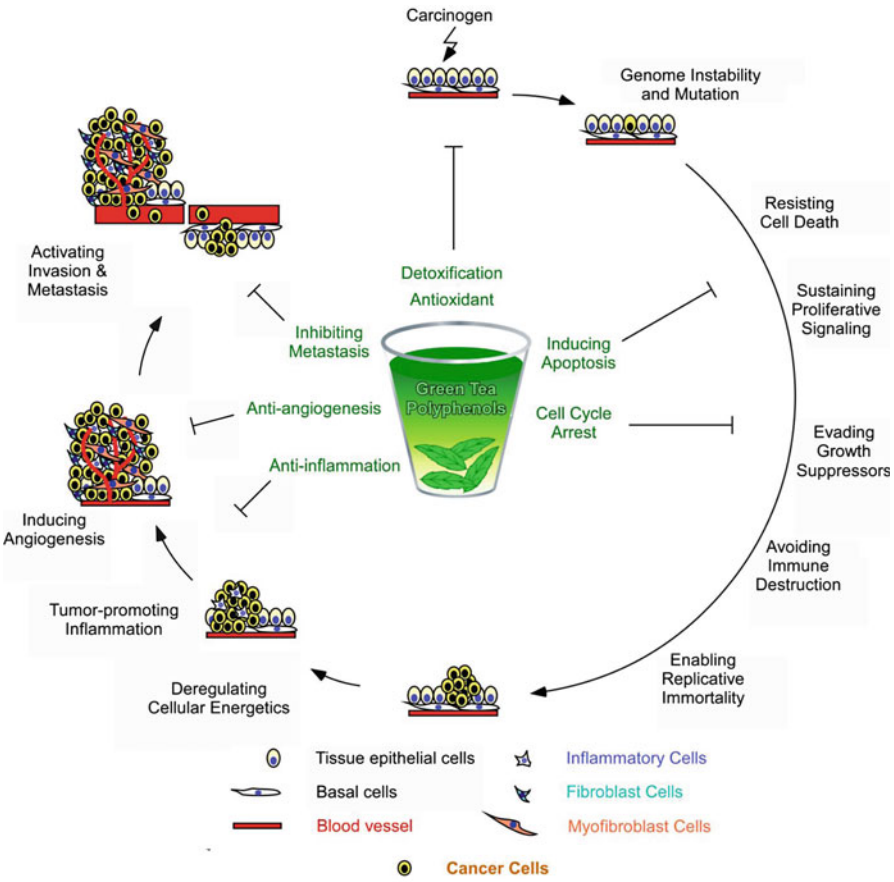

Fig. 4.3 Green tea polyphenols display inhibitory activity on multiple cancer hallmarks

inhibitions on several aspects in carcinogenesis (Fig. 4.3). These aspects are part of the key elements promoting human cancer and also referred to as cancer hallmarks (Hanahan and Weinberg 2011). Compared with the inhibitors designed for targeting specific hallmarks, green tea polyphenols are not potent inhibitors. But, perhaps, it is the inhibitions on multiple hallmarks that lead to the overall anticancer activity of green tea polyphenols albeit most of these inhibitory actions are weak (Fig. 4.3). It is also possible that a specific event/hallmark targeted by EGCG plays dominant role in a specific cancer. To associate any cellular and molecular mechanism to the anticancer activity of EGCG and its application should be carefully evaluated by its effective concentration at the levels compatible to the achievable blood levels in human. In the following, we will discuss the mechanism generally accepted in this field.

4.3.1 Antioxidant Activity

Green tea polyphenols are very sensitive to oxidation reaction. For decades, the antioxidant activity is believed to be the major biological activity of tea polyphenols (Yang et al. 2009a, b; Singh et al. 2011). For example, supplementation of green tea polyphenols (500 mg/day) in diet for healthy individuals for 4 weeks reduces oxidized low-density lipoproteins in blood by 18 % (Inami et al. 2007). In the experimental model, administration of EGCG to aging rats decreases the aging associated oxidative stress and lipid and protein damages (Senthil Kumaran et al. 2008; Srividhya et al. 2008). The similar effect could also protect cells from oxidative DNA damage. It has been reported that the supplement of four cups of green tea with 73.5 mg polyphenols to heavy smokers for 4 months reduced the urinary level of 8-hydroxydeoxy-2′-deoxyguanosine (8-OHdG) by 31 % (Schwartz et al. 2005).

In addition, the indirect antioxidant mechanism, which includes the induction of antioxidant enzymes (i.e. catalase, SOD, etc.) and phase 2 conjugating enzymes (i.e. glutathione-*S*-transferases, glucuronidase, and sulphotransferases), has been proposed for green tea polyphenols (Na and Surh 2008). In a trial with 42 volunteers receiving green tea extract containing 800 mg/day for 4 weeks, glutathione-*S*-transferases activity and glutathione-*S*-transferases-pi (GSTP1) level in blood lymphocytes were increased significantly in individuals with low baseline enzyme activity/level (Chow et al. 2007). Since some of these enzymes are regulated by transcriptional regulator Nrf2, which is responsive to cellular reactive oxygen level, it has been suggested that EGCG enhances Nrf2 activity (Na and Surh 2008). However, this issue remains to be further clarified. EGCG generates ROS *via* auto-oxidation reaction, as discussed above (Fig. 4.2). It is possible that Nrf2 as the ROS sensor is activated by ROS generated by EGCG. In fact, we have found Nrf2 activity is upregulated in lung cancer cell H1299 carrying Nrf2 binding site-luciferase reporter treated by EGCG, but such an upregulation is abolished when catalase and SOD are added to culture medium. Therefore, it is unlikely that the antioxidant activity of green tea polyphenols involves Nrf2. Whether the indirect antioxidant mechanism is activated through other mechanism remains to be determined.

4.3.2 Direct Binding to Proteins

One major mechanism of the cancer preventive activity of green tea polyphenols is attributed to binding to proteins. Phenolic groups, for example in EGCG, offer hydrogen bond donor to mediate interactions with other molecules. To date, EGCG has been demonstrated to bind physically to a panel of proteins with different affinities. These proteins are featured with varieties of functions involved in cellular signaling, proliferation, apoptosis, structure, and etc. (Singh et al. 2011; Yang and

Wang 2011). They underline the involvement of multiple mechanisms, but make it hard to recognize the important one. Sometime, high concentration of EGCG (i.e. ~100 μM) is needed to validate the influence of polyphenols on the functions of these proteins in cells. Such high concentrations raise questions about the physiological relevance of these targets. Indeed, to what degree the binding proteins contributing to the *in vivo* anticancer activity remains to be determined. Here, we briefly review the proteins with the high-affinity binding EGCG.

Using EGCG-sepharose 4B column, Dong and colleagues have identified a group of proteins that include intermediate filament vimentin, non-receptor tyrosine kinases Fyn and ZAP70, cell signaling regulators GRP78, and Ras-GTPase-activating protein SH3 domain-binding protein 1 (Ermakova et al. 2005, 2006; He et al. 2008; Shim et al. 2008, 2010). Since the binding affinities for these proteins range from 3.3 to 0.7 μM, they are generally considered as the high-affinity binding proteins. Among them, EGCG binds vimentin with $K_d = 3.3$ nM. However, the role of EGCG binding remains unclear since there is no phenotypic change found in mice with vimentin null mutation (Colucci-Guyon et al. 1994). EGCG binding to Fyn and ZAP70 results in the loss of kinase activity of Fyn and ZAP70. But the inhibition of Fyn and ZAP70 are unlikely to directly mediate the cancer preventive activity of EGCG because these two protein kinases are hematopoietic-cell specific. On the other hand, SH2 domains of Fyn and ZAP70 are the physical binding sites for EGCG, suggesting that EGCG may target other SH2 domain proteins expressed in cancer cells, a possibility that should be explored further. EGCG was also reported to bind and inhibit IGF-1R but with $K_d = 14$ μM (Li et al. 2007). Although the K_d is higher, it is consistent with the findings of IGF signaling inhibition by EGCG administrated in a colon carcinogenesis model (Shimizu et al. 2008b) and a liver carcinogenesis (Shimizu et al. 2011) in db/db mice and a prostate carcinogenesis in TRAMP mice (Gupta et al. 2001). Thus, both *in vitro* and *in vivo* experimental studies support that IGF signaling is an EGCG target.

A recent important finding is the interaction of EGCG with peptidyl prolyl *cis/trans* isomerase (Pin1). Dong and colleagues demonstrated the physical interaction by X-ray crystal structure of EGCG-Pin1 complex at 1.9 Å resolution (Urusova et al. 2011). This interaction inhibits isomerase activity of Pin1 by preventing the access of its catalytic domain to the substrates (Urusova et al. 2011). Since NF-κB and the AP-1 member c-Jun are Pin1 substrates, this result suggests how EGCG indirectly regulates AP-1 and NF-κB activities, which are critical regulators in both cancer and inflammatory cells. Furthermore, this mechanism is demonstrated to be the mechanism, at least partially, for EGCG to inhibit the growth of colon cancer cells in a xenograft model (Urusova et al. 2011).

Another new finding revealed recently is that EGCG binds and stabilizes HIF-1α and upregulates *miR-210* expression (Wang et al. 2011). There are several studies on the regulation of EGCG on HIF-1α but whether EGCG downregulates or upregulates HIF-1α activity remains controversial. By microRNA expression profile analysis of lung cancer cells treated by EGCG, *miR-210* is found to be the only microRNA upregulated by EGCG treatment (Wang et al. 2011). Further study reveals that EGCG is likely to bind the key Proline residues in the

oxygen-dependent regulatory domain of HIF-1α and prevent the modification of Proline and subsequent proteasome-mediated degradation. Since *miR-210* displays suppressor activity in tumor initiation, presumably by regulating the expressions of more than 50 genes (Huang et al. 2009), the upregulation of *miR-210* provides an additional mechanism for EGCG to target multiple genes indirectly. However, whether this is the case *in vivo* needs to be further investigated in the animal model.

Besides the above mentioned targets, EGCG has been reported to bind other proteins and affect their functions. Some of them, such as 67-LR, glucose-6-phosphate dehydrogenase and HGF receptor/c-met, also provide the mechanism for EGCG to interfere with the cellular signaling, metabolism, and inflammatory regulation (Yang and Wang 2011). In addition, EGCG is found to alter plasma membrane through affecting the protein distribution and function in lipid raft, resulting in indirect influence on the activity of EGFR (Adachi et al. 2007), c-Met (Duhon et al. 2010), and 67-LR (Fujimura et al. 2005) at relatively lower concentrations in different cancer cell lines. Therefore, EGCG can display activities on different pathways through these proteins. A combinatory effect of these activities may lead to inhibition of cancer initiation, progression or metastasis (Pan et al. 2011; Singh et al. 2011; Yang and Wang 2011).

4.3.3 Induction of Cell Cycle Arrest and Apoptosis

The anticancer activity resulted from the treatment of green tea polyphenols has been found to be associated to the reduced cell proliferation and increased apoptosis (Yang et al. 2009b; Singh et al. 2011). For example, in the mouse lung cancer models, treatment of green tea polyphenols leads to the significantly reduction of cell proliferation marker Ki-67, pro-proliferation signaling such as the phosphorylation of Akt and ERK1/2, and the increase of cleaved caspase-3, an apoptosis index (Lu et al. 2006a; Li et al. 2010). In various cancer cells cultured *in vitro*, EGCG has been reported to trigger cell cycle arrest by modulating the levels of cyclin D1, cdk4, cdk6, p21/WAF1/CIP1, and p27/KIP1 as well as p53, and induce apoptosis by increasing levels of pro-apoptotic regulators, Bax, Bak, Bcl-XS, and PUMA, and decreasing levels of anti-apoptotic regulators, Bcl-2 and Bcl-XL (Yang et al. 2009b; Singh et al. 2011). Cell cycle arrest and induction of apoptosis are likely to be resulted from the actions of green tea polyphenols on the targets such as the inhibition on the activities of NF-κB, Ap-1 transcriptional factors, Akt and MAP kinases (Yang et al. 2009b). More, they could be combinatory effects resulted from multiple upstream events targeted by green tea polyphenols.

4.3.4 Anti-angiogenesis

The anticancer activity of green tea polyphenols also involves the inhibition on the growth of tumor-associated blood vessels. Such an anti-angiogenesis activity has been reported to be mediated by the downregulation of VEGF in cancer cells or by direct inhibition on endothelial cells. When cancer cell lines (HeLa, HepG2, and SW837) were treated with EGCG, the hypoxia-induced stabilization of HIF-1α and upregulated expression of VEGF were reduced (Zhang et al. 2006; Shimizu et al. 2010). However, these data are controversial to the results supporting that EGCG can direct bind and stabilize HIF-1α (Thomas and Kim 2005; Weinreb et al. 2007; Wang et al. 2011). Our expression profiles on the EGCG-treated lung cancer H460 cells also show that treatment with EGCG upregulates VEGF and other HIF-1α targets (unpublished data). Thus the anti-angiogenesis *via* the downregulation of VEGF by EGCG may depend on the cells or cell culture conditions. When endothelial cells such as HUVECs were treated with EGCG, the activity of FOXO was upregulated, resulting in the reduced HUVEC migration and capillary tube formation (Shankar et al. 2008), suggesting the direct inhibitory activity of EGCG on endothelial cells. This result is consistent with the finding that EGCG enhances the phosphorylation and phosphorylation-dependent transcriptional activity of FOXO at lower concentration (i.e. 1 μM) (Anton et al. 2007; Bartholome et al. 2010). Thus, the anticancer activity of green tea polyphenols in animal models involves the anti-angiogenesis via directly modulating the activity of FOXO in endothelial cells.

4.3.5 Other Potential Mechanisms

In addition to above discussed mechanisms, experimental results suggest the involvement of other mechanisms such as anti-inflammation and anti-metastasis. The inhibition on NF-κB and AP-1 transcriptional factors supports that green tea polyphenols play regulatory roles in inflammatory response (Singh et al. 2011). For example, EGCG reduces the virus-induced inflammation mediated through quenching the RONS which activates NF-κB (Lee et al. 2004). Inhibitions on AP-1 transcriptional factors are the downstream events of the inhibition on PI3K/Akt and MAP kinases (Singh et al. 2011). More, as discussed above, the negative regulation on NF-κB and AP-1 factors could be mediated through the inhibition on Pin1 by EGCG (Urusova et al. 2011). Green tea polyphenols are also reported to be effective on inhibiting tumor metastasis in the mouse model using Lewis lung carcinoma cells, which mimic lung metastasis after injected through tail veins. The total number of tumor colonies in lung, an index of metastatic Lewis lung carcinoma cells, has been found reduced significantly by oral administration of green tea polyphenols (Sazuka et al. 1995). In an *in vitro* assay to measure the metastasis potentials of cancer cells, treatment with EGCG reduces the invasive

characteristics of B16 melanoma cells (Watanabe et al. 2012), which might be associated to the reduced matrix metalloproteinases in cancer cells after EGCG treatment (Deng and Lin 2011). In addition to these mechanisms discussed, it can be expected that further in-depth studies on each of these specific directions will uncover more details of the action of green tea polyphenols in cancer prevention. Nevertheless, experimental results support that the anticancer activity of green tea polyphenols is mediated through multiple mechanisms.

4.4 Prospective

In summary, preclinical studies using animal models, molecular and cellular approaches demonstrate the anticancer activities of green tea polyphenols. In addition, limited clinical studies support the cancer preventive effect, despite the mixed results yielded from epidemiology studies. Our understanding of the roles of green tea polyphenols remains incomplete. Further clinical study should integrate new advancements and technologies to systematically monitor the intake, blood and urine levels of green tea polyphenols and metabolites in subjects as well as to determine the short-term responsiveness with biomarkers and correlate it to disease causes, progression, and other aspects. Since the "-omic" technologies are ready for better characterization of individual case based on its genetic and epigenetic background, the tissue sample should be ensured and properly preserved for future extraction of DNA/RNA/protein for -omic analyses.

Furthermore, the combination of green tea polyphenols with other agents or medicines also provides an opportunity to explore the potentials for more effective prevention or treatment. In NNK-induced A/J mouse lung carcinogenesis model, green tea polyphenols display significant inhibition on adenoma progression to adenocarcinoma but not on the induction of adenoma (Lu et al. 2006a). When 0.25 % green tea polyphenols was used with 200 ppm atorvastatin (trade name Lipitor) to treat NNK-induced A/J mouse, the tumor multiplicity was reduced by 56 % and the tumor burden was reduced by 55 % at 20 weeks after NNK-treatment (Lu et al. 2008). When 0.25 % GTE or 200 ppm atorvastatin was used alone, there was no effect significant effect on tumor multiplicity at this stage. Higher dose, 0.5 % GTE or 400 ppm atorvastatin, reduced the tumor burden by 22 % compared with the NNK control group, but no effect on tumor multiplicity (Lu et al. 2008). This work strongly suggests the synergistic effect of polyphenols and atorvastatin on cancer prevention. Other preclinical studies support the synergy of EGCG with other agents, such as taxane (Stearns and Wang 2011), curcumin (Yunos et al. 2011), COX-2 inhibitors (Suganuma et al. 2011), doxorubicin (Stearns et al. 2010), luteolin (Amin et al. 2010), erlotinib (Amin et al. 2009), and sulforaphane (Nair et al. 2008). An exciting advance reported recently is that the combination of EGCG with phosphodiesterase 5 inhibitor (i.e. Vardenafil) significantly potentiates the EGCG induced apoptosis of cancer cells expressing high level of 67-LR (Kumazoe et al. 2013). Low dose of EGCG (i.e. 1 μM) which can be reached in plasma is

effective to induce cancer cell death in animal model when administrated with Vardenafil. These combinations effectively inhibit tumor growth in animal model or cancer cell *in vitro* presumably by targeting different aspects of tumor simultaneously, multiple components in one pathways, or same protein through different mechanisms. Such applications show advantages to overcome several limitations of one agent, which is consistent with the concept to treat cancer with multiple agents to improve the effectiveness, to reduce the side effect or toxicity, and to reduce the possibility of drug resistance (Glickman and Sawyers 2012). For example, the synergistic effect of EGCG and taxane can reduce the toxicity of taxane by using lower dose of taxane (Stearns and Wang 2011). More, the combination of EGCG and erlotinib could be more potent in inhibiting lung cancer with EGFR mutations but also make drug resistance resulted from addition EGFR mutation much less possible by simultaneously targeting EGFR with different mechanisms (Amin et al. 2009). These findings will need to be further investigated to uncover the mechanisms and be validated in both animal and clinical studies. Nevertheless, it opens a new page for the potential application of green tea polyphenols against cancer.

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Chapter 5
Soy Foods: Towards the Development of Novel Therapeutics for Breast Cancer

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Abstract The increasing cognizance that diet (and lifestyle) can modify breast cancer risk and progression has motivated many breast cancer patients to take increasing personal control of the direction of their therapies after diagnosis and surgery. While this has certain advantages, including higher compliance to prescribed drugs and improvements in emotional and mental well-being, it predicates the need for increased understanding of the benefits of particular diets and dietary regimen to the treatment programs and for improved translation of data obtained from studies with animal models into clinical settings. Epidemiological studies have linked high consumption of soy-rich foods to the lower incidence of breast

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cancer in Asia relative to that in Western countries. The potential of soy-rich foods as breast cancer protective when dietary exposure occurs early in life, has resulted in driving the use of soy and its associated bioactive components, specifically the isoflavone genistein, as chemopreventive agents or as adjuvants to conventional drug therapies. Bioactive components in soy foods may affect hormone and non-hormone-mediated mechanisms. However, their overall biological outcomes remain not well-understood and at times, contradictory, due to distinct physiological contexts and doses of exposure, multiple targets, and inconsistent measures of relevant endpoints. Here we provide an argument in support of the potential use of soy foods for breast cancer patients based on the review of the current literature as well as raise caveats that must be addressed for its successful application as standard-of-care treatment.

5.1 Introduction

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer deaths among women in the United States (Siegel et al. 2012). Worldwide, more than 450,000 new cases of breast cancer are diagnosed annually, and the numbers of women who succumbed to the disease have tripled within the past three decades. Nevertheless, there is a disparity in the global distribution of breast cancer, a consequence in part of environmental rather than genetic differences among the general population (Hortobagyi et al. 2005). Diet and lifestyle constitute modifiable determinants of breast cancer risk (Blackburn et al. 2003; Brennan et al. 2010; Patterson et al. 2010). The strongest support for the notion that breast cancer susceptibility can be influenced by nutrition and lifestyle has come from epidemiological and case–control studies demonstrating a two- to eight-fold lower occurrence of breast cancer in Asian women, whose early intake of soy foods is 10–20 times higher than their Western counterparts (Shu et al. 2001; Hilakivi-Clarke et al. 2010). Based on the latter and the emerging evidence for diet-mediated regulation of mammary epithelial differentiation, proliferation, and apoptosis, either directly or circuitously (Su et al. 2011), the prospect that soy foods and associated bioactive components may constitute novel therapeutics for breast cancer is a definite possibility. Indeed, the current interest in soybeans and their phytoestrogen components have triggered a number of limited clinical trials (http://www.clinicaltrials.gov) to evaluate the efficacy of these compounds as treatment modalities in women afflicted with the disease.

Natural agents found in foods may, theoretically, confer benefit for breast cancer control in two ways, which are not necessarily exclusive: one, by acting as chemopreventive agents, to inhibit, delay and reverse the development and progression of the disease, and second, as a drug to sensitize tumor cells to conventional therapies (chemotherapy, radiation treatment) and impede further progression, recurrence, and metastasis. The over-arching goal of these interventions is to decrease breast cancer risk, increase patient survival, and improve quality of life after

Table 5.1 Pro-survival and pro-death pathways influenced by exposure to bioactive components in soy foods

Signaling pathway	Pro-survival mediators	Pro-death mediators
Wnt/β-catenin	β-catenin	BAX
	Bcl2	E-cadherin
	C-myc	p21
	Cyclin D1	
PI3K/PKB(AKT)	mTOR	PTEN
NF-κB	Interleukins	p65
	TNFα	
p53	Survivin	p53
IGF-1	PKB (AKT)	IGFBP3
	mTOR	
	PI3K	
	MAPK	
	JNK	
Estrogen receptor mediated	ERα	ERβ
BRCA mediated		BRCA1, BRCA2

BRCA1 breast cancer type 1 susceptibility protein, *ER* estrogen receptor, *IGF-1* insulin-like growth factor 1, *IGFBP* insulin-like growth factor binding protein, *JNK* jun kinase, *MAPK* mitogen activated protein kinase, *mTOR* mammalian target of rapamycin, *NF-κB* nuclear factor-kappa B, *PI3K* phosphoinositide 3-kinase, *PKB* protein kinase B (also known as AKT), *PTEN* phosphatase and tensin homologue, *TNFα* tumor necrosis factor alpha

breast cancer. How cells integrate the cellular signals imposed by dietary factors and respond accordingly under distinct physiological contexts remains unclear. Because the major consequences of these regulatory signals may differ between normal and neoplastic breast cells, it is imperative that clinicians and healthcare professionals with the intent of harnessing the bioactivities of dietary constituents for therapeutic interventions understand the central molecular players that orchestrate response to pro-death and pro-survival signals (Table 5.1) that are induced and repressed, respectively by bioactive soy components.

In this chapter, we discuss the preclinical and clinical studies that provide support for (or against) the use of soy foods as endocrine or local paracrine interventions that may dictate the fate of breast cancer cells to arrest growth and die. We also briefly present here, the biological mechanisms currently considered to mediate dietary effects on distinct mammary compartments.

5.2 Mammary Gland Biology and Mechanisms of Dietary Protection

The mammary gland, structurally and developmentally, is one of the most complex tissues in mammals. It is comprised of myoepithelial and luminal epithelial cells embedded in a complex stromal matrix (so called mammary fat pad), composed

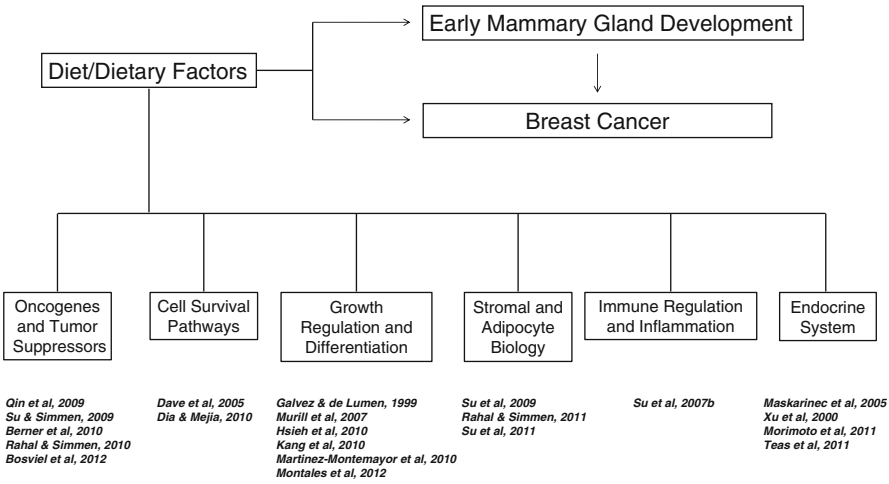

Fig. 5.1 Summary of proposed biological events regulated by soy and associated bioactive components in mammary epithelial and stromal compartments for breast cancer protection. Dietary factors influence numerous processes during distinct stages of early mammary gland development that are subverted due to genetic mutations and epigenetic modifications during breast cancer initiation and progression. Representative publications providing scientific support to signaling pathways that are influenced by diet are cited here and listed under References

predominantly of fibroblasts, adipocytes and macrophages. While the development of the mammary gland differs temporally to some extent in human females and rodents (Hennighausen and Robinson 2001), it is widely acknowledged that the dynamic growth, organization and structuring of the epithelial compartments in both species occur at puberty with the onset of ovarian estrogen synthesis. Nevertheless, the key (and paradigm-shifting) findings that a mammary epithelial hierarchy exists (Shackleton et al. 2006); that the epithelial sub-population 'sitting at the top' of the mammary epithelial hierarchy can serve as initial targets of oncogenic agents (Visvader 2009), and that fetal mammary glands (in mice) contain a higher population of mammary stem cells than in adult mammary tissues (Spike et al. 2012) implicate mammary epithelial cells from which tumors arise and neighboring stromal cells to exhibit remarkable plasticity beginning from the earliest stages of mammary development. Thus, the study of events leading to breast cancer initiation and progression and of how diets can influence breast cancer risk is tightly coupled to the understanding of dietary effects on early mammary gland development. The plethora of local- and endocrine-derived factors that regulate the transcriptional programs in mammary epithelial cells and of the neighboring stromal cells is beyond the scope of this chapter. However, the signaling pathways regulated by dietary factors that allow for normal functions and development of the mammary gland are most likely the same as those that become deregulated leading to breast cancer (Fig. 5.1).

5.3 Soy Food Intake and Breast Cancer Prevention

Evidence suggests that soy food intake during childhood and adolescence is breast cancer protective in later life (Shu et al. 2001; Wu et al. 2008a, b; Korde et al. 2009; Lee et al. 2009). Of importance, the protective effects of early soy intake during childhood were stronger and more consistent than intake at any other life stage (Korde et al. 2009) and found to be equally effective for both pre- and post-menopausal breast cancers (Shu et al. 2001; Lee et al. 2009). These observations are aligned with the concept of developmental plasticity originally proposed by Prof. Barker (2007) based on epidemiological data, suggesting critical periods during very early development that are vulnerable to environmental factors, including diet. Studies on rodent models of breast cancer have provided support for the human observations (Lamartiniere 2002; Hilakivi-Clarke and De Assis 2006; Murill et al. 2007; Su et al. 2007a). Exposure of developing rat mammary glands to soy bioactive components, primarily the soy isoflavone genistein (GEN), reduced the number of terminal end buds and increased the number of differentiated lobules in young adult rat mammary glands, indicative of an early increase in mammary tissue differentiation, a well-accepted mechanism for protection against mammary tumorigenesis. Our own group has shown using chemically-induced (*N*-methyl-nitrosourea) mammary tumor formation in rats, that lifetime dietary exposure (i.e. beginning *in utero* through adulthood) to soy protein isolate (SPI) containing GEN reduced tumor incidence and increased tumor latency (Simmen et al. 2005); this was accompanied by an early increase in tumor suppressor phosphatase and tensin homologue (PTEN) deleted on chromosome ten expression (Dave et al. 2005) and a concomitant decrease in the tumor oncogene β-catenin signaling (Su et al. 2007b). PTEN, next to p53 is the most common tumor suppressor to be lost or inactivated in human cancers, including breast cancer (Li et al. 2002) and functions to antagonize the phosphatidylinositol-3-kinase (PI3K), thus, preventing the activation of the pro-survival protein kinase B/Akt downstream pathway (Stambolic et al. 1998). A consequence of PTEN loss is apoptosis-resistance and decreased differentiation, both hallmarks of cancer cells (Hanahan and Weinberg 2011). On the other hand, defective pathways in Wnt signaling lead to the stabilization of nuclear β-catenin pools, resulting in uncontrolled proliferation, and are associated with >50 % of breast carcinomas (Lin et al. 2000). Studies from our group using mammary epithelial cell lines *in vitro* confirmed the GEN effects noted in the animal studies and provided mechanistic insights for GEN-mediated induction of PTEN expression and activity (Dave et al. 2005; Rahal and Simmen 2010) and inhibition of Wnt-signaling (Su and Simmen 2009). These mechanisms are summarized in Fig. 5.2.

How may early exposure to soy foods and soy isoflavones promote mammary differentiation, leading to breast cancer protection in women at adulthood? Qin and colleagues (2009) in a study of healthy premenopausal women implicated the ability of soy isoflavones to increase the methylation of several cancer-related

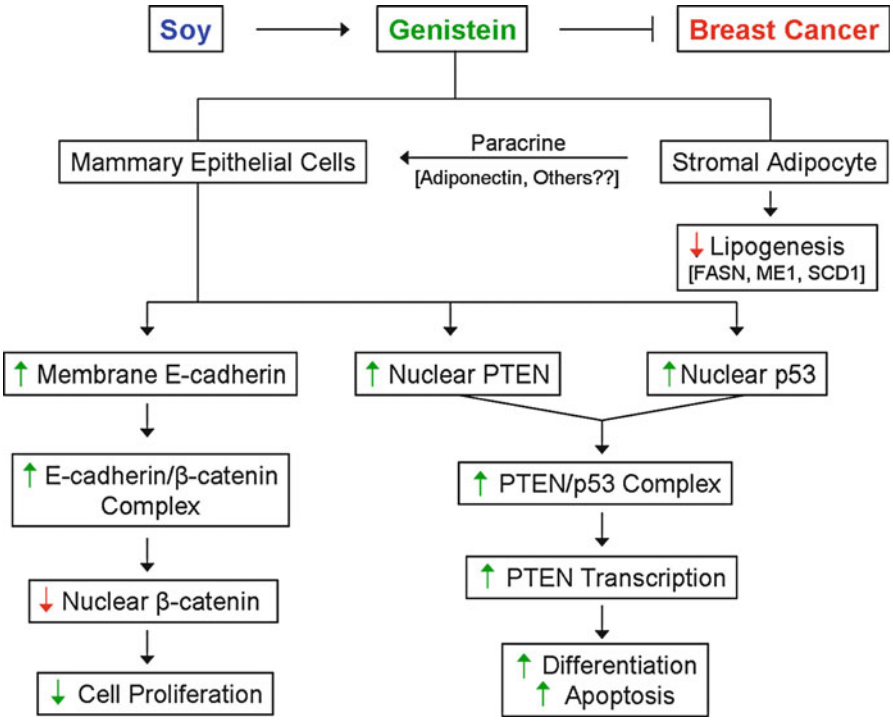

Fig. 5.2 Schematic representation of experimentally-defined mechanisms underlying the protective effects of the soy isoflavone genistein against breast cancer. Human mammary epithelial cells and rat mammary glands were used in these studies, as described in detail in Su and Simmen (2009), Su et al. (2009), and Rahal and Simmen (2010).

genes as potential mechanisms for mammary tumor protection. These include: the cyclin-dependent kinase inhibitor 2A (*p16*), tumor suppressor retinoic acid receptor B2 (*RARB2*), estrogen receptor-α (*ER-α*), and cyclin D2 (*CCND2*), a tumor oncogene by virtue of its inhibition of tumor suppressor retinoblastoma (Rb) protein function. GEN's activity to alter promoter hypermethylation in human breast tissues provides *in vivo* support for epigenetic underpinnings to its anti-breast cancer risk activity. In this regard, transcriptional networks in the mammary gland are widely acknowledged to be regulated by chromatin architecture and promoter DNA modifications (Rijnkels et al. 2010). Soy phytoestrogens GEN and daidzein have been shown to reverse DNA hypermethylation of tumor suppressors *BRCA1* and *BRCA2* in breast cancer cell lines (Bosviel et al. 2012) and GEN, similar to the natural polyphenolic compound resveratrol, increased promoter methylation of *ER-α*, coincident with this gene's increased expression (Berner et al. 2010). Similarly, methylation patterns in mice (Day et al. 2002) and cynomolgus monkeys (Howard et al. 2011) were altered by dietary GEN or soy consumption. GEN, daidzein and equol have also been shown to modify histone marks (by acetylation and demethylation) in target genes, including *BRCA1* to

modify their transcription (Dagdemir et al. 2013). GEN-mediated enhancement of mammary PTEN expression during early mammary gland development coincident with mammary tumor protection in rats (Dave et al. 2005), is likely related to GEN's role in altering promoter methylation, since increased methylation of *PTEN* gene promoter was associated with increased breast cancer invasion and metastasis (Liu and Yang 2011). GEN exposure does not appear to affect global DNA methylation (Vanhees et al. 2011), however, indicating that its selective epigenetic impact in mammary chemoprevention might involve the reversal of adverse epigenetic marks in a minority of mammary epithelial subpopulations. This small subset of cells, designated as mammary stem cells, give rise to functional mammary glands and when deregulated, can initiate mammary tumors (Stingl et al. 2006; Visvader 2009). The elucidation of the effects of bioactive compounds associated with soy foods on this epithelial subpopulation is a major focus of ongoing studies in our group (Montales et al. 2012). In this regard, work from our laboratory suggest that post-wean intake of soy protein isolate (as sole protein source) or of GEN added to control (casein) diet at concentrations approximating those found in soy foods, reduced mammary tumor incidence, relative to casein in a mouse model of human breast cancer. This was accomplished in part, by reducing the mammary stem cell-enriched population and in particular, the cancer stem cell population in hyperplastic tissues of mice overexpressing the Wnt oncogene (e.g. MMTV-Wnt1 transgenic mice) only in mammary tissues.

5.4 Soy Food Intake and Breast Cancer Survival

A lingering question related to soy food intake is whether breast cancer survivors who are receiving adjuvant endocrine therapy should include or exclude soy foods as part of their normal diets. This question stems from the lack of understanding of whether and how the weak estrogenic properties of isoflavones might interfere with conventional therapies (e.g. tamoxifen, anastrozole), leading to the promotion of breast cancer recurrence and mortality. In a 2004 article, Nair presented several case reports of cancer survivors who showed significant improvements in their conditions after dietary supplementation with a fermented soy product Haelan951 either as sole treatment or as adjuvant nutrition. While the reported cancer cases included only one breast cancer patient with infiltrating ductal carcinoma, the data provided support for the benefits associated with the dietary supplementation of fermented soy. The collective review of the more recent literatures (2003 and later) with median follow-up of 3 years or greater, has largely demonstrated the significant reductions in breast cancer risk or recurrence with high dietary intake of soy isoflavones through regular consumption of soy foods (Suzuki et al. 2008; Guha et al. 2009; Shu et al. 2009; Caan et al. 2011; Dong and Qin 2011; Kang et al. 2012; Woo et al. 2012). The specific dietary interventions and findings from a number of such studies with breast cancer patients are summarized in Table 5.2. Differences in outcomes are likely due to differing intervention designs, duration of dietary intake,

Table 5.2 Representative studies on the effects of soy and isoflavone intake on breast cancer incidence, breast cancer recurrence, and mortality in women with breast cancer

	Demographic data					
Study	Status	Total women in study	Length of follow-up	Intake	Outcome (incidence, recurrence, mortality)	Findings
Yamamoto et al. (2003)	With breast cancer	21,852	NR	Iso	Incidence	IA (S)
Nishio et al. (2007)	With breast cancer	30,454	7.6 y	Iso	Incidence	NA
Wu et al. (2008a, b)	With breast cancer	35,303	NR	Iso	Incidence	IA (S)
Guha et al. (2009)	Breast cancer survivor	1,954	6.3 y	Iso	Recurrence	IA (S)
Shu et al. (2009)	Breast cancer survivor	5,042	3.9 y	Soy, Iso	Recurrence, mortality	IA (S)
Kang et al. (2010)	Breast cancer survivor on adjuvant therapy	542	5.1 y	Iso	Recurrence, mortality	IA with recurrence (S); NA with mortality
Caan et al. (2011)	Early stage breast cancer	3,088	7.3 y	Iso	Recurrence, mortality	IA (S)
Kang et al. (2012)	With breast cancer	256	5 y	Soy	Mortality	IA (S)
Nechuta et al. (2012)	Breast cancer survivor	9,514	7.4 y	Iso	Recurrence, mortality	IA (NS)

IA inverse association, *Iso* isoflavone, *NA* no association, *NR* not reported, *NS* non-significant, *S* significant, *y* year

menopausal status, and race (White or Asian). Nevertheless, it is important to note that for these reports, none found increased deaths or breast cancer recurrence with the interventions, suggesting the relative safety of soy food intake for breast cancer patients.

An example of a study providing a definitive message on the positive effect of regular soy food consumption is the report by Shu et al. (2009). Upon adjustments for known clinical predictors and other lifestyle factors, the authors found that intake of soy foods either as soy protein or soy isoflavone was inversely associated with mortality and recurrence. Importantly, the inverse association was found irrespective of estrogen receptor status, and use or non-use of tamoxifen. In other studies however, the benefits of soy isoflavones for decreasing risk of breast cancer recurrence have not proved clear-cut and appear to be highly dependent on physiological context, likely reflecting the complex spectrum of bioactivities of soy components. Guha et al. (2009) reported that the trends for reduction of breast

cancer recurrence among postmenopausal, tamoxifen users were positively associated with increasing intake of daidzein and glycetin, while Kang et al. (2010) found effects of soy isoflavones only among postmenopausal but not premenopausal patients. The improved efficacy of tamoxifen in combination with isoflavone daidzein, relative to tamoxifen alone in reducing mammary tumor formation was previously shown in rat models to be associated with decreased oxidative damage in mammary glands (Constantinous et al. 2005). On the other hand, low-dose GEN abrogated the inhibitory effect of tamoxifen on growth of MCF-7 mammary tumors explanted in ovariectomized athymic nude mice (Du et al. 2012). The study of Suzuki et al. (2008) highlighted receptor status among patients as a modifiable parameter for efficacy of soy dietary intake on reducing breast cancer recurrence. The authors found that reduced risk of breast cancer recurrence was observed only in patients with ER+/PR+/HER2− tumors. The latter is supported by the recent report that high intake of soy isoflavones increased breast cancer recurrence in HER2+ breast cancer patients (Woo et al. 2012). A most restrictive criterion for the benefits of soy and isoflavones in breast cancer came from the study by Dong and Qin (2011). Here, the inverse association between soy isoflavone intake and breast cancer recurrence was only observed in Asian but not in Western populations, suggesting that overall lifestyle differences, of which higher soy consumption is only one parameter, contribute to relative risks.

Given that breast cancer is generally a disease of old age and affects largely postmenopausal women, the finding that menopausal status is an important factor for the therapeutic value of soy food intake implicates endocrine effects of soy foods and isoflavones. The effects of soy consumption on endogenous estrogen metabolism have been reported (Xu et al. 2000; Morimoto et al. 2011). Interestingly, soy isoflavones altered estrogen metabolism in both pre- and post-menopausal women, suggesting that the response of mammary epithelial cells to changes in estrogen levels rather than estrogen levels itself may account for the differential effects. However, serum concentrations of other hormones are also altered by soy intake; these include serum insulin-like growth factor-1 (IGF-1) which is increased in pre- (Gann et al. 2005; Maskarinec et al. 2005) and post-menopausal (Teas et al. 2011) women, and follicle-stimulating hormone and luteinizing hormone which are similarly decreased in premenopausal women, in the absence of effects on menstrual cycle length (Duncan et al. 1999). These results are counter-intuitive and difficult to reconcile as potential mechanisms underlying reduction in breast cancer recurrence, since IGF-1 is pro-proliferative in epithelial cells. In this regard, intake of soy isoflavones has not been demonstrated to modify mammographic density, a strong marker of breast cancer risk, in postmenopausal women (Verheus et al. 2008; Maskarinec et al. 2009). By contrast, a modest increase in mammographic density was noted in premenopausal women (Hooper et al. 2010). Findings suggest that the exploration of specific cellular contexts of premenopausal *vs* postmenopausal breast epithelial cells that alter their steroid and growth factor responses is critical to our understanding of potential therapeutic strategies aimed at utilizing soy intake for improving breast cancer prognosis.

5.5 Bioactive Soy Components and Predictive Biomarkers

The growing repertoire of signaling pathways mediated by soy isoflavones, if validated, could serve as relatively straightforward predictive biomarkers for identifying patient populations potentially responsive to cellular actions of bioactive soy components. Histological analyses of mammary biopsies for proliferative (e.g. Ki-67) and apoptotic (caspase-3, Bcl2) markers, and tumor suppressors (e.g. PTEN) could provide indications of soy effects prior to and after treatments. Levels of estrogens in nipple aspirate fluids, rather than in serum, could be valuable as more direct indications of the impact of dietary soy and/or GEN exposure with tamoxifen therapy, on breast tissue due to changes in estrogen metabolism (Morimoto et al. 2011). In a randomized phase 2 trial involving 126 healthy, high risk adult Western women, fine needle aspiration was used for collection of mammary epithelial cells to evaluate the effects of mixed soy isoflavones or placebo prior to and after dietary supplementation for 6 months. Ki-67 labeling of the cells as well as the expression of genes related to proliferation, apoptosis and estrogenic effects were quantified. Despite the lack of demonstrable significant differences between control and treated groups for these measured parameters, suggesting lack of efficacy of soy isoflavones within the limited exposure time, the methodology highlights the value of these biomarkers to measure response rate in future study populations (Khan et al. 2012).

Given the increasing interest towards personalized therapy for breast and other types of cancer, other strategies are being developed to identify and validate biomarkers for chemotherapy and pathological complete response. Biomarkers identified and currently being assessed, in addition to the classical ER and Ki-67 expression, are the anti-apoptotic protein survivin and pro-proliferative phosphorylated ERK (by immunohistochemistry) in breast tissues (Sanchez-Rovira et al. 2012); apoptotic-related biomarkers (e.g. soluble cell death receptor sFAS, plasminogen activator inhibitor-1) in serum of breast cancer patients undergoing neoadjuvant therapy (Fersching et al. 2012); expression of epidermal growth factor receptor and topoisomerase II alpha (TOP2A) in circulating tumor cells (CTC) isolated using anti-CTC surface antigens (Nadal et al. 2012), and methylation signatures using a functional hypermethylome screen for breast tissue (Jeschke et al. 2012). In the latter study, methylation of tachykinin 1 precursor 1 (*TAC1*) and creatine kinase muscle (*CKM*) singly proved to be highly correlated with poor overall survival in breast cancer patients, and in combination, was strongly associated with poor overall survival independent of age. While studies to investigate soy effects on breast cancer patients using these putative prognostic markers maybe premature, the universal application of these promising technologies in future soy studies might streamline the variables in experimental design and outcome measurements that confound data interpretation in clinical studies carried out under different settings.

5.6 Challenges for Potential Therapeutic Exploitation of Soy Bioactive Components

While there is a paucity of information to directly link soy bioactive components and therapeutic outcome in breast cancer patients, there are sufficient information, as highlighted in recent meta-analysis of prospective and epidemiological studies cited in this chapter (Qin et al. 2006; Trock et al. 2006; Dong and Qin 2011) to support this possibility. However, there are several challenges associated with developing soy components for breast cancer therapy. The first challenge is to identify the specific targets of soy components; this has two aspects, namely the gene targets and the cellular targets. Genes whose expression levels are up- or downregulated with soy food intake are readily identifiable, given the availability, easy access to, and affordability of gene and proteome profiling tools. These analyses allow for the discovery of novel as well as the confirmation of previously identified, pathways that can serve as consistent biomarkers for favorable or poor tumor outcome. In studies from our group using Affymetrix GeneChip microarrays (Su et al. 2007b), we showed that expression of only a very low percentage of mammary epithelial cell transcripts (0.5 % of the total 14,000 genes evaluated) were altered with lifetime dietary exposure of rats to SPI or GEN. These sets of studies could be performed on tumors of breast cancer patients before and after specific drug interventions in the presence of soy (GEN) exposure to allow the identification of breast cancer signatures associated with soy therapeutic activity, in much the same way that an obesity signature for mammary tumors of 103 breast cancer patients was developed (Creighton et al. 2012). Such analyses could be followed-up with proteome profiling, using the same sets of tissues to confirm gain-or-loss-of-functional proteins associated with gene transcriptional changes. While complicated, the identification of which cell compartments are targeted by soy is imperative, given the increasing appreciation that the survival and recurrence rates in breast cancer are dependent on the stromal microenvironment (Polyak and Kalluri 2010; Conklin and Keely 2012). In this regard, our group has shown that adipocytes in the mammary stroma are targets of GEN. We demonstrated mammary adipocyte-specific genomic changes elicited by dietary exposure of rats to SPI *in vivo* that were recapitulated by GEN in the 3T3L1 adipocyte cell line *in vitro* (Su et al. 2009, 2011). Moreover, we showed the cooperative interactions between stromal-derived adipokine adiponectin and GEN to promote differentiation and enhance transcriptional response to estrogen receptor β signaling in mammary epithelial cells (Rahal and Simmen 2011). Consistent with these studies, numerous reports have correlated breast cancer survival with specific aspects of stromal biology (Conklin and Keely 2012).

The second challenge is to identify useful and consistent biomarkers to evaluate therapeutic efficacy. While patient complete response is the most obvious way to demonstrate efficacy, a systematic evaluation of biomarkers during the time course of treatment is useful for the monitoring of partial responses and can be of clinical benefit for prescribing drugs with negative side-effects at high doses. This would

require an understanding of the context of the biological response since expression of biomarkers likely differed with dose and duration of treatment; maybe defined by age, menopausal status and body mass index; and can be unexpectedly influenced by other components present in diets.

The third challenge is to determine which components of soy confer the best therapeutic potential. While isoflavones (predominantly GEN) are the best described and most studied among soy bioactive components, conflicting results obtained from preclinical, case-controlled, and limited phase 2 studies have lessened enthusiasm and support for further studies with isoflavones, using larger patient numbers. Findings that exposure to isoflavone-free soy diets was mammary tumor protective but those containing isoflavones were tumor-promoting in some studies (Martinez-Montemayor et al. 2010; Du et al. 2012), that soybeans contain proteins that are anti-cancer (Galvez et al. 2001; Jeong et al. 2007; Wang et al. 2008; Boué et al. 2009) and in particular mammary tumor-protective (Hsieh et al. 2010a, b), and that soy isoflavones act as weak antiestrogens, raising the potential for adverse effects on the reproductive system (Petrakis et al. 1996), make a compelling case for the testing of soy-associated components, other than isoflavones, for chemotherapeutic modalities.

Partial hydrolysis of the major protein component of soybeans yielded peptides with inhibitory effects on cancer cell lines *in vitro* (Wang et al. 2008; Mochizuki et al. 2009). A β-conglycinin derived peptide from the hydrolysate was found to inhibit growth of leukemia cells, alone and together with GEN (Wang et al. 2008). Saponin, another component of soy was also demonstrated to inhibit growth of human colon cancer cells (Tsai et al. 2010) and reduce colon tumor metastasis in mice, the latter by suppressing the expression of the matrix metalloproteinases (MMP)-2 and MMP-9 and stimulating the expression of tissue inhibitor of metalloproteinase-2 (TIMP-2) (Kang et al. 2008). In this study, mice fed the soybean component saponin prior to vein injection of colon cancer cells had reduced lung metastasis. While similar experiments using these molecules have not been conducted in breast cancer cells *in vitro* and in animal models of breast cancer *in vivo*, such studies demonstrate the potential of factors in soy foods that can selectively arrest tumor growth and metastasis.

Of recent interest is the soybean peptide lunasin, a 43-aa peptide component of post-translationally processed 2S albumin (Galvez and de Lumen 1999), which is also present in barley, wheat and other seeds (Jeong et al. 2010). Lunasin's anti-carcinogenic properties have been demonstrated in rodent models of skin (Galvez et al. 2001) and breast (Hsieh et al. 2010a) cancers and in colon and breast cancer cell lines (Dia and Mejia 2010; Hsieh et al. 2010b). In recent studies using non-malignant (mouse HC11) and malignant (human MCF-7) mammary epithelial cells, we showed that lunasin displayed common and distinct actions from those of GEN. In particular, lunasin induction of cellular apoptosis was mediated by PTEN, akin to GEN, albeit this occurred independent of p53, unlike that for GEN. Moreover, lunasin did not mimic GEN's inhibitory effects on the expansion of the limited cancer stem cell-like/progenitor cell population in MCF-7 cells (Pabona et al. 2013). The analyses of genomic signatures associated with lunasin signaling by

whole genome-array profiling and evaluation of whether serum levels of this peptide is associated with good prognosis in patients consuming soy foods will be required to begin to understand its clinical benefits.

5.7 Implications and Future Directions

Cancer remains a major global killer. Despite the seemingly positive report (http://www.cancer.org/Research/CancerFactsFigures/ACSPC-031941) that the annual rate of new cancer cases and the overall cancer death rate in the United States had dropped for the 10-year period between 1999 and 2008, the nation's health outlook remains problematic. The growing awareness of the association between obesity and cancer (Simmen and Simmen 2011), and the skyrocketing of the overweight and obese population, currently estimated at ~36 %, predict that this reduction in cancer cases will not be sustained, and that Americans (and by extension, globally) will be faced with higher cancer risks at adulthood. Thus, the impetus for dietary interventions for decreasing breast cancer susceptibility, beginning at early life, and improving outcome of breast cancer patients should be considered a priority rather than simply an option. While the use of soy foods is still a relatively under-appreciated treatment strategy, given the uncertainties regarding their role in mammary tumorigenesis, efforts by academicians in their respective laboratories and health care professionals in clinical settings should be enhanced to bring these new strategies to fruition. For academicians, the identification of the contextually-regulated environment wherein soy components can exert their most beneficial effects is crucial to maximizing their therapeutic potential. This is true not only for breast cancer but also for other cancer types like colorectal (Xiao et al. 2007, 2008; Yang et al. 2009; Yan et al. 2010) and prostate (Colli and Amling 2009) where the benefits of soy food intake have been reported but remain controversial (Adams et al. 2005). For clinicians, the task is to carefully screen for patients based on their contextual qualities with predicted favorable outcomes and determine at what point in therapy soy foods should be incorporated, as initial steps to determine its practical option and eventually as part of standard-of-care regimen.

The notion that soy food intake is a meaningful adjuvant strategy for conventional breast cancer therapies originally emerged from epidemiological reports that were subsequently evaluated by studies using animal models, leading to limited early phase clinical trials. Although soy isoflavones are considered the major targeting agents, they have not been conclusively associated with improved clinical outcomes. Given the complex system of the mammary environment, the recent discoveries pointing to the involvement of tumor-propagating cancer stem cells in programming breast cancer (Wicha et al. 2006; Damonte et al. 2008) might allow the streamlining of dietary effects directly to fetal and adult mammary stem cells to alter their behavior and inhibit neoplastic transformations, in the absence of confounding endocrine effects (Ablett et al. 2012). Based on the above, we propose a model wherein mammary stem/progenitor cells and when deregulated, cancer

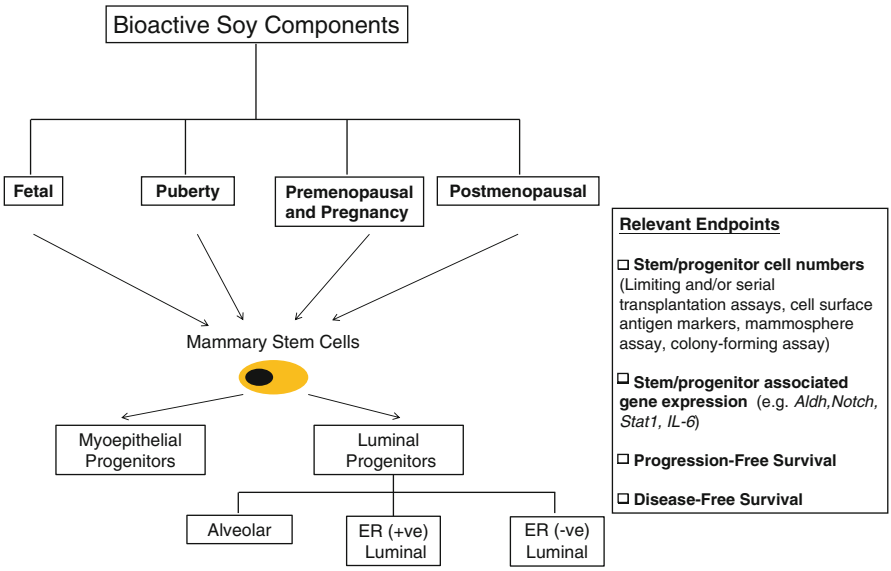

Fig. 5.3 A proposed model on mammary stem/progenitor cells as biological targets of soy food-associated bioactive components at various life stages. The actions of bioactive components on mammary stem and progenitor cells can be validated using relevant biological, molecular and survival endpoints in genetically engineered mouse models to inform future clinical trial designs and eventually, standard-of-care treatments. *Aldh* aldehyde dehydrogenase, *ER*(+) estrogen receptor-positive, *ER*(−) estrogen receptor-negative, *IL6* interleukin 6, *Stat1* signal transducers and activators of transcription-1

stem cells within the spectrum of a women's life (fetal stage, puberty, pregnancy, postmenopausal) may constitute targets of soy effects on immune/inflammatory, proliferation, and self-renewal processes (Fig. 5.3). The relevance of soy bioactive components in targeting mammary stem cells at different life stages could be initially tested using genetically engineered mouse models of breast cancer (Vaillant et al. 2008), which can recapitulate the distinct histopathological and molecular subtypes that characterize the human disease (Sorlie et al. 2001), to inform future clinical trial designs. While the paucity of tools to effectively target these cells remains a major challenge, this approach if successful could pave the way for novel therapeutic opportunities to eradicate cancer of the breast and other cancers.

In conclusion, it is apparent from multiple investigations cited here, that soy foods and soy isoflavone intake have the potential for becoming part of the standard-of-care treatments for breast cancer patients and survivors. A better understanding of their diverse effects under more defined and well-controlled clinical settings is warranted to yield definitive indications of the value of this strategy in the successful management of cancer.

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Chapter 6
Association Between High Intake of Lycopene-rich Foods and Reduced Risk of Cancer

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Abstract The consumption of lycopene and lycopene-rich foods, such as tomato, papaya, watermelon and grapefruit, has been associated with decreased risk of several cancers, including prostatic, lung and gastrointestinal cancers. *In vitro* studies have demonstrated that lycopene may inhibit the growth of several types of cancer cells and provided valuable insights into the mechanisms by which lycopene exert their cellular and intracellular effects. Mechanisms implicated in the prevention of cancer incidence and progression by lycopene-rich foods include: modulation of redox activity, enzyme detoxyfication, inhibition of cell proliferation and apoptosis induction, regulation of growth factor and hormone signaling, inhibition of cell adhesion and angiogenesis, inhibition of cholesterol synthesis, immunomodulation and enhancement of gap junction communication. A number of animal studies indicate a protective effect of pure lycopene or lycopene-rich foods on prostatic, gastro-intestinal and lung tumorigenesis. Although numerous epidemiological studies demonstrate that lycopene and lycopene-rich foods may reduce cancer risk, intervention trials establishing a direct link between lycopene and/or lycopene-rich foods and cancer prevention are still few and controversial. This chapter examines the experimental and clinical evidences for the preventive role of lycopene and lycopene-rich foods on cancer as well as the implicated mechanisms of action. In addition, it speculates on the interactions existing between lycopene and other bioactive food components in cancer prevention.

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6.1 Introduction

Increasing evidences suggest that lycopene and lycopene-rich food consumption can be associated with decreased risk of several cancers, including prostatic (Giovannucci 1999, 2002; Giovannucci et al. 1995, 1999, 2007), lung (Palozza et al. 2011a) and gastrointestinal cancers (Liu and Russell 2008). Moreover, recent data evidence that lycopene supplementation may inhibit the progression of benign prostate hyperplasia, a risk factor for prostate cancer (Schwarz et al. 2008). Interest in lycopene and lycopene-rich foods as possible modulators of cancer risk, is also based on the fact that lycopene is present in plasma and breast milk of Western population at levels as high or higher than other carotenoids or other bioactive food components, normally contained in fruits and vegetables (Steinmetz and Potter 1991).

Lycopene, one of more than 600 carotenoids synthesized by plants and microorganisms, is a tetraterpene hydrocarbon containing 40 carbon atoms and 56 hydrogen atoms. It is a highly unsaturated molecule containing 11 conjugated and 2 unconjugated double bonds. Lycopene from natural plant sources exists predominantly in an all-*trans* configuration. In human plasma, lycopene is present as an isomeric mixture, with 50 % as *cis* isomers. Some lycopene metabolites, including 5,6-dihydroxy-5,6-dihydro-lycopene, and apo-lycopenals have been also detected in human plasma (Khachik et al. 1997). Apo-6′and apo-8′-lycopenals were also reported to be present in raw tomatoes (Kopec et al. 2010). Lycopene absorption from dietary sources is influenced by several factors including the break up of the food matrix containing lycopene, cooking temperatures and the presence of lipids and other lipid soluble compounds, including other carotenoids. Absorption of lycopene is similar to that of other lipid soluble compounds. Low density lipoprotein is the primary carrier of lycopene. In general, 10–30 % of the dietary lycopene is absorbed by humans. It is absorbed equally efficiently from different sources of lycopene including tomato sauce, tomato juice and tomato oleoresin capsules (Fang et al. 2003). Lycopene is also found to concentrate *in vivo* in the adrenal gland, testes, liver and prostate gland, where it is the most prominent carotenoid (Zaripheh and Erdman 2005).

Tomato, papaya, watermelon and pink grapefruit are highly rich in lycopene (Table 6.1). Although gac has the highest content of lycopene of any known fruit or vegetable, up to 70 times more than tomatoes for example, due to gac's rarity outside its native region of Southeast Asia, tomatoes and tomato-based sauces, juices, and ketchup account for more than 85 % of the dietary intake of lycopene for most people. The lycopene content of tomatoes depends on species and increases as the fruit ripens. Lycopene in tomato paste is four times more bioavailable than in fresh tomatoes. Processed tomato products such as pasteurized tomato juice, soup, sauce and ketchup contain the highest concentrations of bioavailable lycopene from tomato-based sources.

This chapter summarizes the most current knowledge with respect to lycopene and lycopene-rich foods in the prevention of cancer risk. In particular, it examines

Table 6.1 Lycopene-rich foods

Source	Lycopene (μg/g wet weight)
Gac	2,000–2,300
Raw tomato	8.8–42
Tomato juice	86–100
Tomato sauce	63–131
Tomato ketchup	124
Watermelon	23–72
Pink grapefruit	3.6–34
Papaya	20–53

the experimental and clinical evidences for a preventive role of lycopene and lycopene-rich foods on cancer as well as the mechanisms of action implicated. In addition, it speculates on the interactions existing between lycopene and other bioactive food components in cancer prevention.

6.2 Evidences for Anti-tumoral Effects of Lycopene and Lycopene-rich Foods

6.2.1 In Vitro Studies

It has been recently reported that lycopene is able to inhibit the growth of several types of cancer cells in culture conditions. Inhibitory effects of lycopene in mammary, endometrial, lung, colon leukaemia and liver cancer cell growth have been reported (Levy et al. 1995; Karas et al. 2000; Chalabi et al. 2007; Tang et al. 2008). Physiologically concentrations of lycopene have been also shown to induce mitochondrial apoptosis in LNCaP cells (Hantz et al. 2005). In addition, tomato extracts have been reported to reduce prostate cancer cell survival in a dose-dependent manner (Hwang and Bowen 2005). Recently, Korean tomato varieties and processed tomato products have been shown to influence differently the growth of normal and tumor cells (Choi et al. 2011). When the antiproliferative effects of several aqueous extracts of plant foods consumed in Mexico was compared in breast cancer cells, only the papaya extract had a significant growth-inhibitory effect. Interestingly, such an effect was not related to the phenolic content (Garcia-Solis et al. 2009).

6.2.2 Animal Studies

Pure lycopene or tomato extracts were both able to inhibit non-alcoholic steatohepatitis-promoted hepatocarcinogenesis in a rat model (Wang et al. 2010). In prostate cancer models (Siler et al. 2004; Konijeti et al. 2010), lycopene

supplementation caused a significant reduction of tumor tissue. Tomato lycopene supplementation has been also shown to prevent the change in p53 target gene induced by cigarette smoke exposure in gastric mucosa of ferrets (Liu et al. 2006), suggesting a protective effect of lycopene against the development of gastric cancer. On the other hand, a recent finding evidences that tomato puree revealed a much stronger, antimutagenic effect compared with the corresponding doses of pure lycopene in mice treated with aflatoxin B or *N*-nitroso-*N*-methylurea (Polívková et al. 2010). Moreover, Boileau et al. (2003) reported a significant inhibition of *N*-methy-*N*-nitrosourea-testosterone-induced carcinogenesis in rats following consumption of tomato powder, but not following consumption of pure lycopene.

A reduction of azoxymethane-induced aberrant crypt foci by lycopene-rich foods, including watermelon juice, has been also reported in rats (Boateng et al. 2007). Such an effect was also accompanied by an increase of total glutathione-*S*-transferases activity in rat liver.

6.2.3 Human Studies

The Mediterranean diet, which is rich in vegetables and fruits, including tomatoes, has been suggested to be responsible for the lower cancer rates in that region. Dietary intake of tomatoes and tomato products (Giovannucci 1999) as well as of papaya, watermelon and grapefruit, has been found to be associated with a lower risk of a variety of cancers in several epidemiological studies, as shown in Table 6.2. A high intake of tomatoes was linked to protective effects against digestive tract cancers in a case-control study (Franceschi et al. 1994) and a 50 % reduction in rates of death from cancers at all sites in an elderly US population (Colditz et al. 1985). The most impressive results come from the US Health Professionals Follow-up Study, which evaluated the intake of various carotenoids and retinol, from a food-frequency questionnaire, in relation to risk of prostate cancer (Giovannucci et al. 1995). The estimated intake of lycopene from various tomato products was inversely related to the risk of prostate cancer. This result was not observed with any other carotenoid. A reduction in risk of almost 35 % was observed for a consumption frequency of 10 or more servings of tomato products per week, and the protective effects were even stronger with more advanced or aggressive prostate cancer. In recent studies serum and tissue levels of lycopene were shown to be inversely associated with the risk of breast cancer (Dorgan et al. 1998) and prostate cancer (Rao and Agarwal 1999) while no significant association with other important carotenoids, including β-carotene, was observed (Rao and Agarwal 1999). Giovannucci (1999) reviewed 72 epidemiological studies, including ecological, case–control, dietary and blood-specimen-based investigations of tomatoes, tomato-based products, lycopene and cancer. In 57 studies there was an inverse association between tomato intake or circulating lycopene levels and risk of several types of cancer; in 35 cases the association was statistically significant. None of the studies showed adverse effects of high tomato intake or

Table 6.2 Epidemiological studies on the effects of tomato, papaya, watermelon and grapefruit and their derivatives on cancer risk and progression in human subjects

Diet	Population	Cancer	End point	Effect[a,b]	References
Tomato sauce	USA. 1,560 subjects (non-metastatic cancer)	Prostate cancer	Cancer progression	↓ progression[b]	Richmann et al. (2012)
Tomato and derivatives	Iran. 194 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Salem et al. (2011)
Tomato	335 subjects	Kidney cancer	Cancer risk	↓ risk[a]	Grieb et al. (2009)
Tomato and derivatives	Canada. 752 subjects	Prostate cancer	Cancer risk	↑ risk[a]	Darlington et al. (2007)
Tomato	Korean. 359 subjects	Breast cancer	Cancer risk	↓ risk in pre-menopause[a]	Do et al. (2007)
Tomato sauce	USA. (Health Professionals Follow-up Study)	Prostate cancer	Cancer risk	↓ risk[a]	Giovannucci et al. (2007)
Tomato and derivatives	Hawaii. 183,522 subjects (529 cancer)	Pancreas cancer	Cancer risk	↓ risk[b]	Nöthlings et al. (2007)
Tomato sauce	Italy. 8,861 subjects (238 cancer)	Breast cancer	Cancer risk	↓ risk[b]	Sant et al. (2007)
Tomato sauce	USA. (HPFS: 1,202 subjects with local cancer. 392 cancer in progression)	Prostate cancer	Cancer progression	↓ progression (tomato sauce)[a] ↑ progression (tomato)[b]	Chan et al. (2006)
Pizza	North USA. 2,569 breast cancer, 1,031 ovary cancer, 1,294 prostate cancer	Breast, ovary and prostate cancers	Cancer risk	↓ risk[a]	Gallus et al. (2006)
Tomato (≥5 times/week	California. 13,210 subjects	Ovary cancer	Cancer risk	↓ risk[a]	Kiani et al. (2006)
Tomato derivatives	USA. 29,361 subjects	Prostate cancer	Cancer risk	↓ risk (only in familiar cancers)[a]	Kirsh et al. (2006)
Tomato and derivatives	Canada. 462 subjects	Pancreas cancer	Cancer risk	↓ risk[b]	Nkondjock et al. (2005)
Tomato and derivatives	USA. 39,876 subjects	Breast cancer	Cancer risk	↓ risk[a]	Sesso et al. (2005)

(continued)

Table 6.2 (continued)

Diet	Population	Cancer	End point	Effect[a,b]	References
Tomato foods	858 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Hodge et al. (2004)
Tomato	Japan. 47,997 males and 66,520 females	Ureter cancer	Cancer risk	↓ risk[b]	Sakauchi et al. (2004)
Tomato sauce	Italy. 8,489 females	Breast cancer	Cancer risk	↓ risk[b]	Sieri et al. (2004)
Tomato	Japan. 140 subjects	Prostate cancer	Cancer risk	↓ risk[b]	Sonoda et al. (2004)
Tomato and derivatives	USA. (HPSF): 47,365 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Giovannucci et al. (2002)
Tomato and derivatives	27,084 male smokers. 1,644 cancer subjects	Lung cancer	Cancer risk	↓ risk[a]	Holick et al. (2002)
Tomato	Italy. 754 oral cancer subjects, 304 oesophagus cancer subjects, 1,953 colon-rectal cancer subjects, 2,529 breast cancer subjects, 1,031 ovary cancer subjects	Oral, oesophagus, colon-rectal, breast and ovary cancers	Cancer risk	↓ colorectal cancer risk[a]	La Vecchia (2002)
Tomato sauce	England. 982 subjects	Lung cancer	Cancer risk	↓ risk[a]	Darby et al. (2001)
Cooked tomato	Greece. 112 healthy subjects	Prostate cancer	IGF-I, IGFBG-3	↓ risk[a]	Mucci et al. (2001)
Fresh and cooked tomato	Greece	Prostate cancer	Cancer risk	↓ risk[a]	Bosetti et al. (2000)
Tomato	506 non-smokers subjects	Lung cancer	Cancer risk	↓ risk[a]	Brennan et al. (2000)
Tomato and derivatives	Uruguay. 238 subjects	Oral and respiratory cancers	Cancer risk	↓ risk[a]	De Stefani et al. (2000)
Tomato	Hawaii, USA, Colombia, Canada. 1,619 subjects	Prostate cancer	Cancer risk	↓ risk[b]	Kolonel et al. (2000)

Tomato food	New Zealand. 317 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Norrish et al. (2000)
Tomato	Study on 41 countries	Prostate cancer	Cancer risk	↓ risk[a]	Grant (1999)
Tomato	Greece. 320 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Tzonou et al. (1999)
Tomato	Canada. 1,623 subjects	Prostate cancer	Cancer risk	↓ risk[b]	Villeneuve et al. (1999)
Tomato	Spain. 103 subjects	Lung cancer	Cancer risk	↓ risk[a]	Agudo et al. (1997)
Tomato and derivatives	USA. 47,894 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Giovannucci et al. (1995)
Fresh tomato	Italy. 314 subjects with oral and pharynx cancers, 85 subjects with oesophagus cancer, 723 subjects with stomach cancer, 955 subjects with colon cancer, 629 subjects with rectal cancer	Oral, pharynx oesophagus, stomach, colon-rectal cancers	Cancer risk	↓ risk of all cancers[a]	Franceschi et al. (1994)
Tomato	China. 183 miners (95 % smokers)	Lung cancer	Cancer risk	↓ risk[a]	Forman et al. (1992)
Tomato	Hawaii: 675 subjects	Lung cancer	Cancer progression	↓ progression[a]	Goodman et al. (1992)
Tomato	Netherlands. 164 subjects	Pancreas cancer	Cancer risk	↓ risk[a]	Bueno de Mesquita et al. (1991)
Fresh tomato	USA. 293 subjects	Gastric cancer	Cancer risk	↓ risk[a]	Graham et al. (1990)
Tomato	Hawaii. 332 subjects	Lung cancer	Cancer risk	↓ risk[a]	Le Marchand et al. (1989)
Tomato	California. 14,000 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Mills et al. (1989)
Grapefruit	Oxford. EPIC Study: 114,504 female with risk factors	Breast cancer	Cancer risk	↓ risk[b]	Spencer et al. (2009)
	California. 232 subjects	Childhood leucemia	Cancer risk	↓ risk[b]	

(continued)

Table 6.2 (continued)

Diet	Population	Cancer	End point	Effect[a,b]	References
Grapefruit and grapefruit juice					Peters et al. (1994)
Grapefruit and tomato juices	384 subjects	Pancreas cancer	Cancer risk	↓ risk[a]	Jansen et al. (2011)
Watermelon and tomato	China. 130 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Jian et al. (2005)
Watermelon	Korea. 539 adenoma subjects and 162 colon-rectal cancer subjects	Colon-rectal adenoma and cancer	Cancer risk	↓ risk in males[a]	Lee et al. (2005)
Papaia and tomato sauce	Malaysia. 35 subjects	Prostate cancer	Cancer risk	↓ risk[a]	Shahar et al. (2011)
Papaya (one or †more/ week)	Arizona. HPV positive (185 subjects with persistent infection, 248 subjects with temporary infection)	Uterine cervix cancer	Persistent HPV infection risk	↓ risk[a]	Giuliano et al. (2003)
Papaya	India. 64 subjects	Bile duct cancer	Cancer risk	↓ risk[a]	Pandey and Shukla (2002)
Papaya and tomato	Hawaii. 452 subjects	Prostate cancer	Cancer risk	↑ risk in elderly subjects (papaya)[a] (tomato)[b]	Le Marchand et al. (1991)

EPIC European Prospective Investigation into Cancer and Nutrition, *HPFS* Health Professionals Follow-Up Study

[a]Indicating the effect is statistically significant

[b]Indicating the effect is not statistically significant

high lycopene levels. As part of the Health Professionals Follow-up Study, the relationship between tomato consumption, including the measurement of lycopene plasma levels, and prostate cancer risk was investigated in more than 40,000 men (Giovannucci et al. 1995). High estimated lycopene intake was inversely related to risk of prostate cancer while the estimated intakes of total carotenoids, β-carotene, α-carotene, lutein and β-cryptoxanthin were not associated with a risk of prostate cancer. The inverse correlation was stronger for more advanced or aggressive prostate cancer (relative risk 0.47) (Giovannucci and Clinton 1998). In a prospective study of the same population and analysis of all data from 1986 through 1998, lycopene intake was again determined to reduce the risk of prostate cancer (Giovannucci et al. 2002). In two more recent but smaller prospective epidemiological studies reported in 2006 (Kirsh et al. 2006) and 2007 (Peters et al. 2007), no association was detected between lycopene intake and prostate cancer risk. The epidemiological studies on the preventive effects of papaya, watermelon and grapefruit intake on cancer risk are little. However, they show beneficial effects in the prevention of prostate, breast, colon and gastric cancer risk. Although the epidemiological evidence of the role of such fruits and vegetables in cancer prevention is persuasive, this role still remains to be proven.

Table 6.3 summarizes some clinical trials showing beneficial effects of tomato, papaya, watermelon, grapefruit and their derivatives on cancer. As evidenced from the table, most of the studies clearly show an inverse association between administration of lycopene-rich fruits and vegetables and cancer progression or elevated levels of cancer biomarkers. Compared to the epidemiological studies of tomato consumption and cancer risk, there have been few clinical trials of lycopene intervention and cancer risk that are randomized, placebo controlled and double blind. However, there have been several studies that lack one or more of these design features such as placebo control, blinding, randomization or adequate sample size to be significant. Based on the epidemiological evidence, it is not surprising that most of the intervention studies to date have concerned prostate cancer. Usually, these intervention trials have been of relatively short duration (weeks instead of years), and as a result, the outcomes that have been measured have usually been intermediate endpoints or markers of risk such as oxidative stress instead of cancer incidence. The most significant of these studies will be discussed. Chen et al. (2001) examined the effects of 30 mg lycopene per day for 3 weeks in the form of tomato sauce on 32 men with localized prostate adenocarcinoma preceding scheduled radical prostatectomy. Although this study was not blinded and not placebo controlled, serum lycopene levels increased approximately two-fold and prostate levels of lycopene increased almost three-fold compared to baseline. In addition, total serum levels of prostate-specific antigen (PSA) levels, which was used as a surrogate endpoint for prostate cancer progression, decreased by about 20 % and DNA oxidation in leukocytes (measured as 8-oxo-dG released from DNA) decreased by about 21 % compared to baseline. Kucuk et al. (2001) reported on a randomized, placebo controlled but unblinded study in which 26 men recently diagnosed with prostate cancer were administered 30 mg of lycopene or placebo daily for 21 days before radical

Table 6.3 Clinical trials on the anticancer effects of supplementations with tomato, papaya, watermelon and grapefruit and their derivatives

Supplementation	Population	Cancer	End point	Effect[a,b]	References
Tomato derivatives for 10 weeks	High-risk females	Breast cancer	IGF-I, IGFBP-3, estrogen, SHBG, insulin, C-peptide	No difference[b]	McLaughlin et al. (2011)
Red tomato paste (16 mg/day lycopene), yellow tomato paste (0 mg), purified lycopene (16 mg/day), for a week	30 healthy subjects, 50–70 years	Prostate cancer	Antioxidant status, PSA, IGF-I, IGFBP-3 and other markers	↓ risk for both tomato and purified lycopene[a]	Talvas et al. (2010)
Tomato derivatives (25 mg/day lycopene) for 4 weeks	41 healthy subjects	Prostate cancer	PSA	↓ risk[a]	Grainger et al. (2008)
Tomato juice (330 mL/day) for 2 weeks	22 young healthy subjects	Colon cancer	Faecal markers	No difference[b]	Schnabele et al. (2008)
Lycopene-rich tomato (30 mg/day lycopene) for 16 months	46 prostate cancer subjects	Prostate cancer	Cancer progression	No difference[b]	Jatoi et al. (2007)
Tomato paste (50 g/day) for 10 weeks	43 subjects, 45–75 years, benign prostate hyperplasia and PSA 4-10 ng/mL	Prostate cancer	PSA	↓ risk[a]	Edinger and Koff (2006)
Tomato drinks (5.7 mg lycopene) for 26 days	26 young healthy subjects		Oxidative stress, immunity and inflammatory markers	↓ TNF-alpha[a] ↓other parameters[b]	Riso et al. (2006)
Watermelon juice (150 mL)	10 young healthy subjects		Plasma antioxidant status	↓ ROS production[a]	Ko et al. (2005)
Tomato sauce for 3 weeks	Prostate cancer subjects	Prostate cancer	DNA oxidation of leukocytes and prostatic tissue, PSA, cell proliferation	↓ risk[a]	Stacewicz-Sapuntzakis and Bowen (2005)

Fermented Papaya (6 g/day) for 6 months	70 subjects at high risk for gastric cancer	Gastric cancer	Assay of XO, MAD, ODG, 8-OhdG gastric biopsy	↓ parameters analysed[a]	Marotta et al. (2004)
Food with tomato sauce (30 mg/day lycopene) for 3 weeks	66 cancer subjects	Prostate cancer	Apoptosis on prostatic tissue	↓ cancer progression[a]	Kim et al. (2003)
Food with tomato sauce (30 mg/day lycopene) for 3 weeks	32 local cancer subjects	Prostate cancer	PSA, apoptosis, 8OHdG DNA on leukocytes and prostatic tissue	↓ parameters analysed[a]	Bowen et al. (2002)
Food with tomato sauce (30 mg/day lycopene) for 3 weeks	32 local cancer subjects	Prostate cancer	DNA oxidation of leukocytes and prostatic tissue, PSA	↓ cancer progression[a]	Chen et al. (2001)
Tomato puree (25 g/day: 7 mg/day lycopene) for 2 weeks	9 women		DNA oxidation of leukocytes	↓ oxidation[a]	Porrini et al. (2000)

[a]Indicating the effect is statistically significant
[b]Indicating the effect is not statistically significant

prostatectomy. In this study, lycopene levels were not measured and no conclusions could be drawn due to the small sample size of this study. Clark et al. (2006) on a dose escalating trial of lycopene from 15 to 120 mg/day in 36 men with biochemical relapse of prostate cancer. There was no randomization or placebo group, and this study was unblinded. Lycopene levels in serum were similar after 3-month dosages of 15, 30, 45, 60, or 90 mg/day, and no change in serum PSA was detected as a result of intervention. In an unblinded, randomized intervention study of 81 men with high-grade prostatic intraepithelial neoplasia neoplasia, Bunker et al. (2007) administered 30 mg/day lycopene (in the form of a tomato oleoresin) plus a multivitamin or just a multivitamin for 4 months. After 1 month of intervention, total serum PSA declined, but PSA levels were identical in both groups after 4 months. In an unblinded intervention study without a control group reported, Jatoi et al. (2007) evaluated 46 men with androgenin-dependent prostate cancer who received 30 mg lycopene/day for 16 months. Lycopene was not effective in preventing the progression of prostate cancer in this group. Although the outcomes of these intervention trials were negative, lycopene was well tolerated and no significant side effects were observed.

An increased number of clinical trials are needed to evaluate the efficacy of lycopene as a chemoprevention agent. The trials should be placebo controlled, randomized and double-blind. Moreover, they should take into consideration the population, the modality and dose of lycopene and lycopene-rich food administration as well as the different types of cancer and the end points of the studies.

6.3 Mechanisms of Action

The biological activities of carotenoids such as β-carotene are related in general to their ability to form vitamin A within the body. Since lycopene lacks the β-ionone ring structure, it cannot form vitamin A. Its biological effects in humans have therefore been attributed to mechanisms other than vitamin A. The proposed mechanisms for the role of lycopene in cancer prevention are briefly discussed above and summarized in Fig. 6.1.

6.3.1 Antioxidant Activity

Increasing evidence suggests that lycopene may inhibit cancer cell growth by directly protecting critical biomolecules, including lipids, proteins and DNA from reactive oxygen species attack, as recently reviewed by Palozza et al. (2011b). The carotenoid has been reported to inhibit oxidative DNA damage in the Hep3B human hepatoma cell line. Interestingly, in the same model, it also blocked cell growth in a

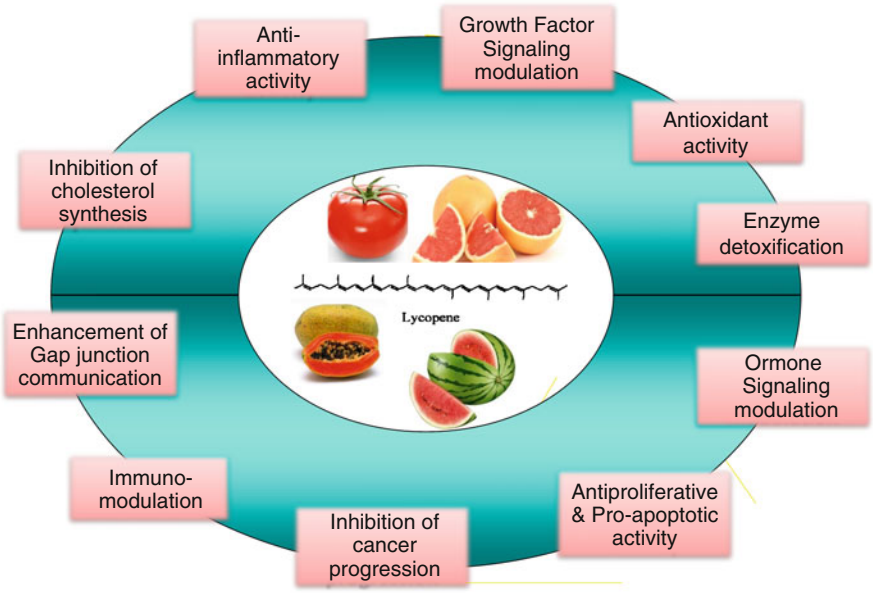

Fig. 6.1 Potential mechanisms by which lycopene and the main lycopene-rich foods, including tomato, papaya, watermelon and pink grapefruit and their derivatives, may prevent cancer risk

dose-dependent manner by inducing G0/G1 arrest and S phase arrest. Scolastici et al. also suggested that lycopene is a suitable agent for preventing chemically-induced DNA and chromosome damage in a human hepatoma cell line. Both lycopene beadlets and tomato paste were able to reduce oxidative DNA damage in the livers of TRAMP mice (Konijeti et al. 2010). Rats supplemented with lycopene for 5 days prior ferric nitrilotriacetate treatment showed a reduction of oxidative DNA damage, measured as 8-oxodGuo levels, and MDA production in prostate (Matos et al. 2006). In addition, it has been recently demonstrated that lycopene and grape seed extracts show high scavenging capacity against gas phase cigarette smoke-produced free radicals, which are considered as an important group of carcinogens (Yu et al. 2012). Several studies have shown the antioxidant effects of supplementation of tomato products or purified lycopene (providing 6–17 mg lycopene/day), on cellular DNA, in healthy human volunteers (Porrini and Riso 2000; Porrini et al. 2005; Zhao et al. 2006). However, effects on lipid peroxidation have been somewhat conflicting. In healthy human subjects, lycopene- or tomato free diets resulted in loss of lycopene and increased lipid oxidation (Rao and Agarwal 1998, 1999), whereas dietary supplementation with lycopene increased serum lycopene levels and reduced endogenous levels of oxidation products (Rao and Agarwal 1998). Rao and Shen (2002) also reported a significant decrease in serum lipid peroxidation and protein oxidation in healthy volunteers, following a 2-week consumption of tomato ketchup or oleoresin capsules, with baseline serum lycopene levels less than 0.2 mmol/L. On the other hand, Riso et al. (2004) observed no effects on lymphocyte resistance from lipid oxidation, following a

3-week supplementation of tomato products (8 mg lycopene/day). Briviba et al. (2004) also reported null effects on lipid peroxidation in plasma and feces in healthy men following a 2-week supplementation of 330 mL/day of tomato juice. These baseline plasma lycopene levels in Rao and Shen study were lower than those reported by Riso et al. (1999) and Briviba et al. (2004) in their studies (0.34 and 0.2 mmol/L, respectively). Thus, there may be a possibility that a depleted baseline lycopene level shows a better response to tomato antioxidant supplementation, than subjects with higher values. Kiokias and Gordon (2003) reported a significant decrease in biomarkers of oxidative stress in young healthy volunteers, following a 3-week supplementation of lycopene, in combination with other natural carotenoids. Tomato lycopene consumption in patients before prostatectomy has been reported in few studies to lower prostate DNA oxidative damage, serum prostate-specific antigen, and cause an overall reduction in disease aggressiveness (Chen et al. 2001; Kucuk et al. 2001, 2002; Bowen et al. 2002). Patients with prostate cancer were found to have low levels of lycopene and high levels of oxidation of serum lipids and proteins (Rao and Agarwal 1999).

Several lycopene-rich foods, including ketchup, fresh tomatoes, tomato paste, tomato sauce, tomato soup, tomato juice, vegetable juice, canned tomato and watermelon, have been reported to exhibit a potent total antioxidant capacity (Djuric and Powell 2001). Supplementation with tomato extracts has been also reported to ameliorate tissue damage and oxidative stress parameters against acetaminophen-induced acute hepatotoxicity and against amiodarone-induced lung toxicity in rats (Jamshidzadeh et al. 2008). Moreover, a small amount of tomato puree added to the diet has been reported to increase carotenoid concentration and the resistance of lymphocytes to oxidative stress (Porrini and Riso 2000).

It has been recently reported that papaya epicarp extract acted as a potent free radical scavenger and provided neuroprotection against H_2O_2-induced oxidative stress (Guizani et al. 2011). Such an extract also ameliorated glutathione depletion, restored total antioxidant capacity and augmented the inhibition of antioxidant enzymes. Fermented papaya preparations have been also shown to possess high free radical scavenging ability *in vivo* (Imao et al. 1998) and *in vitro* (Zhang and Omaye 2001; Aruoma et al. 2006). In addition, they reduced H_2O_2-induced DNA damage and benzo[a]pyrene-induced genotoxicity (Aruoma et al. 2006). Moreover, the oral administration of fermented papaya for 4 weeks decreased iron-induced lipid peroxide levels and increased superoxide dismutase activity in rat cortex (Imao et al. 1998).

The consumption of grapefruit has been also associated with protection against DNA damage induced by several chemical agents. In particular, a marked inhibition of ifosfamide-induced chromosome damage by grapefruit juice has been observed in mice (Alvarez-González et al. 2010).

In addition, the juices from several fruits and vegetables, including watermelon, grapefruit and tomato, showed protection against the genotoxicity of heterocyclic aromatic amines activated by human xenobiotic enzymes expressed in mammalian cells (Platt et al. 2010). Several fruit juices, including grape and watermelon juices, exhibited potent antioxidant effects in human plasma (Ko et al. 2005).

Numerous studies suggest that lycopene and lycopene-rich foods can also interfere with several redox-sensitive signaling pathways involved in cell proliferation and apoptosis (Palozza et al. 2011b).

6.3.2 Enzyme Modulation and Enhancement of Detoxification

Induction of phase 2 enzymes, through their ability to conjugate reactive electrophiles and to act as indirect antioxidants, may represent potential means for achieving protection against a variety of carcinogens. The expression of such enzymes at transcriptional level is mediated, at least in part, by the antioxidant response element (ARE) which is found in the regulatory regions of their genes. The nuclear factor E2-related factor 2 (Nrf2), which binds to ARE, appears to be essential for the induction of phase II enzymes (Ramos-Gomez et al. 2001). It has been reported that oxidation products of lycopene, including apo-10′-lycopenal, apo-10′-lycopenol and apo-10′-lycopenoic acid, can induce the nuclear accumulation of transcription factor Nrf2 protein in human bronchial epithelial cells (Lian and Wang 2008). Lycopene has been also reported to influence the expression of drug detoxification enzymes such as cytochromes P450 (Gradelet et al. 1996). The administration of lycopene to rats was shown to induce liver CYP types 1A1/2, 2B1/2 and 3A (Breinholt et al. 2000). The observation that these enzymatic activities were induced at very low lycopene plasma levels suggests that this mechanism may be relevant to humans.

6.3.3 Inhibition of Cell Proliferation and Apoptosis Induction

Lycopene is hypothesized to stop cell division at the G0-G1 cell cycle phase (Park et al. 2005; Ivanov et al. 2007; Ford et al. 2011). Park et al. (2005) reported that the growth of Hep3B human hepatoma cells was inhibited 20–50 % by lycopene at physiologically significant concentrations as low as 0.2 μM. In a similar study with the human prostate cancer cell lines LNCaP and PC3, Ivanov et al. (2007) also found that lycopene induced mitotic arrest at the G0/G1 phase mediated by decreased levels of cyclins D1 and E and cyclin dependent kinase 4. Using human breast MCF-7 and endometrial ECC-1 cancer cells, Nahum et al. (2006) showed that lycopene inhibits cell cycle progression in the G0/G1 phase through reduction of cyclin D1. Through suppression of phosphorylation of p53 and Rb anti-oncogenes, Matsushima-Nishiwaki et al. (1995) reported that lycopene inhibited cell division of mouse hepatocytes at the G0G1 cell cycle phase. The carotenoid is also able to induce apoptosis through the modulation of apoptosis-related proteins (Palozza et al. 2011b).

6.3.4 Regulation of Growth Factor and Hormone Signalling

Evidence also indicates that lycopene may interfere with growth factor and hormone signalling. In particular, Siler et al. (2004) suggested that the consistent evidence from epidemiological studies, which associates high intakes of vitamin E or lycopene with a reduced risk in prostate cancer, might be due to interferences with the prostate-specific endocrine loop of local testosterone activation and androgen signalling. Vitamin E contributed in an additive manner to the repression of androgen target genes by lycopene. This additive effect of both nutrients might be explained by their interaction at different levels of androgen signaling: whereas vitamin E inhibits the androgen receptor, lycopene suppresses androgen activation. Moreover, lycopene significantly reduced local expression of IGF-I and of IL-6.

Recent findings suggest that dietary lycopene may modulate the levels of testosterone, which has been highly implicated in prostate cancer incidence and development. In fact, mice lacking the expression of carotene-15,15′-monooxygenase fed with 10 % tomato powder or lycopene (248 nmol/g diet) for 4 days, showed a reduction of serum and testicular testosterone concentrations (Ford et al. 2010). It has been shown that a subset of 391 genes was found to be differentially modulated by lycopene between estrogen-positive and estrogen-negative breast cancer cells (Chalabi et al. 2007). Modified gene expression was observed in various molecular pathways, such as apoptosis, MAPK and cell cycle, as well as xenobiotic metabolism, fatty acid biosynthesis and gap junctional intercellular communication (Chalabi et al. 2007). These data suggest that hormones may modify lycopene response in cancer cells.

Increasing evidence suggests that lycopene may prevent cancer by a mechanism involving an inhibition of IGF signaling (Wang et al. 2003). In several experimental studies, this seems to occur through a downregulation of IGF expression, a downregulation of IGF-IR and/or through an upregulation of IGFBPs (Vrieling et al. 2007). Cross-sectional studies have investigated the relationship between tomato consumption (Signorello et al. 2000; Mucci et al. 2001; Gunnell et al. 2003) or lycopene intake (Holmes et al. 2002), IGF-I and IGFBP-3. In three of these studies, higher intake of cooked or processed tomatoes or lycopene was associated with lower IGF-I levels, and higher IGFBP-3 levels, or a lower IGF-I/IGFBP-3 molar ratio. Human studies (colon cancer patients or healthy subjects) reported a positive impact of lycopene in decreasing IGF-1, IGF-1R, or increasing IGFBP-1 or IGFBP-3 (Walfisch et al. 2007). There was also a decrease in IGF-1 in women at high familial breast cancer risk, but no effect on IGF-1 and IGFBP-3 levels with lycopene consumption in premenopausal breast cancer survivors (Voskuil et al. 2008). In a small human intervention study, IGF-I was decreased both in the lycopene intervention as in the control group (Kucuk et al. 2001).

6.3.5 Inhibition of Cell Adhesion, Invasion and Angiogenesis

In studies of the highly invasive human hepatoma cell line SK-Hep-1, lycopene was shown to have antimetastatic and anti-invasion activity. Hwang and Lee (2006) showed that lycopene at 5 and 10 μM (higher than physiologically relevant concentrations) could decrease the gelatinolytic activities of the matrix metalloproteinases MMP-2 and MMP-9 and inhibit the adhesion, invasion and migration of SK-Hep1 cells. Recent data seem to confirm the ability of lycopene to inhibit metalloproteinase expression in cultured cells even at lower concentrations (0.5–2 μM) (Palozza et al. 2012a) as well as in animal models (Huang et al. 2008). Huang et al. (2007) confirmed that MMP-9 expression was suppressed in SK-Hep-1 cells and found that the metastasis suppressor gene nm23-H1 was induced.

6.3.6 Inhibition of Cholesterol Synthesis and Ras Prenylation

It is known that cancer cells have abnormal cholesterol biosynthetic pathways that are resistant to downregulation by cholesterol. The committed step in the biosynthesis of cholesterol and isoprenoids is catalyzed by 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which promotes the reductive deacylation of HMG-CoA to mevalonate. This pathway produces numerous bioactive signalling molecules, which are responsible for the prenylation of many low molecular weight GTPases, including the Ras superfamily proteins. An iper-activated prenylation of Ras has been associated to tumorigenesis (Palozza et al. 2010a). In this context, lycopene has been shown to inhibit HMG-CoA reductase (Palozza et al. 2011c) and to inactivate Ras in several cancer cells (Palozza et al. 2010a).

6.3.7 Immunomodulation

Regulation of intrathymic T cell differentiation was suggested to be the mechanism for suppression of mammary tumor growth by lycopene treatments in SHN retired mice (Nagasawa et al. 1995).

6.3.8 Anti-inflammatory Effects

Epidemiological studies show that populations consuming a tomato-rich diet, containing high levels of lycopene, exhibit lower incidence of chronic inflammation-related diseases, such as coronary heart disease and certain types of cancers (Rao and

Agarwal 1999). Moreover, increasing investigations have proven that lycopene molecule possesses anti-inflammatory activity in various cellular and animal models of inflammation (Palozza et al. 2010b).

6.3.9 Enhancement of Gap Junction Communication

The anti-carcinogenic effects of lycopene have been suggested to be due to regulation of gap-junction communications in mouse embryo fibroblast cells (Zhang et al. 1992). This mechanism involves the upregulation of connexin-43.

6.4 Synergistic Interactions Between Lycopene and Other Bioactive Food Components in Cancer Prevention

The evidence that health benefits of tomato are greater than those of pure lycopene has been recently reviewed (Basu and Imrhan 2007). It is known that tomatoes contain a matrix of many bioactive components, including vitamin C, vitamin E, other carotenoids (α-, β-, γ-carotene, lutein) and flavonoids, and their synergistic interactions may be responsible for the observed beneficial effects of tomato-based products. It has been recently shown that tomato digestate is able to inhibit the growth of colon cancer cells by modulating the expression of regulators of cell cycle and apoptosis (Palozza et al. 2007). Such an effect was remarkable greater than that observed using purified carotenoids.

Increasing evidence suggests that the redox effects of lycopene in biological models may be influenced by the presence of other antioxidants, which may enhance the redox properties of the carotenoid as well as its anticarcinogenic functions. Antioxidant synergistic interactions have been reported among carotenoids, tocopherols/tocotrienols, ascorbic acid and flavonoids (Palozza et al. 2012b). On the other hand, lycopene only in combination with α-tocopherol inhibited the growth of prostate cancer cells (Pastori et al. 1998).

It has been recently suggested that papaya possesses chemopreventive effects against certain types of cancer. Although this can be due to the high lycopene content, it should be underlined that papaya also contains isothiocyanates, which are effective chemopreventive agents against chemical carcinogenesis (Nakamura 2009). The antitumoral activity seems to occur through different mechanisms, including apoptosis induction by phosphorylation of the antiapoptotic protein Bcl-2 (Nakamura 2009). On the other hand, limonoids are important constituents of the grapefruit and they may act as anticancer agents independently of lycopene (Vikram et al. 2010). Bergamottin, a cytochrome P450 inhibitor from grapefruit, may account for the inhibitory effects of grapefruit on tumor invasion and migration by a mechanism involving a downregulation of MMP-9 (Hwang et al. 2010).

Moreover, naringin, naringenin and quercitin, flavonoids found in grapefruit, acted as antitumoral agents (Miller et al. 2008) and they may cooperate with lycopene in cancer prevention. These molecules share with lycopene antioxidant, pro-apoptotic and antiproliferative properties. According with this, combinations of flavonoids with carotenoids (Yeh et al. 2006) or combinations of flavonoids with tocotrienols (Guthrie and Carroll 1998) inhibited oxidative damage or proliferation of the cells more effectively than the individual compounds.

Most of the experimental and clinical trials with lycopene-rich foods suggest a synergistic action of lycopene with other nutrients, in lowering biomarkers of oxidative stress and carcinogenesis. Lycopene contributes to this effect, but its role *per se*, at least in clinical trials, remains to be investigated.

6.5 Conclusions and Future Directions

Here, we have summarized several *in vitro* and *in vivo* studies in which lycopene and/or lycopene-rich foods have been shown to prevent cancer risk. *In vitro* studies demonstrated that lycopene may inhibit the growth of several types of cancer cells and provided valuable insights into the mechanisms by which lycopene exert their cellular and intracellular effects. The mechanisms by which lycopene may exert its anticancer activity include: antioxidant activity, modulation of redox activity, enzyme detoxyfication, inhibition of cell proliferation and apoptosis induction, regulation of growth factor and hormone signalling, inhibition of cell adhesion and angiogenesis, inhibition of cholesterol synthesis, immunomodulation and enhancement of gap junction communication. However, the results of the *in vitro* studies could be criticized since their relatively artificial nature. This is due to the lack of adequate *in vitro* methods of solubilizing and delivering lycopene to cells. We need more physiological methods of carotenoid delivery to cultured cells. In fact, the high hydrophobicity of lycopene makes it insoluble in aqueous systems and therefore poorly available for cell cultures. At the moment, in most *in vitro* studies, lycopene was provided as detergent solutions, or diluted in various solvents, such as tetrahydrofuran and dimethyl sulfoxide. These methods allowed the evaluation of the potential effects of the pigment but non-specific uptake and problems of miscibility, crystallization and toxicity could mislead the physiological significance of the observed phenomena.

A good number, although not all, of animal studies indicates a protective effect of pure lycopene or lycopene-rich foods on prostatic, gastro-intestinal and lung tumorigenesis. However, these studies are relatively few. Moreover, because of differences in routes of administration (gavage, intraperitoneal injection, intra-rectal instillation, drinking water and diet supplementation), species and strain differences, form of lycopene administration (pure, mixed carotenoid suspension or contained in lycopene-rich foods), no a real comparison is possible among the different studies.

Although numerous epidemiological studies demonstrate that lycopene and lycopene-rich foods may reduce cancer risk, intervention trials establishing a direct link between lycopene and/or lycopene-rich foods and cancer prevention are few and controversial. This is probably due to the multitude of interactions among lycopene and other nutrients with the intracellular signal pathways and to inter-individual polymorphisms which can mask the response to lycopene. Further research is critical to elucidate the anticancer role of lycopene and/or lycopene-rich foods. Although some human clinical trials are beginning to be undertaken, there is a great need for well-designed human intervention studies that take into consideration study designs including subject selection, specific markers of analysis, the levels of carotenoids being tested, metabolism and isomerization of lycopene and their biological significance, interaction with other carotenoids and antioxidants.

It is only through such studies that our understanding of the anticancer role played by lycopene will be enhanced and help us to develop complementary strategies for the prevention, treatment and management of cancer.

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Chapter 7
Effect of Antioxidant-rich Foods and Supplements on Cancer Risk

Xiaolin Zi and Anne R. Simoneau

Abstract Diet and health have been linked throughout the centuries. Which components of the diet are responsible for health is not clear. Using epidemiological studies, case control, and cohort studies, potential nutrients have been identified which may act to promote health. Early studies identified yellow green vegetables and fruits associated with less cancer in populations which consumed more of these foods. Antioxidants are a class of nutrients that have been identified through these types of studies and theoretically their ability to scavenge reactive oxygen species caused by cellular stress may lead to less DNA damage, mutagenesis, and cancer. Randomized controlled trials (RCTs) stringently test the hypothesis that adding antioxidants to a well nourished or over-nourished populace do not demonstrate the desired reduction in cancer or mortality in most studies. To illustrate the difficulty in translating epidemiological associations to successful RCTs, one of the first nutrients identified with fewer cancers, β-carotene, will be reviewed as the compound that initially identified as a potential anticancer agent and subsequently shown in RCTs to increase the risk of cancer. In addition, attempts to prevent prostate cancer with supplements will be evaluated. Review of the laboratory evidence and human studies will be performed and the discrepancies in results in RCTs are discussed. These discrepancies may be due to the baseline nutritional status of the populace, lifestyle factors such as smoking, as well as the status of the organ of interest in addition to the type and amount of agent.

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7.1 Introduction

One should eat to live, not live to eat.

–Moliere

"*Dis-moi ce que tu manges, je te dirai ce que tu es.*"
Tell me what you eat and I shall tell you what you are.

–Anthelme Brillat-Savarin

Though the link between food and health has been recognized throughout the ages, in the twentieth century the awareness of molecular nutrition moved into a new realm with scientific discoveries leading to both individual and societal behavioral changes regarding food. Knowledge of nutrition and what one should eat occurred at the same time agricultural produce was being taken down to its molecular level and then rebuilt into foods that were tasty but full of fats, sugars, salt and devoid of fiber. Consumption, even over consumption, was encouraged by effective marketing campaign. Intrinsically people are aware they make poor food choices, which is why they try to counter their poor choices with supplements or modified consumables; thus the laundry lists of daily supplements or the manufacturing of counter-intuitive products such as sodas enriched with vitamins and antioxidants. In 2010 in the US, 28 billion dollars were spent on nutritional supplements (Wang 2011). The food industry is enriching common foods with vitamins and minerals and promoting these products as heart, breast, prostate healthy, as well as other claims. Agriculture has modified its crops to produce more lycopene in a tomato, more β-carotene in a cucumber, which are then marketed to the consumer.

But what is really known about the addition of antioxidants to an otherwise non-restricted diet? The associations made between health and antioxidants were made from observations that people who ate more foods with particular compounds were healthier, but food choices are often linked to other behaviors, such as exercise and tobacco, which may also influence health. It is hard to determine if it is the ingestion of the antioxidants or the fiber found in vegetables, *vs* the absence of dietary animal proteins, or fewer overall calories that is more contributory to health. Or is it possible that people who eat vegetables are more likely to exercise, not to smoke, and not to drink alcohol in excess.

In this chapter, we will review the epidemiological data between antioxidants and cancer, and the efforts that have been made to isolate the particular components felt to be responsible in preventing cancer, and then efforts to study supplements on the diet to prevent cancer. Emphasis will be on randomized controlled trials (RCTs). The picture is somewhat murky, and it may be that individuals' genetic polymorphisms, baseline nutritional status, lifestyle behaviors (i.e. smoking) in addition to the doses of individual antioxidants which will eventually explain the variations in trial results. As the authors research urological cancers, trial examples will lean heavily toward prostate cancer, which in 1997 was already hypothesized to be a nutritional disease (Fair et al. 1997).

7.2 What Are Antioxidants?

7.2.1 Overall In Vivo Hypothesis of Antioxidants and Cancer

Normal oxidative metabolism produces free radicals, which can cause damage to cellular components such as DNA, proteins and lipids or death to the cell. Antioxidants are defensive agents that prevent free-radical-induced cell and tissue damage by reducing the formation of free radicals, scavenging them or by promoting their decomposition (Sies 1997). In normal physiology, there is a delicate balance between pro- and antioxidants. There are different antioxidants (e.g. glutathione, ubiquinone, uric acid) that are present in body fluids and tissues at a wide range of concentrations. However, a poor diet, pollution, toxins, medications, alcohol, smoking, sedentary lifestyle, stress, trauma, aging, infections, radiation, cancer cells, conventional cancer treatments, and other factors often produce excessive reactive oxygen species (ROS), which in turn lead to the disruption of the delicate balance between pro- and antioxidants, and then result in oxidative stress (Sies 1997). Therefore, an eminently plausible hypothesis for carcinogenesis is that oxidative stress damages RNA and DNA in cells, predisposing these cells to malignant alternations, and that antioxidants can prevent cell damage by neutralizing free radicals and oxidants, thus preventing subsequent development of cancer.

7.2.2 Different Types of Antioxidants

In general, there are two different types of antioxidants, including water-soluble (hydrophilic) and lipid-soluble (hydrophobic) (Vertuani et al. 2004). Water-soluble antioxidants react with oxidants in the cell cytosol and the blood plasma, whereas lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Vertuani et al. 2004). Antioxidant can be synthesized in the body or obtained from the diet. Antioxidants can also be classified as (1) antioxidant nutrients: vitamin E, C, β-carotene, selenium, manganese, zinc, etc.; (2) antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx); and (3) other antioxidants: coenzyme Q10, uric acid (a product of DNA metabolism), melatonin, astaxanthin, canthaxanthin, lutein, lycopene, zeaxanthin, natural phenols (flavonoids, stilbenoids, resveratrol, phenolic acids and their esters, other non-flavonoid phenolics), citric acid, oxalic acid, and phytic acid, n-acetylcysteine, eugenol bilirubin, r-α-lipoic acid, and others (Vertuani et al. 2004).

The National Academy of Sciences in releasing dietary reference intakes (DRI) for antioxidants proposed the definition of a “dietary antioxidant is a substance in foods that significantly decreases the adverse effects of reactive oxygen species, reactive nitrogen species, or both on normal physiological function in humans”.

Though there are several antioxidants many did not meet the rigor to determine DRI as their particular role in human physiological function *in vivo* has yet to be determined. The Academy's proposed DRI can be found at their website, Institute of Medicine of the National Academies, but currently only selenium, vitamins C and E have meet the rigor to have DRI calculated (Institute of Medicine 2000).

7.2.3 Ranking of Efficacy

There are several *in vitro* assays (Corral-Aguayo et al. 2008; Wu et al. 2004) including the oxygen radical absorbance capacity (ORAC) assay, the total oxyradical scavenging capacity (TOSC) assay, the ferric reducing/antioxidant power (FRAP) assay, the Trolox equivalent antioxidant capacity (TEAC) assay, and the total radical-trapping antioxidant parameter (TRAP) assay for testing chemical antioxidant activity. These assays have been used for generating relative ranking of efficacy of antioxidants. The most popular one is the list of ORAC values for plant foods commonly consumed by the US population (fruits, vegetables, nuts, seeds, spices, grains, etc.), which was published by scientists from the United States Department of Agriculture (USDA) (Wu et al. 2004). ORAC values are presented as the sum of the lipid soluble (e.g. carotenoid) and water-soluble (e.g. phenolic) antioxidant fractions (i.e. total ORAC) using micromoles Trolox equivalents (TE) per 100 g sample as a unit. These values are also compared to assessments of total polyphenol content in the samples. Table 7.1 was adopted from a published paper to rank antioxidant capacity in foods (Corral-Aguayo et al. 2008). The highest ranked foods in four major categories are as follows: fruits: blueberries, cranberries, and blackberries; vegetables: beans, artichoke hearts, and russet potatoes; nuts: pecans, walnuts, and hazelnuts; spices: cinnamon, oregano, and ground cloves. Although these chemical assays give the same relative ranking of efficacy of antioxidants, there exist some variations that depend on how the radicals react with food components. The ORAC method uses the peroxyl radical, the most common free radical in the human body. In addition, the actual ability of these chemical assays for predicting the actual efficacy of antioxidants in the body remains unknown (Wolfe and Liu 2007; Wu et al. 2004). Therefore, the ranking of antioxidants should be explained with caution. In addition, raw chocolate and goji berries are two foods that are extremely rich in antioxidants but were not evaluated in the list.

Recently, Liu and his associates (Wolfe and Liu 2007) have developed a cell-based method, named the cellular antioxidant activity (CAA) assay, for the antioxidant activity of various phytochemicals and fruit extracts. Among the pure compounds tested, quercetin had the highest CAA value, followed by kaempferol, epigallocatechin gallate (EGCG), myricetin, and luteolin. Among 25 fruits tested, blueberry had the highest CAA value, followed by pomegranate, blackberry, strawberry, raspberry, and cranberry.

Table 7.1 Ranking of antioxidant capacity in foods

Food	Serving size	ORAC (M TE/100 g)	Rank
Small red beans	1/2 cup dried	13,427	1
Wild blueberries	1 cup	13,427	2
Red kidney bean	Half cup dried	13,259	3
Pinto bean	Half cup	11,864	4
Blueberry (cultivated)	1 cup	9,019	5
Cranberry	1 cup (whole)	8,983	6
Artichoke (cooked)	1 cup (hearts)	7,904	7
Blackberry	1 cup	7,701	8
Prune	Half cup	7,291	9
Raspberry	1 cup	6,058	10
Strawberry	1 cup	5,938	11
Red delicious apple	One	5,900	12
Granny Smith apple	One	5,381	13
Pecan	1 ounce	5,095	14
Sweet cherry	1 cup	4,873	15
Black plum	One	4,844	16
Russet potato (cooked)	One	4,649	17
Black bean (dried)	Half cup	4,181	18
Plum	One	4,118	19
Gala apple	One	3,903	20

7.2.4 Food Sources

Many supplements and foods, particularly plant foods that have bright colors, are rich in antioxidants. Different types of antioxidants can protect different types of oxidative damages in tissues. Therefore, eating a wide range of antioxidant-rich foods has been considered to be important for health. The Table 7.2 is the list of antioxidant compounds and their food resources.

7.2.5 In Vivo Testing of the Antioxidant Theory in Preventing Cancer

One theory for the anticancer effects of antioxidants was that antioxidants prevent oxidative DNA damage and then inhibits tumor initiation and promotion during the process of carcinogenesis. However, antioxidant compound vitamin C (ascorbate) exhibited conflicting results in carcinogenesis and tumor growth in animal experiments. Millimolar concentrations of vitamin C can be achieved in humans by i.v. infusion but not by diet or supplements (Levine et al. 2011). Vitamin C at these pharmacologic doses decreases the growth and weight of tumor xenografts in nude mice (Chen et al. 2008, 2011; Levine et al. 2011). At these pharmacologic concentrations, vitamin C has been shown to induce a non-caspase-mediated cancer

Table 7.2 Antioxidant compounds and their food sources

Antioxidant compounds	Foods with high contents
Vitamin C (ascorbic acid)	Cantaloupe, citrus fruits, kiwi fruit, broccoli, brussels sprouts, etc.
Vitamin E	Cereals, sunflower seed kernels, and tomato products
Carotenoids (carotenes, lutein)	Sweet potatoes and carrots
Flavonoids	Tea, coffee, soy, and berries
Astaxanthin	Red algae and animals higher in the marine food chain, crustacean shells, and salmon flesh/roe
β-carotene	Butternut squash, carrots, orange bell peppers, pumpkins, kale, peaches, apricots, mango, turnip greens, broccoli, spinach, and sweet potatoes
Lutein	Spinach, kale, Swiss chard, collard greens, beet and mustard greens, endive, red pepper, and okra
Lycopene	Cooked red tomato products like canned tomatoes, tomato sauce, tomato juice and garden cocktails, guava, and watermelons
Myricetin	Walnuts
Isoflavone phytoestrogens	Soy, peanuts, and other members of the Fabaceae family
Resveratrol	The skins of dark-colored grapes, and concentrated in red wine
Pterostilbene	Vaccinium berries
Chicoric acid	Echinacea purpurea
Chlorogenic acid	Coffee, blueberries, and tomatoes
Cinnamic acid and its derivatives, such as ferulic acid	Seeds of plants such as brown rice, whole wheat and oats, as well as coffee, apple, artichoke, peanut, orange, and pineapple
Gallic acid	Gallnuts, sumac, witch hazel, tea leaves, and oak
Rosmarinic acid	Rosemary, oregano, lemon balm, sage, and marjoram
Salicylic acid	Vegetables, fruits, herbs, and the bark of willow trees
Flavonolignans	Milk thistle
Xanthones	Mangosteen
Capsaicin	Chili peppers

cell death but not normal cells in a hydrogen peroxide (H_2O_2)-dependent manner (Du et al. 2010; Takemura et al. 2010). This result is consistent with other reports that some agents that generate free radicals selectively killed cancer cells while sparing normal cells (Hahm et al. 2011; Raj et al. 2011; Yang et al. 2011). In contrast, several other studies provided evidence that vitamin C may enhance both spontaneous and chemically induced mutations in prokaryotes and eukaryotes and increase the yield of chemically induced tumors in animals (Shamberger 1984; Lee et al. 2003; D'Agostini et al. 2005; Heaney et al. 2008). These results suggested that vitamin C may work as both a prooxidant and as an antioxidant, depending on its concentrations. Other antioxidants, like vitamin E and polyphenols, also produced varying degrees of anti-tumor and anti-carcinogenesis efficacy in a wide variety of animal models at higher doses (Attia and Wilding 2006; Wang and Russell 1999). At lower doses, they may be ineffective or stimulate the

growth of cancer cells. Another emerging theory for the anticancer effect of antioxidants was that the increased production of free radicals by defective or aberrant cancer cell mitochondria stabilized HIF-1α levels, and that antioxidants vitamin C and *N*-acetyl-cysteine (NAC) normalized the activity of the HIF-1α-destroying enzyme for its degradation, inhibiting cancer growth in an animal model (D'Agostini et al. 2005). Almost all antioxidants have pleiotropic effects. Up to now, there is no sufficient evidence in animals to support that a single antioxidant mechanism would be an efficient way in prevention or treatment of cancers in animals.

7.3 Global Epidemiology to RCTs

Cancer statistics are a window into the health of the global community. Not always a clear window as several factors can skew how the number of cancer cases is reported. Not all countries have available access to care, or fund the collection of cancer statistics. Different medical practices can alter cancer statistics. In the US, the wide spread use of prostate-specific antigen (PSA) screening has lead to a spike in prostate cancer incidence compared to other countries which do not use PSA screening, mortality rates in this instance are a better reflection of the impact of the disease. Despite these limitations, several epidemiological observations have been made between cancer and nutrition. The seminal paper by Doll and Peto (1981) stressed the importance of lifestyle factors – tobacco control, but additionally stressed nutrition as a factor in cancer promotion and recommended a diet reduced in fat, increased in yellow green vegetables and fiber and some micronutrients, and improved food preservation (Doll 1992). Some of the nascent dietary literature grouped food as either dietary deficiencies in nutrients leading to cancer, or dietary excesses in fats as causing cancer (van der Linde 1976).

Some of the earliest and strongest association between cancer and nutrition were made with excesses of dietary fat/calories and increases in the hormonal tumors such as breast and prostate. A strong correlation was seen between starchy foods in developing nations with stomach cancer *vs* fatty foods and colon cancer as seen in developed nations (Hirayama 1975). It was proposed that reduction in energy intake could lead to cancer reduction, and estimated that 35 % of cancers could be prevented with such intervention (Doll 1992).

Associations between dietary deficiency and disease are numerous as with vitamin C and scurry, vitamin D and rickets, folate and myelomenigocele. Dietary intervention has been proven to treat such diseases (Meyskens and Szabo 2005). Defining specific deficiencies in micronutrients to a type of cancer and proving reversal with supplementation has been more difficult as outlined below in the β-carotene and prostate stories.

7.3.1 β-carotene

β-carotene best illustrates a repeated scenario of favorable epidemiology studies, intriguing case control and cohort studies leading to disappointing clinical trials. β-carotene is a carotenoid and a provitamin found in plants that is converted to vitamin A (retinol). β-carotene had the earliest venture into large scale trials, but there are similar stories for selenium, vitamin E, and vitamin C. In the 1980s, reports of levels of blood retinol and dietary intakes of green yellow vegetables were inversely proportional to cancer incidences (Peto et al. 1981). Several epidemiological investigations were published with β-carotene and vitamin A being strong contenders for protective effects on lung and gastrointestinal tumors. These investigations led to several case control studies. Lung cancer was inversely associated with vitamin A consumption in men who smoked heavily (Mettlin et al. 1979) in a study comprised of 292 cases and 801 controls, and vitamin A was associated with squamous but not adenocarcinoma of the lung (Byers et al. 1984) in an expanded study by the same group. Smith and Jick (1978) reported on 800 patients with malignancy compared to 3,433 controls and found men had a cancer risk ratio of 0.54 with higher vitamin A consumption, though women had a relative risk (RR) of 1.11. A prospective study of 8,278 Norwegian men found an inverse relationship between dietary vitamin A and lung cancer (Bjelke 1975). In a large cohort study of the Japanese population, 265,118 adults over the age of 40 years recruited in 1965 and followed until 1982 found an inverse association with green yellow vegetables rich in fiber, carotene and vitamin C and all types of cancers (Hirayama 1982, 1986). As reviewed by British Medical Journal in 1980 of these and other studies there was an accumulation of evidence for vitamin A, retinol and carotene with a modest doubling of the relative risk for cancer risk in groups with lowest intake of these nutrients (1980). These observations led to design of the first RCTs with nutrients to prevent cancer. Although it was noted that tobacco and dietary fat had stronger associations with cancer initiation or promotion, it was recognized smoking cessation and dietary limitations were less acceptable to the general population than nutritional supplementation in preventing cancer.

In 1982, a large prospective trial addressing the effect of β-carotene supplementation on cancer and cardiovascular mortality was undertaken (Hennekens et al. 1996). This Physicians' Health Study was composed of 22,071 well nourished men ages 40–84 years in a two by two design of 50 mg of β-carotene every other day and/or 325 mg of aspirin *vs* placebo controls. The every other day dosing was felt to give better plasma carotene levels by fourfold and is roughly equal to two carrots a day. After 12 years, there were no differences in cancer rates (RR 0.98) or deaths (RR 1.02) with β-carotene. The arm with aspirin did show a cardiovascular protective effect, and that arm of the study was closed early and aspirin was offered to all men.

A study directly addressing lung cancer prevention was begun in 1985. A joint venture by the Finnish and US governments, 29,133 Finnish male smokers were randomized in a two by two fashion with 20 mg of β-carotene and/or 50 mg of

vitamin E (α-tocopherol) *vs* placebo. At 6 years, the overall mortality was 8 % greater with β-carotene and 2 % higher with vitamin E. The β-carotene arm showed an increase of lung cancer (RR 1.16) and death (RR 1.08), in addition there were more prostate cancers, and more ischemic heart and stroke deaths. Vitamin E demonstrated an intriguing decrease in prostate cancer incidence by 30 %, and smaller decrease in colon cancer, but an overall increase in cancers outside the lung, genito-urinary and gastrointestinal tracks. There was also an increase in deaths from hemorrhagic stroke though there was improvement in ischemic heart and stroke deaths (1994).

Another study for adults at risk for lung cancer by smoking or asbestos history was begun in 1988. This Carotene and Retinol Efficacy Trial randomized 18,314 men and women ages 45–69 years to a trial of combination 30 mg of β-carotene and 25,000 IU of retinyl palmitate *vs* placebo control (Omenn et al. 1996). The trial proceeded for 4 years but was stopped 21 months early as the lack of benefit, and the evidence of possible harm, with intervention was accumulating. There was a 28 % increase in lung cancer, a 17 % increase in death and more cardiovascular disease in the intervention group. Six years after termination of the study, the effects of intervention showed a persistence in lung cancer and all cause mortality (RR 1.08) (Goodman et al. 2004). The observation that women were more affected post intervention (lung cancer, mortality, and cardiovascular disease) than men may be due to persistent fat storage of the micronutrients compared to men.

The Womens' Health Study initially began as a trial studying β-carotene 50 mg with 100 mg aspirin and 600 IU vitamin E all on alternate days, there were eight treatment arms in this two by two by two RCTs, but the carotene supplement was stopped at 2 years in response to the results of the previously addressed studies. In a 1999 analysis of the carotene arm, there appeared to have been no adverse events during the 2 years of intervention and subsequent 2 years of observation. All cause mortality showed RR 1.07, death from cancer was RR 1.11 (Lee et al. 1999).

The Women's Antioxidants Cardiovascular Trial studied antioxidants' effects on 18,000 women with a minimum of three cardiovascular risk factors. Vitamin E 600 IU, β-carotene 50 mg both every other day and vitamin C 500 mg given every day in a two by two by two trial design, and found over no difference in the groups after a mean follow up of 9.4 years (Lin et al. 2009).

One population identified as being deficient in micronutrients is Linxian China, located in North Central China. This area of the world has the highest rates of esophageal and stomach cancers. Greater than 85 % of the cancers seen here are these two types. Previous studies had linked fruit and vegetable deficiencies to these cancers (Hirayama 1986; Mettlin et al. 1981). Nutrient intervention to prevent cancer was felt to be possible. The trial consisted of 29,584 men and women between 40 and 69 years. Several combinations of micronutrients were utilized. (A) Retinol 5,000 IU, zinc oxide 22.5 mg; (B) Riboflavin 3.2 mg, niacin 40 mg; (C) Ascorbic acid 120 mg, molybdenum 30 g; (D) β-carotene 15 mg, selenium 50 mg, α-tocopherol 30 mg. Doses were set at twice the daily recommendations (Table 7.3). There were a total of eight research arms (Blot et al. 1993). After 5 years of intervention, group D was the only arm with a positive effect. In those treated

Table 7.3 Large randomized control trials with β-carotene or vitamin A supplements

Study	Date	Number of participants	Study group	Intervention	Duration of supplementation	Primary endpoint outcome
Physicians' Health Study (Hennekens et al. 1996)	1982–1995	22,071	Male physicians 40–84 years old Well nourished	Two by two β-carotene 50 mg every other day Aspirin 325 mg every other day	12 years	No difference in cancer rates (RR 0.98)/ deaths (RR 1.02) or cardiovascular disease with β-carotene
α-Tocopherol β-carotene Study (1994)	1985–1993	29,133	Male smokers Well nourished	Two by two β-carotene 20 mg α-Tocopherol 50 mg	6.1 years	β-carotene increased risk of lung cancer (RR 1.16), increased death (RR 1.08) α-Tocopherol no effect on lung cancer, higher risk of hemorrhagic stroke
Linxian Nutrition Intervention Trial (Blot et al. 1993, 1995; Qiao et al. 2009)	1986–1991	29,584	Male/female 40–69 years old Poorly nourished	8 arms: ABCD, AB, AC, BC, BD, CD, placebo A: retinol 5,000 IU, zinc oxide 22.5 mg B: riboflavin 3.2 mg, niacin 40 mg C: ascorbic acid 120 mg, molybdenum 30 μg D: β-carotene 15 mg, selenium 50 mg, α-tocopherol 30 mg	5 years	In those treated with carotene, selenium and tocopherol, there was a lower cancer mortality (RR 0.87) and lower overall mortality (RR 0.91), no differences in other treatment arms After 10 years, group D still had lower mortality (HR 0.95) more so in those younger than 55 years; there was an associated increase mortality in group A and increase in strokes; In group C an increase in esophageal/gastric cancer deaths in older participants but a decrease in CV deaths
Carotene and Retinol Efficacy Trial (Omenn et al. 1996; Goodman et al. 2004)	1988–1996	18,314	Male/female 45–69 years old High risk lung cancer	β-carotene 30 mg Retinyl palmitate 25,000 IU	4 years	Trial stopped early, treatment increased risk of lung cancer (RR 1.28) and increased death (RR 1.08), cancer risk remained elevated at 4 years post intervention

Women's Health Study (Lee et al. 1999, 2005)	1993–2004	39,876	Female ≥45 years old	Two by two by two 8 treatment arms Vitamin E 600 IU every other day Aspirin 100 mg every other day β-carotene 50 mg every other day	10.1 years	No significant difference with vitamin E on cancer incidence (RR 1.01), cancer deaths, or mortality (RR 1.04); carotene arm stopped at 2 years, analysis at four showed no effect on cancer or cardiovascular disease
Women's Antioxidants Cardiovascular Trial WACS (Lin et al. 2009)	1995–2005	8,171	Female ≥40 years or old ≥3 cardiovascular risk factors	Two by two by two Vitamin E 600 IU every other day β-carotene 50 mg every other day Vitamin C 500 mg every day Later 4th arm of folic acid and B6, B12 added	9.4 years	No significant decrease in cancer incidence or mortality with the vitamins C, E or β-carotene Subcategories showed that with vitamin E a decrease in colon cancer and decreased cancer in previous and current smokers compared to nonsmokers

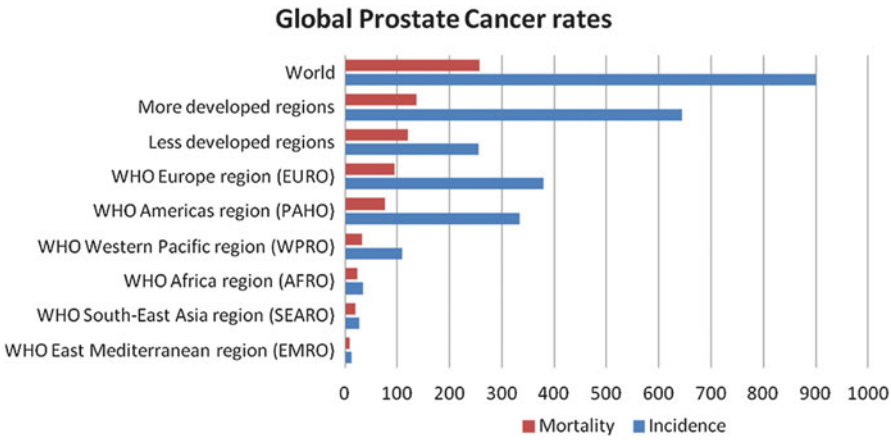

Fig. 7.1 2008 estimates of world prostate cancer incidence and mortality by region reported by Globocan, International Association of Cancer Research and accessed on website in spring 2012 (Ferlay et al. 2010)

with carotene, selenium and tocopherol there was a lower cancer mortality (RR 0.87), and lower overall mortality (RR 0.91). There was no difference in other treatment arms. After 10 years, group D still had lower mortality (HR 0.95), more so in those younger than 55 years. There was an associated increase of mortality in group A, with an increase in strokes. In group C, there was an increase in esophageal/gastric cancer deaths in older participants, but a decrease in cardiovascular deaths (Qiao et al. 2009).

In summary, despite several epidemiological, case control and cohort studies showing a strong association between foods with β-carotene and vitamin A with diminished cancer risk, the one positive trial was in a population known to be nutrient deficient.

7.3.2 Prostate Cancer

Prostate cancer is common as men age. Latent or incidental prostate cancer increases to 60 % in men in their seventh decade (Sakr et al. 1994). Global incidental prostate cancer rates are the same throughout the world, but clinical cancer rates with morbidity and mortality differ greatly throughout the world (Ferlay et al. 2004) (Fig. 7.1). Rose et al. (1986) studied prostate, breast, ovary and colon cancer mortality from the late 1970s and compared it to world food consumption as reported to the United Nations, and observed decreases in cancer mortality with vegetable and cereal consumption and increased cancer mortality with milk, animal fat and calories from animals. Hebert et al. (1998) looked

particularly at prostate cancer and diet and noted similar observations – areas of low prostate cancer mortality had more consumption of vegetables, soy, cereals, nuts, and fish. Areas of high prostate cancer mortality had high levels of consumption of animal products, fat and calories. Of course there is more than nutrition when comparing geography and cancer incidence – genetic factors, and environmental factors such as sunlight exposure can also play a role. With prostate cancer there have been several studies comparing the effect of migration and changing food consumption on prostate cancer incidence. Historically Asian countries have had low rates of prostate cancer mortality. It has been noted that when men move to the USA their rates of prostate cancer increase to that of the native population by 2–3 generations (Muir et al. 1991). There have been correlations between the adaptation of the western diet by Asian men who migrate to the US to increased prostate cancer incidence when compared to those who continue with an Asian diet (Whittemore et al. 1995). The reverse has also been noted, as Asian countries import Western style foods the incidence of prostate cancer has also increased (Lee et al. 1998; Pu et al. 2004; Sim and Cheng 2005). This gives rise to theories that the initiation of prostate cancer occurs with age, but the promotion to clinical disease is influenced by environmental, dietary or genetic influences (Kolonel et al. 2000). The epidemiology of prostate cancer with its global pattern, nutritional associations, the prevalence of prostate cancer, and its long latency period make prostate cancer an ideal tumor to try to intervene with dietary supplements.

Two trials performed for lung cancer prevention and skin cancer prevention are notable for their instigation of the role of antioxidants in prostate cancer prevention. One was the α-tocopherol β-carotene Study (ATBC). To recap male smokers who received 50 mg of vitamin E showed a decreased prostate cancer risk, 99 prostate cancers in the intervention arm *vs* 151 in the placebo group. This was a secondary endpoint. The trial was randomized for lung cancer risk.

A second trial, Nutritional Prevention of Cancer (NPC) was a trial with 200 μg selenium in a yeast form in adults at risk for recurrent skin cancer. Studies had linked populations living in areas of low soil selenium concentration to risk for cancer (Fleet 1997). The trial was stratified for history of squamous and basal cell carcinoma (Clark et al. 1996). The initial publication reported on non-significant decreases in all cause mortality (RR 0.83), and a significant decrease in cancer incidence (RR 0.63), all cause cancer mortality (RR 0.55). The main contributor to this effect was seen in the decrease in prostate cancer incidence and mortality, but there were in addition, decreases in colorectal and lung cancer. The trial was stopped early based on these positive findings. Interestingly there was no effect seen on either basal or squamous cell carcinomas, the primary endpoint (Clark et al. 1996). Duffield-Lillico et al. (2003b) reported on subsequent follow-up that the incidence of squamous cell carcinoma did increase in the treatment arm (HR 1.25).

Analysis of the secondary endpoint for prostate cancer reported by Clark et al. (1998) revealed that men who most benefited from selenium supplementation were men with initial PSA levels less than 4 ng/ml (RR 0.26).

In a large cohort study, the Physicians' Health Study II, a prospective study of 51,529 male health professionals ages 40–75 years, authors reviewed the

correlation between toenail selenium concentration as a reflection of long term selenium exposure and advanced prostate cancer and found an inverse relationship, collaborating the NPC trial (Yoshizawa et al. 1998).

With the results of these two secondary endpoints and collaborating smaller studies the Selenium and Vitamin E Trial (SELECT) was designed. Debate over dose and type of vitamin E and selenium was rigorous and eventually the trial proceeded with 400 mg of α-tocopherol and 200 μg of selenomethionine (Lippman et al. 2005). Accrual was rapid and 35,534 men in 428 study sites throughout North America and Caribbean were randomized to either selenium, vitamin E, both agents, or neither agents (placebo). The trial was stratified for prostate cancer risk, median age 62 years, 15 % African Americans, and 5 % Latino. The trial was stopped early in 2008 for a non-significant increase of prostate cancer in men receiving vitamin E and increased risk of diabetes mellitus in men receiving selenium (Lippman et al. 2009). Subsequent reports confirmed the increase risk of prostate cancer in men receiving vitamin E (Klein et al. 2011).

In the same JAMA journal as the initial report for SELECT was the publication by Gaziano et al. on the results of Physicians' Health Study II RTC, a study of 14,641 male physicians, 50 years or older who were randomized to either vitamin E 400 IU every other day or vitamin C 500 mg daily in the same two by two trial design as SELECT. After 10 years, there was no significant change in prostate cancer or all cancer incidences or mortality. Vitamin E demonstrated 9.1 *vs* 9.5 prostate cancer cases per 1,000 person years, and 17.8 *vs* 17.3 all cancers compared to placebo. For vitamin C, there were 9.4 *vs* 9.2 per 1,000 person years for prostate cancer and 17.6 *vs* 17.5 all cancers compared to placebo (Gaziano et al. 2009).

Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI. MAX) is a European trial of 12,741 men and women ages 45–60 randomized to a multivitamin with multiple smaller doses of nutrients or placebo for 8 years. The supplement included vitamin C 120 mg, vitamin E 30 mg, β-carotene 6 mg, and selenium 100 μg. The primary end points of cancer and cardiovascular disease showed no improvement in women, but for men there was decreased cancer rates and mortality. In reporting the effect on prostate cancer risk, overall HR was 0.88 with supplementation, but as will be addressed later the effect of supplementation was not consistent between different ranges of baseline PSA levels. Table 7.4 summarizes the notable large RCTs with supplements in prostate chemoprevention.

7.4 Investigating the Discrepancies

The information is confusing, one week β-carotene is the answer, followed by selenium, and then lycopene, now vitamin D as preliminary investigations are not confirmed on subsequent trials. A recent Cochrane review in 2012 for antioxidant supplements in disease reviewed 78 RCTs with a total of 296,707 participants, 46 % were women, and mean age of participants was 63 years. The reviewed focused on β-carotene, vitamin A, vitamin C, vitamin E, and selenium studies. Supplements were

Table 7.4 Notable large randomized control trials with supplements in prostate cancer prevention

Study	Date	Number of participants	Study group	Intervention	Duration of supplementation	Primary endpoint outcome
α-Tocopherol β-carotene Study (1994)	1985–1993	29,133	Male smokers	Two by two	6.1 years	β-carotene increased risk of lung cancer (RR 1.16), increased death (RR 1.08)
			Well nourished	β-carotene 20 mg α-Tocopherol 50 mg		α-Tocopherol had no effect on lung cancer, higher risk of hemorrhagic stroke
Nutritional Prevention of Cancer (Clark et al. 1996, 1998)	1983–1991	1,312	Men and women with skin cancer risk in area of low selenium soil levels	Double blind Selenium 200 μg	4.5 years	No difference in skin cancer RR 0.37 reduction in prostate cancer
Supplémentation en Vitamines et Minéraux Antioxydants (Meyer et al. 2005)	1994–2002	12,741	Adults	Double blind Vitamin C 120 mg Vitamin E 30 mg	8 years	No effect seen in women
			45–60 years old	β-carotene 6 mg Selenium 100 μg Zinc 20 mg		5,141 men: decrease cancer (HR 0.69) and decrease mortality (HR 0.63), supplement decreased prostate cancer HR 0.88
Physicians' Health Study II (Gaziano et al. 2009)	1997–2007	14,641	Male ≥50 years old Well nourished	Two by two Vitamin E 400 IU every other day Vitamin C 500 mg every day	8 years	No difference in prostate cancer or other cancer rates with either vitamins C or E compared to placebo
SELECT (Klein et al. 2011; Lippman et al. 2009)	2001–2008	35,533	Male	Two by two	5.5 years	Stopped early for trend in increased prostate cancer in men receiving vitamin E
			≥55 years old	Vitamin E 400 mg every day		Trend in diabetes in men on selenium follow-up
			Well nourished	Selenium 200 μg		

taken orally and intake ranged from 28 days to 12 years, with a median of 2 years and an average of 3 years. Overall supplement use did not improve mortality. In the supplement arms, there were 21,484 dead of 183,749 participants (11.7 %) *vs* the placebo arms 11,479 dead of 112,958 participants (10.2 %). The fixed effect relative risk was 1.03 (95 % CI 1.01–1.05). In reviewing the trials with low bias, there were 56 trials and the risk of mortality increased with supplements; (18,833 dead/146,320 (12.9 %) *vs* 10,320 dead/97,736 (10.6 %); RR 1.04, 95 % CI 1.01–1.07). After removing potentially confounding factorial trials from this low bias group, there were 38 trials remaining with risk of mortality with supplementation increasing (2,822 dead/26,903 (10.5 %) *vs* 2,473 dead/26,052 (9.5 %); RR 1.10, 95 % CI 1.05–1.15). Review of the low bias trials for individual agents showed: β-carotene (26 trials) (RR 1.05, 95 % CI 1.01–1.09); vitamin E (46 trials) (RR 1.03, 95 % CI 1.00–1.05); vitamin A (12 trials) (RR 1.07, 95 % CI 0.97–1.18), and there was noted to be a dose effect; vitamin C (29 trials) (RR 1.02, 95 % CI 0.98–1.07); only selenium did not significantly affect mortality (17 trials) (RR 0.97, 95 % CI 0.91–1.03). The authors concluded that there was no evidence that antioxidants were useful in primary or secondary disease prevention and that β-carotene, vitamin E, and possibly higher dose vitamin A increased mortality (Bjelakovic et al. 2012).

To date, the evidence does not justify a recommendation of a specific antioxidant treatment for a disease process to prevent cancer. The closest accepted use of an antioxidant supplement in an otherwise healthy population is for macular degeneration, though only for moderate to severe disease, results from the Age-related Eye Disease (ARED) Study (Evans 2006). The current recommendations by the National Cancer Institute (National Cancer Institute 2011), the (American Cancer Society 2012), the American Institute for Cancer Research (AICR) (American Institute for Cancer Research 2012a, b) is to eat a healthy diet with fruits and vegetables.

There may be several reasons for the discrepancies: (1) the characteristics of the population studied – underlying nutritional status, lifestyle factors, baseline organ health, and individual polymorphisms; (2) the agents utilized and dosages given.

7.4.1 Baseline Nutrient Status of an Individual

Clark et al.'s study demonstrating selenium decreased the prostate cancer risk in men with supplementation was further evaluated by Duffield et al. (2003a) and substratification by baseline selenium levels. As outlined in Table 7.5, the improvement with selenium was seen in the lower two quartiles for plasma selenium.

In the analysis of the SU MAX results, men but not women benefited from supplementation, but men also had lower baseline levels of vitamin C and β-carotene than women, and men with the lowest baseline levels of these nutrients along with lowest baseline vitamin E who received placebo were at the highest risk for cancer. Supplements improved cancer risk in men with low baselines in these nutrients. Interestingly, baseline selenium and zinc (high or low) levels did not influence the outcome of supplementation on cancer risk (Galan et al. 2005).

Table 7.5 Correlation between baseline plasma selenium (PSA) status with selenium supplementation and prostate cancer diagnosis in the Nutritional Prevention of Cancer Trial (Duffield-Lillico et al. 2003a)

	Selenium/placebo cases	Adjusted hazard ratio
Overall – 1983–1993	13/35	0.35
Overall – 1983–1996	22/42	0.48
Baseline PSA <106.4	2/5	0.14
Baseline PSA 106.8–123.2	7/16	0.33
Baseline PSA >123.2	13/11	1.14

Kirsh et al. (2006) reeviewed the baseline dietary/food intake of antioxidants, vitamin E, C, β-carotene, and 12 different supplements usage of men participating in a large screening trial, the Prostate, Colon, Lung, Ovary (PCLO) Screening Tiral and correalted with prostate cancer risk. Overall there was no association between prostate cancer risk and dietary or supplemental intake of vitamin E, β-carotene, or vitamin C, but men with below the median baseline levels of dietary β-carotene had reduced prostate cancer risk when supplemental β-carotene was used.

Bjelakovic et al. (2007) reviewed and performed a meta-analysis of 68 RCTs with oral antioxidants (β-carotene, vitamins A, C, E, and selenium) published between 1966 and October 2005 and found increased mortality in nonbiased trials with β-carotene, vitamins E and A. Biesalski et al. (2010) recently re-examined those same trials, the research team felt nutrient studies with expected smaller effects, goals for disease prevention, and inability to ethically deprive a population of vitamins and minerals differed from drug studies where disease cure rates and large effects are noted. They divided the trials into primary prevention in healthy populations, secondary prevention in populations already at risk and therapeutic trials in populations currently experiencing disease states. They then rated if there was a positive effect, a null effect or a negative effect seen in the RTC. Sixty percent of the studies were null, with neither benefit nor harm. One area where nutrients were beneficial was in populations where baseline nutritional deficiencies were found. In eight RCTs for primary cancer prevention which the authors rated as a beneficial in outcome, seven of those trials were in populations where baseline nutritional depletion was present.

7.4.2 Population Lifestyle

It is beyond the scope to review all aspects of lifestyle, but will briefly look at the impact of smoking and antioxidant usage on cancer risk. In the ATBC trial, a trial of smokers, vitamin E was found to be protective for prostate cancer (1994) *vs* the SELECT trial, with few smokers, vitamin E was found to be a hazard (Lippman et al. 2009). Since then several investigators have looked at this synergism. Kirsh et al. (2006) using his PCLO study population reviewed smoking status, (never *vs* current or recent smokers) and usage of vitamin E supplements (dosage and years) with prostate cancer risk, and as there is some discussion on the meaning of a prostate cancer diagnosis in a country where PSA screening is common further stratified for advanced prostate cancer, i.e.

Table 7.6 Advanced prostate cancer cases from the Prostate, Lung, Colorectal, and Ovarian Screening Trial in relationship to supplemental vitamin E usage, dose and years of supplementation, relative risk in men with smoking *vs* never smoking history (Kirsh et al. 2006)

Vitamin E supplementation dosage or years of use	Advanced prostate cancer cases in men who never smoked	Advanced prostate cancer cases in men with current or recent smoking history
None	1 referent	1 referent
>0–30 IU	1.34	0.67
>30–400 IU	1.16	0.72
>400 IU	1.29	0.29
None	1 referent	1 referent
>0–2 years	1.14	0.69
3–4 years	0.97	0.71
5–9 years	0.97	0.17
>10 years	1.11	0.30

Gleason score of 7 or more, or stage III, IV. They found that in restricting the analysis to advanced prostate cancers nonsmokers were at higher RR when taking vitamin E supplements, but smokers had a decrease in RR with a benefit seen with higher doses and duration. Table 7.6 shows the relationship of advanced prostate cancer cases and supplemental vitamin E usage, dose and years of supplementation.

When the group looked at smokers and evaluated the type of prostate cancer cases that were seen they found vitamin E usage did not improve the RR of non-advanced prostate cancer, but did improve the RR for advanced prostate cancer. Table 7.7 shows the association between smokers from PCLO stratified by vitamin E supplement use and the detection of advanced Gleason Score ≥ 7 or Stage ≥ 3 *vs* non-advanced prostate cancer.

The Cancer Prevention Study II consist of 1.2 million participants (Rodriguez et al. 2004). The nutrition cohort of this study included 184,192 US men and women, 86,404 of which were men and 72,704 men with known vitamin E use. The authors found with regular vitamin E use (>4 pills per week), there was no change in prostate cancer risk. Current smokers had slightly reduced risk with regular Vitamin E use RR 0.87 (0.67–1.11).

7.4.3 Baseline Organ Health

The NPC trial was studying the effects of selenium on secondary cancer prevention, preventing skin cancers in participants with previous skin cancer, otherwise the participants were considered healthy. In the SU MAX study, the participants were considered healthy and the goal was primary prevention of cancer and cardiovascular disease. But both of these studies reporting beneficial outcomes for overall prostate cancer prevention did report that those men who seemed to benefit most were men with the lowest risk for prostate cancer if PSA is used as a biomarker. It raises the question if occult disease was present at the time of randomization in

Table 7.7 Smokers from Prostate, Lung, Colorectal, and Ovarian Screening Trial stratified by vitamin E supplement use and the detection of non-advanced prostate cancer *vs* advanced Gleason score >7 or stage >3 (Kirsh et al. 2006)

Vitamin E supplementation dosage or years of use	Non-advanced prostate cancer cases in men with current or recent smoking history	Advanced prostate cancer cases in men with current or recent smoking history
None	1 referent	1 referent
>0–30 IU	1.67	0.67
>30–400 IU	1.46	0.72
>400 IU	1.47	0.29
None	1 referent	1 referent
>0–2 years	1.21	0.69
3–4 years	1.08	0.71
5–9 years	2.19	0.17
>10 years	1.73	0.30

Table 7.8 Baseline plasma selenium (PSA) and risk of prostate cancer with supplement intervention from the Nutritional Prevention of Cancer Trial (Duffield-Lillico et al. 2003a) and Supplémentation en Vitamines et Minéraux Antioxydants Trial (Meyer et al. 2005)

	Selenium/placebo cancer cases	Adjusted hazard ratio
Nutritional Prevention of Cancer Trial (selenium)		
Overall – 1983–1993	13/35	0.35
Overall – 1983–1996	22/42	0.48
Baseline PSA $\leq$ 4	7/20	0.33
Baseline PSA >4	11/13	0.95
Supplémentation en Vitamines et Minéraux Antioxydants (multivitamin)		
Overall	49/54	0.88
Baseline PSA <3	18/33	0.52
Baseline PSA $\geq$3	31/19	1.54

which the antioxidants not only did not prevent disease promotion, but perhaps sped up the promotion process. Table 7.8 shows the association of baseline PSA and risk of prostate cancer with supplement intervention.

7.4.4 Individual Polymorphisms

As more is being learned about antioxidants, the importance of the role of individual polymorphic variation is becoming understood. One example, as reported by Chan et al. (2009), is with SOD, a mitochondrial enzyme, which takes ROS and converts it to oxygen and hydrogen peroxide. The hydrogen peroxide subsequently is broken down by another enzyme, GPX into water. Both enzymes involve selenium. There is a polymorphism in the SOD gene, a valine to alanine at rs4880. Not all is known about its function, but it is hypothesized that the valine allele is less effective in transporting SOD into mitochondria and thus less efficient at neutralizing free radicals. Chan et al.

Table 7.9 Relationship between plasma selenium levels quintiles and polymorphism in the manganese superoxide dismutase (SOD2) gene (AA *vs* VA/VV) with aggressive prostate cancer risk (Chan et al. 2009)

	1st quintile	2nd quintile	3rd quintile	4th quintile	5th quintile
SOD2 VV/VA	1	0.98	1.29	1.32	1.82
SOD2 AA	1	0.95	0.65	0.71	0.60

(2009) previous work showed increasing selenium levels were protective for men with the polymorphism AA, and for VV there was a protective trend. To further study, their group took 778 men from the PHS, 489 men with prostate cancer and assessed the relationship between selenium and SOD2 and prostate cancer. They found in this enlarged group that for men in the highest quintile for selenium concentration, the presence of the valine polymorphism increased the RR for aggressive prostate cancer. The authors reported that for the 25 % of the population with an AA allele selenium supplementation maybe beneficial, but for the 75 % of the population with a V allele it may not be appropriate. Table 7.9 shows an association of plasma selenium and manganese SOD2 with aggressive prostate cancer.

7.5 Supplements

Due to the complexity and cost of RCTs, only one or two supplements have been evaluated at a time in the majority of these studies. When beginning trial design and when trials are being analyzed after completion, especially if a negative study, there is much debate on the natural *vs* synthetic sources and dosage. Those who believe in supplementation will point to inappropriate supplement selection as to the cause for an unwanted outcome. But a reminder that even though a healthy lifestyle (weight control, exercise, tobacco cessation, fruits and vegetables) is protective from chronic diseases, the fruit and vegetable component have shown minor associations in isolation. In trying to supplement a poor diet or lifestyle with antioxidants two thoughts should be considered.

7.5.1 A Tomato Is More Than Lycopene

The above reviewed studies for β-carotene, selenium, vitamin A, C, and E. In addition, lycopene (a carotenoid) also has much preliminary evidence but few large RCTs to review. Briefly, for completion lycopene (ψ, ψ-carotene) is responsible for the red color in tomato, watermelon, papaya, apricot, guava, and pink grapefruit (Lee et al. 2011). Lycopene as an antioxidant has shown to be a highly efficient scavenger of singlet oxygen and an excellent singlet oxygen quencher in biological membrane models, as well as a trap for other reactive oxygen species (e.g. peroxynitrite) (Woodall et al. 1997; Pannala et al. 1998; Stahl et al. 1998;

Cantrell et al. 2003). In addition, lycopene has the potential in improving oxidative stress defense by increasing the activity of the phase II enzymes (Wertz et al. 2004). Several clinical studies have shown that lycopene-rich diet reduced oxidative DNA damage in the prostate and protected human leukocytes against oxidative DNA damage (Pool-Zobel et al. 1997; Rehman et al. 1999; Porrini and Riso 2000; Bowen et al. 2002). At physiological concentrations (1 μM or less), lycopene has been shown to alter multiple signaling pathways that regulate cell cycle progression and signaling of cytokines, hormones, and growth factors (Wertz et al. 2004). It is yet unclear whether lycopene mediates these effects through its antioxidant function or through other mechanisms. Lycopene inhibited the growth of prostate (Hall 1996; Pool-Zobel et al. 1997; Pastori et al. 1998; Rehman et al. 1999; Porrini and Riso 2000; Kotake-Nara et al. 2001; Bowen et al. 2002; Kim et al. 2002; Obermuller-Jevic et al. 2003), mammary (Karas et al. 2000; Prakash et al. 2001), endometrial cancer cells (Nahum et al. 2001), and promyelocytic leukemia cells (Amir et al. 1999). Lycopene appears to inhibit cancer cell growth by interfering with growth factor receptor signaling, with little evidence of apoptosis or direct cellular toxicity. Lycopene inhibited cell proliferation by interference with IGF-I signaling (Karas et al. 2000). In addition, lycopene has other documented mechanisms of action, including: (1) modulation of intracellular communication by upregulation of connexin 43, as well as improving gap-junctional cell communication (Zhang et al. 1991; Kucuk et al. 2001); (2) inhibition of androgen activation and signaling (Herzog et al. 2005); and (3) inhibition of IL-6 expression (Siler et al. 2005; Nantz et al. 2006). In summary, multiple mechanisms of lycopene action lead to reduced cell proliferation, reduced DNA damage and increased oxidative defense, thus providing explanations for lycopene contributing to a reduced cancer risk.

Despite fruits and vegetables being identified in most epidemiological studies as protective of cancer, efforts to tease out specific compounds and test in a RCT have been generally unsuccessful. Meyskens and Szabo (2005) reviewed the possible reasons for the discrepancy. They identify fruits and vegetables as a "biological action package". Several nutrients traveling together, it is difficult to tease out a single agent, and it maybe more important for the interaction between several agents than a single agent on its own. Giovannucci et al. (2002) proposed tomato products as protective for prostate cancer, and identified lycopene as a potential protective agent, but as reviewed by Ilic et al. (2011) for Cochran in the three RCTs, to date there was no protective effect of lycopene. A tomato is more than just lycopene as outlined in Table 7.10, listing just the top five compounds found in tomato products, and it may be the concert of chemicals *vs* just one chemical that accounts for associations of fruit and vegetables and health, but not seen in RCTs of one or two large dose supplements.

7.5.2 Too Much Is Too Much

Antioxidants have shown *in vivo* that at higher doses they become pro-oxidants. As outlined earlier *in vivo* testing demonstrates this duality of effect in the lab. Within

Table 7.10 Top five nutrients found in tomato or tomato products (Campbell et al. 2004)

	Raw tomato	Catsup	Tomato juice	Tomato sauce	Tomato soup
Lycopene μg/100 g	2,573	17,007	9,037	15,152	5,084
β-carotene μg/100 g	449	560	270	290	75
Potassium mg/100 g	237	382	229	331	181
Folate μg/100 g	15	15	20	9	7
Vitamin C mg/100 g	12.7	15.1	18.3	7	27.3

the body the complexity increases. Chan et al. (2009) in their evaluation of their results on polymorphisms in SOD and selenium levels discuss several theories for their findings, all complex (as the importance of cells to use ROS for apoptosis to control mutagenesis) is offset by ROS causing mutations. As reviewed by Rayman (2012), selenium has an "inextricable U shaped link with status" where supplementation is beneficial only in those with underlying baseline deficiency. Predicting the ability of normal, precancerous or cancerous cells' response to changing the levels of antioxidants is difficult and the lab may not be able to place on the variables found within the body and only tested through RCTs.

7.6 Antioxidants and Cancer Therapy

Though it would seem intuitive to take supplements for enhanced nutrition during times of chemotherapy and radiation to promote the health of the individual, unless there has been a specific trial with a specific therapy and supplement, it is not recommended to take supplements during treatment. An example, as reported by Bairati et al. (2005), vitamin E was studied to determine if during radiation treatment there would be less damage to the skin in adults receiving treatment for head and neck tumors. There was a decrease in acute side effects during treatment, but subsequently an increase in local recurrence HR 1.37. AICR concludes supplements over the dietary reference intake cannot be recommended as safe or effective. Recommend are five fruits and vegetables a day (Norman et al. 2003).

7.7 Summary and Prospective

Though a diet, full of fruits, vegetables, fiber, low in excessive calories, combined with exercise and carcinogen avoidance is linked to health (Ford et al. 2009, 2012); and fruits and vegetables are good sources of antioxidants; and antioxidants have been shown in the laboratory to protect cells and animals from chronic diseases; efforts to supplement the diet of populations without overt dietary deficiencies with antioxidants to prevent cancer and chronic disease have not yet been proven effective. This perhaps should not be surprising. At the start of this road, the role of fruits and vegetables in disease prevention was

acknowledged to be minor (1980), but the thought was it would be easier to give a supplement in efforts to prevent disease than change poor dietary or lifestyle habits. An editorial accompanying the results of the ABTC trial made the observation that the most effect prevention of lung cancer was not β-carotene or vitamin E, but smoking cessation (Hennekens et al. 1994). And little was known about the *in vivo* effect of large doses of a single nutrient *vs* the natural packaging in fruits or vegetables of multiple smaller amounts of vitamins and minerals, but the supposition was doses higher than found in food would improve the efficacy of supplements. Since then, much time and money has been spent on efforts to prevent disease with antioxidant supplements with few positive results. We now recognize more is not always better. The hope that health could be achieved by a balance – not a balance of moderate behaviors as stressed by our wiser elders – but a balance as marketed by commerce where supplements would balance or counteract the poor nutritional and lifestyle choices we make has proven to be wrong.

Currently, there is much work being done in the laboratories across the world on personalized medicine. The belief is that knowledge of an individuals' genetic, nutritional and disease status along with a better understanding of the nutrients themselves will allow supplementation or drugs tailored for that individual to improve one's health. This chapter has outlined examples where genetic differences, dietary or tobacco use alters the disease outcome in supplement use. There is promise in this approach. The skeptic may ask if all this investigation is truly necessary. Just as we embarked in vitamin supplementation in smokers to prevent lung cancer with agents showing at best modest effects on cancer incidence in non-RCTs, especially when compared to the multitude increased cancer risk with the act of smoking, with hopeful, if perhaps misguided efforts, and poor results; the current embrace of personalized medicine to improve health may be a misguided use of time and resources. We already know what activities (exercise, diet of fruits and vegetables, weight maintenance and tobacco avoidance) lead to health in the general public, and it is ignored. It is difficult to believe that investing in genotyping, baseline nutritional and health studies would lead to such a radically different prescription for health that it would alter health outcomes different from following the basic good health advice. Would it be better to fund education and facilities for diet, exercise and tobacco cessation for many than determining the genetic and nutritional status for supplemental use for some? On a personal level maintaining one's weight, exercising daily, avoiding carcinogens and eating a colorful diet of fruit and vegetables has been associated with healthy populations and is prudent for us all.

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Chapter 8
Effect of the Mediterranean Diet on Cancer Reduction

Lisa S. Brown and Teresa T. Fung

Abstract The Mediterranean diet is a plant-based dietary pattern characterized by high intake of olive oil, legumes, whole grains, fruit, vegetables, nuts, seeds, fish, and red wine. The diet has been linked to a decreased risk of developing many non-communicable diseases including several types of cancer. Although findings have been somewhat inconsistent, several large prospective cohort studies have shown an association between greater adherence to a traditional Mediterranean diet and lower overall cancer incidence. When specific forms of cancer are examined, current research suggests a stronger association with some types and a weaker or non-existent relationship with others. Existing literature is equivocal on the association between the Mediterranean diet and breast cancer, but studies are heterogeneous in menopausal status and tumor hormone receptor status. For colorectal cancer, data is reasonably consistent to suggest that adherence to the Mediterranean diet is associated with a reduced risk, although more studies are needed to confirm and refine the relationship. Evidence for a protective association of a Mediterranean dietary pattern on upper gastrointestinal cancers is quite consistent and suggests that the Mediterranean diet is associated with a reduction of risk. It should be noted that due to the small number of studies, more data, especially from non-European countries, is needed. Suggested mechanisms through which the Mediterranean diet may impact cancer initiation and proliferation include increased insulin sensitivity and reduction of excess insulin production, anti-inflammatory and antioxidant effects of the diet, high fiber content and an association with reduced risk of excess weight gain and obesity.

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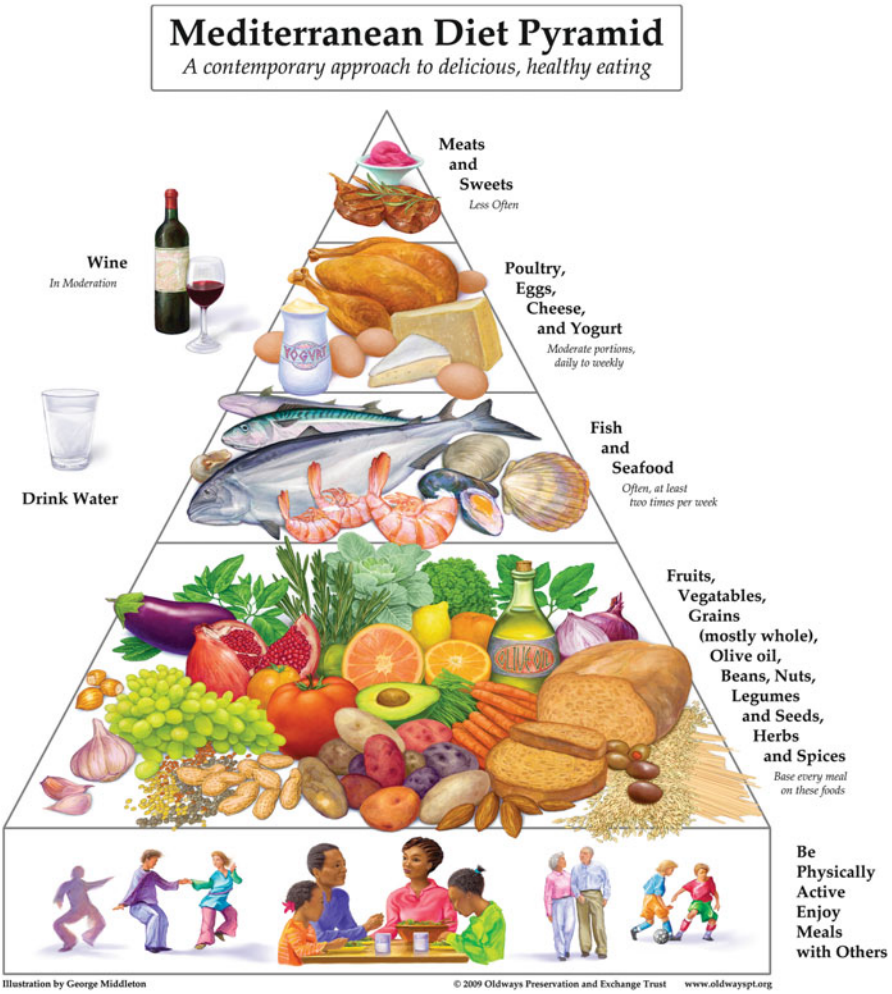

Fig. 8.1 Mediterranean diet pyramid: a contemporary approach to delicious, healthy eating

8.1 Introduction

The Mediterranean diet is perhaps the best known and most comprehensively studied dietary pattern in the world. Despite the common perception that the Mediterranean diet refers to a single entity, the term actually refers to several traditional dietary patterns that have emerged from countries boarding the Mediterranean Sea, including Spain, Italy, Greece, Turkey, Lebanon, Israel, Egypt, Libya, Algeria, and Morocco. Even within each Mediterranean country traditional diets vary, with coastal areas more strongly associated with a Mediterranean dietary pattern (Noah and Truswell 2001) (Fig. 8.1).

Despite regional differences, there are common features that characterize a Mediterranean dietary pattern. All variations of the diet contain relatively high amounts of legumes, whole grains, fruit, vegetables, nuts and seeds, fish, and olive oil. In many areas alcohol is also regularly consumed in moderation, primarily in the form of wine (da Silva et al. 2009). The diet has been linked to a decreased risk of developing many non-communicable diseases (also referred to as chronic diseases) associated with westernized countries, including cardiovascular diseases, type 2 diabetes mellitus, obesity, Alzheimer's disease, and cancer (Sofi et al. 2010).

This chapter explores the makeup of traditional Mediterranean diets, examine variations within the dietary pattern, and review the association between the Mediterranean diet and development of cancer. In addition, possible biological mechanisms by which the dietary pattern may influence development of cancer are discussed.

8.2 Overview of the Mediterranean Diet

The Mediterranean diet was first described as a unique dietary pattern and linked to reduced risk of cardiovascular disease by Ancel Keys in the 1950s and 1960s. In 1960, Keys published a best-selling cookbook describing a Mediterranean diet based on local patterns that Keys and his wife observed as they resided in Southern Italy during the 1950s (Keys and Keys 1960). The diet was characterized by relatively high intake of carbohydrates from sources including pasta, fresh bread, fruits and vegetables. Intake of protein was moderate and came primarily from beans and nuts, with minimal amounts of animal protein from cheese, seafood, and limited servings of meat (approximately two meals per week). Fat intake was very low within the 1950s Italian diet, estimated at approximately 20 % of total calories (Nestle 1995).

Based on his own experience and observational data following World War II suggesting that rates of cardiovascular disease were much lower in Italy and Greece compared to wealthy business men in the United States, Keys initiated a long term prospective cohort study in 1958, known as the Seven Countries Study. The Seven Countries Study was initially comprised of 12,763 men, aged 40–59 years, residing in seven countries representing different regions of the world (Keys 1980). Countries included in the study were the United States, Finland, the Netherlands, Italy, the former country of Yugoslavia (now, Croatia and Serbia), Greece, and Japan.

Findings from the ongoing Seven Countries Study confirmed that incidence of cardiovascular disease in Greece and Italy was comparatively low, while the United States and Finland had the highest rates of cardiovascular disease. Keys concluded that the Mediterranean dietary pattern and the associated lifestyle were responsible for this difference in risk and promoted the diet throughout the rest of his 100 years of life (Vanitallie 2005).

In the 1990s, researchers at the Harvard School of Public Health also began to investigate and promote the benefits of a Mediterranean diet. The diet recommended by Willet et al. (1995) was based less on the Italian diet and more on a Greek version of the diet, one with roots both in the Seven Countries Study and a Cretan study done by the Rockefeller Foundation in the late 1940s to early 1950s. The Cretan form of the Mediterranean diet was similar in many ways to the Italian version described by Keys, but contained significantly higher amounts of fat. The primary fat source was olive oil and fat content in the diet ranged between 30 and 40 % of calories. The Cretan diet also contained less dairy compared to Italy and some other areas of Greece. This version now is perhaps now the best known version of the Mediterranean diet and the one most commonly associated with the term (Hu 2003).

8.2.1 Components of Mediterranean Diet

Given that many countries boarder the Mediterranean Sea including several in Southern Europe, the Middle East, and Northern Africa, the term Mediterranean diet actually refers to a fairly broad range of dietary patterns. There are however, certain commonalities within the different versions of the diet as summarized below and in Table 8.1.

8.2.1.1 Protein Sources

The Mediterranean dietary pattern is a primarily plant-based diet, with the majority of protein coming from plant sources, such as beans, nuts, and grains. Fish comprises the main source of animal protein within the traditional Mediterranean diet, with small portions consumed regularly (Simopoulos 2001).

Meat including lamb, beef and pork is used sparely, often as a side dish, condiment, or flavoring or for special occasions and celebrations (Helsing 1995). Poultry and small game meats such as rabbit are used within some regions and cultures, but are generally not an emphasis of the diet.

Dairy is traditionally utilized in a fermented or processed form, generally as either yogurt or cheese, rather than consumed as milk (Hinrichs 2004). Milk is often obtained from goat, sheep, water buffalo, and even camels rather than from cows. Eggs are also used, but portions are limited with up to four eggs per week observed within various regional definitions of the traditional Mediterranean diet (Willett et al. 1995).

8.2.1.2 Carbohydrate Sources

Vegetables and whole grains make up the base of the diet, while sugar and refined grains are extremely limited within the dietary pattern. Fresh fruit is typically eaten as dessert with sweet desserts reserved for special occasions. Due to the warmer

Table 8.1 Food sources of macronutrients around the Mediterranean

Country	Protein sources plant	Protein sources animal	Dairy	Carbs/grain	Fat sources	Beverages
Spain	Legumes; lentils, chickpeas, beans, almonds, hazelnuts, walnuts	Eggs, chicken, pork, cured ham (prosciutto), beef, lamb, rabbit, sausages, oily fish, shellfish, anchovies, tuna, sardines, dried and salted cod (bacalao), calamari, shellfish, other seafood	Milk, sheep's milk cheese (manchego), milk and egg custard (flan)	Bread, rice, potatoes, sweet potatoes, pasta, corn, churros	Olive oil, olives, vegetable oil, mayonnaise, lard	Wine, sangria, sherry, beer, coffee, hot chocolate
Italy	Legumes; beans (white, fava), almonds, hazelnuts	Veal, lamb, smoked/preserved meat, goat (kid), pork, cured pork (prosciutto), pancetta, small game, sausages, dried salted cod (baccala), swordfish, tuna, sardines, anchovies, shellfish, squid, cod, flounder, whitefish, other seafood	Cheeses; (romano, provolone, parmesan, pecorino, gorgonzola, mozzarella, fontina, ricotta, mascarpone), gelato Cheeses made with milk from cows, sheep, and water buffalo	Pastas: made with eggs in the north, often stuffed and topped with cream sauce, without egg in the south, unfilled with tomato sauce, rice, bread, polenta (cornmeal), pizza dough, couscous, potato, potato dumplings (gnocchi)	Olive oil, butter, lard/bacon fat, heavy cream	Wine (red), coffee, espresso, bottled mineral water, juice, sparkling wine, amaro, Sambuca, limoncello
Greece	Legumes; white beans, chickpeas, fava beans, black beans, almonds and other nuts, sesame and other seeds	Eggs, grilled or roasted meats, beef, veal, pork, mutton/lamb, goat, chicken, hen, turkey, duck, goose, rabbit, game, fish (fresh, dried, or salted), crustaceans, mollusks, and other seafood	Yogurt, feta cheese (cow's milk)	Pita bread, leavened loaf bread, Rice, bulgur, potatoes, pasta, filo dough	Olives, olive oil, vegetable seed oils, butter	Wine, coffee, tea, beer, Anise-flavored alcoholic beverages (ouzo)

(continued)

Table 8.1 (continued)

Country	Protein sources plant	Protein sources animal	Dairy	Carbs/grain	Fat sources	Beverages
Turkey	Legumes; white beans, chickpeas, lentils, nuts, almonds, hazelnuts, pistachios, sesame seeds	Eggs, grilled meats, mutton, lamb, beef, chicken, organ meat (liver, kidney, brain), calamari, mussels, fish	Feta cheese, yogurt, milk	Bread, couscous, bulgur, barley, rice pilaf, pastas, pita bread, filo dough, pizza dough, potatoes	Olive oil, butter, vegetable oils, the tail fat of sheep	Coffee, black tea, herbal teas, yogurt drink (ayran), raki (alcoholic, distilled from grape residue, flavored with anise)
Israel	Beans (chickpeas, fava), almonds, sesame seeds	Lamb, chicken, beef, veal, venison, goat, ox, sheep, chicken, duck, goose, turkey, eggs, fish (herring, salmon)	Cheese, cottage cheese, sour cream	Bread (pita, challah, bagels), matzah (in bread, crackers, dumplings), rice pilaf, potato, crepes	Chicken fat, olive oil, olives, vegetable oils, butter	
Egypt	Beans (fava, chickpeas), lentils, sesame seeds, nuts	Eggs, chicken, fish (fresh or salted and dried), lamb, rabbit, pigeon	Cheese (feta, labna, gebna)	Bulgur, rice, pita bread, barley, potato, macaroni pasta,	Olives, olive oil	Beer, mint tea, coffee
Algeria	Lentils, chickpeas, fava beans, nuts; walnuts, hazelnuts, almonds, pistachios, pine nuts	Beef, chicken, sheep, goat, lamb, pheasant, duck, goose, quail, pigeon, seafood; bass, swordfish, tuna, monkfish, sardines, mullet, crustaceans, and shellfish, eggs	Milk, cheese, yogurt	Couscous, breads, filo dough, potato, corn, pulses	Olive oil, olives, butter, clarified butter (smen)	Coffee, tea, mint green tea
Morocco	Legumes; chickpeas, almonds and other nuts	Eggs, lamb, poultry, organ meats, lamb and kid (spice-rubbed and spit-roasted), pigeon, camel, fish, and other seafood	Fermented dairy products (lben, smen and jben), buttermilk, some cheeses (feta), puddings	Couscous, rice, bread, potato, cereal products	Sour fresh butter (zebeda), preserved clarified butter (smen), olives, olive oil, peanut oil, sesame oil	Coffee, Mint-flavored green tea Culturally inappropriate to consume sodas and alcoholic beverages

(Salaman 1991; Balkwill 1994; Robertson 1996; Mackley 1998; Productions 1999; Randall 1999; Çlik et al. 2004; Kittler and Sucher 2004; McWilliams 2007; Metallinos-Katsaras 2011)

climate of the Mediterranean region, fresh fruits and vegetables are available throughout most of the year. Heartier vegetables such as eggplant and root vegetables make up the base of many meals, while onion and garlic are common for flavoring dishes throughout the region. Use of potato became more prevalent over the latter half of the twentieth century, but is still less frequently eaten than in other parts of Europe and the Americas. Soups and stews are a common meal throughout the region and are generally comprised primarily of vegetables and beans with some meat for flavoring.

While grain sources vary, grain is almost always consumed as a whole grain, often with minimal milling and processing (Trichopoulou 2001). Wheat plays a large role in all countries surrounding the Mediterranean and is commonly used to make different pasta dishes from spaghetti to couscous, as well as, different forms of bread, including challah, pita and lavash. Wheat is also regularly used in an unprocessed form, such as bulger and wheatberries. Other grains used include barley, teff, and buckwheat. Rice and corn are eaten in some areas, but are more common in cuisines associated with areas of Mediterranean countries that are further from the coast.

8.2.1.3 Fat Sources

Olive oil serves as the primary source of fat within the traditional Mediterranean diet. Total fat in the traditional diet depends on region and varied from approximately 14–32 % of calories according to data from the early 1960s (Helsing 1995). More current data shows fat intake as high as 40 % of total energy associated with a reduced risk of cardiovascular disease for individuals consuming a traditional Mediterranean diet. No matter what the percent of total fat, saturated fat is relatively low at 8 % or less of total calories (Willett et al. 1995).

8.2.1.4 Alcohol Sources

In a traditional Mediterranean dietary pattern, wine is generally consumed in low to moderate amounts with meals in areas of Southern Europe that boarder the Mediterranean (Rimm and Ellison 1995). Historical data indicate that wine was often watered down, and more frequently consumed by men than women. In North African areas around the Mediterranean, little to no alcohol was historically consumed by the population, especially in Islamic areas.

8.2.1.5 Lifestyle

Several other environmental and lifestyle factors have been associated with the Mediterranean dietary pattern, notably regular physical activity. Regular physical activity, primarily in the form of lifestyle activity including walking as transportation and physical labor expended in farming and household chores is often observed to accompany traditional Mediterranean diets (Willett et al. 1995).

The Mediterranean lifestyle has also been associated with a distinct meal pattern through the day. Most cultures throughout the region traditionally consume a small breakfast meal comprised of a bread product and either coffee or tea (Nestle 1995). In most Mediterranean cultures the mid-day meal is the primary meal, and the evening meal is lighter and less often contains a meat component.

Given limited ability to separate out the effects of the diet from the effects of other behavior that accompany the diet, it may be more appropriate to characterize this dietary pattern as a lifestyle pattern.

8.2.2 Measurement and Classification of the Mediterranean Diet

Research conducted between the 1950s and 1980s described features of a Mediterranean diet and compared morbidity and mortality in populations who consume Mediterranean diets to those who do not, as seen in the Seven Countries Study. However, beginning in the 1980s and 1990s dietary patterns in countries that had traditionally consumed a Mediterranean diet began to shift to a more Westernized pattern (Balanza et al. 2007). In contrast to a Mediterranean diet, the Westernized diet included significantly more animal protein in the form of meat and dairy, as well as a significant increase in refined carbohydrate from both food and beverage sources, and significantly lower fruit and vegetable intake. This shift presented the opportunity to compare the effect of dietary differences in individuals who come from the same population, effectively controlling for genetics and other non-modifiable factors.

Early research into the Mediterranean dietary patterns of Southern Italy, Greece and Turkey measured dietary intake within the population by compiling food recall data collected in person by trained interviewers. In the 1980s, a new manner of dietary assessment was piloted that allowed researchers to expand data collection to larger populations. The Food Frequency Questionnaire (FFQ) is currently the most commonly used tool to measure diet in large cohort studies. The FFQ is a self administered instrument designed to assess usual food intake over the past year. Study participants are provided with a list of foods frequently consumed by the particular population they are identified with and asked to assign their personal consumption of those items (Cade et al. 2002). Although it cannot measure intake precisely, it is sufficiently comprehensive to assess the entire diet and to distinguish individuals with different levels of intake of key foods and nutrients.

Researchers have created scores derived from FFQs to quantify adherence with some of the primary components of the Mediterranean diet. The majority of research either directly uses or adapts a score developed by Antonia Trichopoulou et al. (2003) and first published in 2003. The Mediterranean Diet Scale combines a food- and nutrient-based scoring algorithm quantifying the intake of nine items:vegetables, legumes, fruit and nuts, dairy, cereals, meat and meat products, fish, alcohol, and the ratio of monounsaturated to saturated fat. For items determined to be positively associated with a traditional Mediterranean pattern, intakes above the median of the cohort receive one point while all other intakes receive zero point. Items determined to be negatively associated with a traditional Mediterranean pattern including meat

and dairy product consumption receive one point if they were less than the median. The objective of this scoring system is to identify those with more traditional Mediterranean patterns of dietary consumption *vs* those who have adopted a more traditionally Westernized manner of eating.

Due to large variation in the type and quantities of food consumed in different populations adaptation of the original scale is often necessary to measure adherence to the primary components of a traditional Mediterranean diet. For example, olive oil is a component of the Trichopolou score, but because it is not consumed in meaningful quantities outside the Mediterranean region, other investigators used the ratio of monounsaturated-to-polyunsaturated fat intake to capture the characteristics of this component. Points are awarded for each food group based on consumption level compared with the study sample, with one point awarded for each food group if consumption is above the median intake for beneficial food groups, and one point for consumption below median intake if for undesirable food groups (e.g. red meat).

8.3 Mediterranean Diet and Cancer

A substantial number of studies have examined the association between adherence to the Mediterranean diet and overall risk of cancer occurrence or death. Research in this area has been robust with many studies utilizing a prospective design and a considerable follow-up period. Large sample sizes and long follow-up periods have provided adequate data on cancer development and mortality. This design has allowed for sufficient statistical power to detect a moderate to strong relationship between Mediterranean diet adherence and either cancer incidence or mortality. Participants in these studies included age ranges from young to elderly and are drawn from mostly Caucasian populations in Western Europe or the United States.

8.3.1 Overall Cancer Risk and Mortality

Two European follow-up studies examined the relationship between a Mediterranean diet and overall cancer risk. In the Greek component of the European Prospective Investigation into Cancer (EPIC) and nutrition study, adherence to a ten-point Mediterranean Diet Scale among middle aged men and women was associated with a lower risk for overall cancer incidence (Benetou et al. 2008). A 12 % reduction ($p < 0.05$) of risk was observed with each two-point increase in score and the result did not differ by gender. A larger study utilizing data from the entire EPIC cohort was later conduced, but to accommodate different food intake from ten countries in Western Europe, the Mediterranean Diet Scale was slightly modified (Couto et al. 2011). A modest, but significant risk reduction (4 %) was noted with each two-point increase in score. The most substantial risk reduction was observed in Greece, in a magnitude similar to the earlier Greek-only study. The differing results between the two studies is likely due to the difference in food consumed within each food group,

as well as the score not being sensitive in capturing consumption quantities when a population-based median was used to determine points.

For total cancer mortality, a 24 % reduction was observed in the Greek segment of the EPIC cohort (consisting of young and old adults of both genders) for each two point increase in the Mediterranean diet score over a median follow-up of nearly 4 years (Trichopoulou et al. 2003). Using the Mediterranean diet score modified for the EPIC cohort, however, no reduction in death was observed for the Spanish segment of the study with a mean follow-up of over 13 years (Buckland et al. 2011). Of note, the Spanish adult men and women consumed fewer vegetables and less olive oil than the Greek participants, but consumption of the other Mediterranean food groups were comparable. Among young women (age 30–49) in Sweden, a higher Mediterranean diet score (as used in the Greek EPIC study) was also not associated with a lower cancer death over a follow-up of 12 years (Lagiou et al. 2006). In this study, the participants consumed fewer fruits and vegetables compared with the Spanish or Greek EPIC participants; therefore, those with a high Mediterranean diet score did not have similar consumption level as the Southern Europeans. It should also be noted that the lack of association may also be attributed to the low baseline risk of cancer death in young people. On the other hand, in an elderly population (age $>$ 70) in Finland, Italy, and the Netherlands, using a score modified from Trichopoulou et al. (2003), a higher Mediterranean diet score was also not associated with a 10-year risk of cancer mortality (Knoops et al. 2004). This study, however, did not include alcohol intake as a component of the score. In a large cohort of men and women age 30–60 years from Northern Sweden, a higher Mediterranean diet score was inversely associated with total cancer mortality in men over a 10-year mean follow-up period, but not in women (Tognon et al. 2012). This analysis found that alcohol intake was significantly associated with lower risk of all cause mortality in this cohort, as well as several lifestyle factors including a BMI of less than 30, being a non-smoker, and higher levels of physical activity. In the US, among individuals (age $>$ 50), closer adherence to the Mediterranean diet as measured by the Alternate Mediterranean Diet Score (aMed) was associated with a lower cancer mortality (21 % for men, 14 % for women comparing the top tertile to the bottom tertile) during 5 years of follow-up (Mitrou et al. 2007).

Available data on the association between the Mediterranean diet and cancer risk or mortality is not entirely consistent, although it appears to point toward a lower risk for cancer death among older people. Using the meta-analysis approach to combine data from observational studies, a modest but significant 6 % reduction of cancer risk or mortality was found (Sofi et al. 2010). Most studies that have looked at the relationship between the Mediterranean diet and cancer development have included development of any type of cancer due to the relatively rare nature of the disease. It has been hypothesized that lack of consistency of findings and weaker than expected associations may be because cancers from different sites are actually very different diseases with unique etiologies. It is possible that the Mediterranean diet reduces risk for some, but may not have influence on all of them. Studies that investigated all cancers may, therefore miss association that is specific to particular cancer sites (Tables 8.2 and 8.3).

Table 8.2 Mediterranean diet and overall cancer incidence

Authors last name (year)	Study type	Number of cases	Population	Scoring criteria characteristic	Major results
Tognon et al. (2012)	Prospective cohort	493 incident cancers in men and 481 incident cancers in women	Men (n = 37,546) and women (n = 39,605) from the Västerbotten Intervention Program (VIP) population study in Northern Sweden	Adherence to Mediterranean diet with a 0–8 point score comprised of eight components: vegetables and potatoes; fruit and juices; whole-grain cereals; fish and fish products; ratio of MUFA + PUFA to SFA; alcohol intake; meat and meat products; and dairy products	[a]Lower overall cancer risk in men, but not in women with higher adherence (HR = 0.92, 95 % CI 0.87–0.98) [a]Of specific cancer types pancreatic cancer was significantly associated with lower risk in men (HR = 0.82, 95 % CI 0.68–0.99)
Cuoto (2011)	Prospective cohort	9,669 incident cancers in men and 21,062 incident cancers in women	Men (n = 142, 605) and women (n = 335, 873) from various European countries (EPIC cohort)	Adherence to Mediterranean diet with a score ranging from 0 to 9 – combined intake of fruits and nuts, vegetables, legumes, cereals, lipids, fish, dairy products and meat products and alcohol	[a]Lower overall cancer risk with greater adherence to the Mediterranean diet for a two-point increase in Med-diet score (HR = 0.96, 95 % CI 0.95–0.98) [a]Inverse association stronger for smoking-related cancers than cancers not known to be related to smoking (P(heterogeneity) = 0.008)
Benetou (2008)	Cohort study	851 incidences of cancer (421 men; 430 women)	25,623 participants (10,582 men; 15,041 women) of Greek segment of EPIC	Traditional Mediterranean diet adherence based on Trichopoulou et al.[a] (zero = minimal adherence; nine = maximal adherence)	[a]Higher degree of MD adherence associated with lower overall cancer incidence; two-point increase corresponded to 12 % reduction in cancer incidence (adjusted HR = 0.88, 95 % CI 0.80–0.95) [a]Exposure-dependent association stronger in women

[a]Trichopoulou Mediterranean diet score: Conformity to the traditional Mediterranean diet was assessed through a score ranging from 0 to 9. Score relies on 9 dietary components. Frequent consumption of traditional components (vegetables, legumes, fruits and nuts, cereals, fish and seafood, high ratio of MUFA:SFA) received a value of 1, but consumption below the median were assigned a score of 0; for components less frequently consumed (dairy and meat and meat products) consumption below the median was assigned a 1 and otherwise 0. Moderate alcohol consumption also received a score of 1 and 0 otherwise. Total score ranged from 0 (minimal adherence to the traditional Mediterranean diet) and 9 (maximal adherence)

Table 8.3 Mediterranean diet and overall cancer mortality

Authors last name (year)	Study type	Number of deaths	Population	Scoring criteria characteristics	Major results
Buckland (2011)	Cohort study (EPIC-Spain)	1,855 deaths (913 from cancer)	Spanish cohort from five Spanish regions (n = 40, 622 – 37.7 % males)	rMED score (18-unit scale including nine dietary components)	[a]Two-unit increase in rMED score associated with significant reduction in all cause mortality (6 % reduction; $P < 0.001$), but not from overall cancer (HR 0.92; 95 % CI 0.75, 1.12)
Mitrou (2007)	Prospective cohort	27,799 deaths (5,985 cancer deaths)	214,284 men and 166,012 women from NIH-AARP study in US	Nine-point scale to assess conformity to Mediterranean dietary pattern (components included vegetables, legumes, fruits, nuts, whole grains, fish, MUFA:SFA ratio, alcohol and meat)	[a]Men: inverse association found comparing high to low conformity of Med-diet for cancer mortality (multivariate HR 0.83, 95 % CI 0.76–0.91) [a]Women: inverse association seen with high conformity; 12 % decrease risk for cancer mortality (P = 0.04 for trend)
Lagiou (2006)	Cohort	572 deaths (76 from cancer)	42,237 young women ages 30–49 years in the Uppsala Health Care Region, Sweden	Mediterranean diet assessed by ten-point score incorporating the characteristics of this diet (according to Trichopoulou et al.)	[a]Women less than 40: no association between Mediterranean diet and cancer mortality Women over 40: two-point increase in score was associated with reduction in cancer mortality (16 %; 95 % CI −1 %, 29 %; P approximately 0.06)

Knoops (2004)	Cohort	935 deaths total (233 cancer)	1,507 men and 832 women ages 70–90 years in 11 European countries	Modified Mediterranean diet: eight components with a score ranging from 0 (low-quality diet) to 8 (high-quality diet)	[a]Adhering to a Mediterranean diet, moderate alcohol use, physical activity and non smoking were associated with a lower risk of overall mortality [a]Combination of four low risk factors lowered all-cause mortality rate to 0.35 (95 % CI = 0.28–0.44) similar results observed for cancer mortality; low risk pattern was associated with a population attributable risk of 60 % of deaths from cancer.
Trichopoulou (2003)	Prospective cohort	275 deaths	22,043 adults in Greece	Traditional Mediterranean diet assessed by ten-point Mediterranean diet scale (range of scores 0–9 with higher scores indicating greater adherence)	[a]Inverse association with greater adherence to diet and death due to cancer (adjusted HR = 0.76 95 % CI 0.59–0.98). [a]Individual food groups of Med. diet and death generally not significantly associated

[a]Trichopoulou Mediterranean diet score: Conformity to the traditional Mediterranean diet was assessed through a score ranging from 0 to 9. Score relies on 9 dietary components. Frequent consumption of traditional components (vegetables, legumes, fruits and nuts, cereals, fish and seafood, high ratio of MUFA:SFA) received a value of 1, but consumption below the median were assigned a score of 0; for components less frequently consumed (dairy and meat and meat products) consumption below the median was assigned a 1 and otherwise 0. Moderate alcohol consumption also received a score of 1 and 0 otherwise. Total score ranged from 0 (minimal adherence to the traditional Mediterranean diet) and 9 (maximal adherence)

8.3.2 Mediterranean Diet and Breast Cancer Risk

Epidemiological research on female breast cancer usually separately considers pre- and post-menopausal breast cancer due to possible difference in etiology. Among studies that focused on post-menopausal cancer, the Greek segment of the EPIC cohort with a mean follow-up of close to 10 years had a 22 % reduction of risk with a two-point increase of the Trichopolou Mediterranean Diet score (Trichopoulou et al. 2003). However, no association was observed in a study in Britain which used a score slightly modified in the meat and lipid components and with a similar follow-up duration (Cade et al. 2011). In an American study with up to 18 years of follow-up, the aMed was not associated with post-menopausal breast cancer risk. However, when estrogen receptor status of the tumor was considered, women in the top 20 % of the aMed score was associated with a lower risk of estrogen receptor negative (ER−) tumors than those in the bottom 20 % of the score, but not estrogen receptor positive tumors (ER+) (Fung et al. 2006). In the EPIC study with 11 years of follow-up, the 16-point adapted relative Mediterranean diet score was more strongly associated ER- tumors among post-menopausal and with a 20 % risk reduction comparing women with at least 10 points *vs* those with 5 points or less (Buckland et al. 2012). However, in a large Swedish study with 16 years of follow-up, no association was observed between a slight variant of the Trichopolou (Trichopolou et al. 2003) and any kind of breast cancer risk (Couto et al. 2013). Because ER- tumors are not as strongly influenced by estrogen levels, it was proposed that the influence of lifestyle factors would be easier to detect in ER- tumors.

Four case-control studies combined both pre- and post-menopausal tumors in the analysis. In a French-Canadian study among women with *BRCA1* and *BRCA2* mutations, the Alternate Mediterranean Diet Score showed no association was noted (Nkondjock and Ghadirian 2007). In the only non-Caucasian study, two Mediterranean diet scores were computed for Asian-American women (Wu et al. 2009), one using all foods that fit into the usual Mediterranean food groups, and one excluding soy foods which would have been counted as legumes. An inverse association between breast cancer risk and diet score was only observed for the ten-point soy containing score, and high lifetime soy intake was strongly associated with a lower risk of breast cancer (30 % risk reduction comparing eight + *vs* three or less points). High soy intake was likely associated with higher intake of healthy foods such as fruits and vegetables, and represented an intake pattern closer to a traditional Asian pattern than typical American pattern. Therefore, even though other aspects of the diet may be similar to a Mediterranean diet, such as higher fruits and vegetables intake, there may be substantial other differences in food choices that accounted for the observed results. A Greek study based in Cyprus, an area known to have high adherence to a traditional Greek dietary pattern, found no association between Mediterranean dietary pattern scores and breast cancer risk, although single food analyses found higher intake of olive oil, fish and vegetables was associated with decreased risk (Demetriou et al. 2012). There was also no association found in a study in southern France with a Mediterranean diet pattern statistically derived using the principal component procedure (Bessaoud et al. 2012).

Among the four studies on pre-menopausal breast cancer, neither the EPIC group (Trichopoulou et al. 2010) that used the Trichopolou score nor the British group (Cade et al. 2011) that used a modified Trichopolou score noted any significant association between adherence to the Mediterranean diet and breast cancer risk. Existing literature is equivocal on the association between Mediterranean diet and breast cancer. Although a number of studies have been conducted, they are heterogeneous in menopausal status and tumor hormone receptor status. It is possible that the Mediterranean diet may influence post-menopausal breast cancer, as some studies have suggested (Fung 2006; Trichopolou 2010), but more studies are needed to draw a more confident conclusion (Table 8.4).

8.3.3 Mediterranean Diet and Colorectal Cancer

Several studies examined the association of adherence to the Mediterranean diet and colorectal cancer risk. In a 5 years follow-up study of Americans age 50 or greater, men scoring in the top 20 % of a nine-point Mediterranean diet score had a 28 % reduction of colorectal cancer risk and women had an 11 % reduction (Reedy et al. 2008) compared to those in the bottom 20 %. The risk reduction was observed for tumors in the colon or rectum. In another American study of middle-aged men and women with up to 26 years of follow-up, the aMed was only marginally associated with a reduction of risk (11 %, $p = 0.06$) (Fung et al. 2010). More recently, two European studies (Kontou et al. 2012; Agnoli et al. 2013) both showed a decreased risk of CRC. In the Italian arm of the EPIC study, a 11-point index adapted to Italians (Italian Mediterranean Index) was shown to reduce risk by 50 % (95 % CI = 0.35–0.71) comparing individuals with 6 points or above and those with 1 point or less (Agnoli et al. 2012). This index emphasized higher intake of pasta, typical Mediterranean vegetables, fruits, legumes, olive oil, fish, and lower intake of soft drinks, butter, red meat, potatoes, and alcohol. A Greek case-control study with 250 cases and controls each used a 15-component adherence score with a maximum of 75 points. For each one point increase in this Modified Mediterranean Diet score, a 12 % reduction in odds of CRC was observed (95 % CI = 0.84–0.92).

Another approach to studying colorectal cancer is to examine the precursor lesion adenomatous polyps, also known as adenomas. Using the Trichopolou score, a case-control study in Americans 55–74 years old showed that high adherence to the Mediterranean diet was associated with 21 % lower odds of colorectal adenomas in men (Dixon et al. 2007). Interestingly, no association was observed in women. In a multi-country European study, women with a history of colorectal adenoma who also had diets high in olive oil and fruit and vegetables, were at a lower risk for recurrence after a 3 years follow-up (Cottet et al. 2005). The odds of adenoma were reduced by 70 % comparing the top tertile to bottom tertile of a Mediterranean eating pattern. However, no association was found among men. The dramatically reduced odds in women need to be interpreted carefully. The analysis was not adequately controlled for lifestyle factors that also influence colorectal cancer development, such as physical activity. Therefore, the apparent protective association for adenoma development may not be as great as it appears without taking relevant lifestyle factors into account.

Table 8.4 Mediterranean diet and specific cancer sites

Authors last name (year)	Study type	Outcome	# of cases	Population	Scoring criteria characteristics	Major results
Cade (2011)	Cohort study	Breast cancer	828 incident cases of breast cancer	UK women's cohort ($n = 33{,}731$)	217-item FFQ used to generate Mediterranean dietary pattern and a dietary pattern conforming with the WHO Healthy Diet Index	[a]Maximal adherence to the Mediterranean diet was associated with HR 0.65 (95 % CI 0.42–1.02, *P* trend 0.09) compared with minimal adherence [a]No statistically significant association between either the WHO HDI or a Mediterranean dietary pattern on risk of breast cancer
Trichopoulo (2010)	Cohort	Breast cancer	240 incident cases of breast cancer	14,807 women in Greece (EPIC cohort)	Traditional Mediterranean diet evaluated through a score (range 0–9) incorporating characteristics of the diet (zero-minimal adherence; nine-maximal adherence)	[a]Increasing adherence to Med. diet was not associated with a decrease risk of breast cancer in the entire cohort (HR = 0.88 for every two points; 95 % CI 0.75–1.03) or in premenopausal women (HR = 1.01 for every two points; 95 % CI 0.80–1.28) [a]Marginally significant inverse association between greater adherence to the Med. diet and breast cancer risk in postmenopausal women (HR = 0.78 for every two points; 95 % CI 0.62–0.98; *P* for interaction by menopausal status 0.05)

Wu (2009)	Population-based case-control	Breast cancer	1,248 incident cases of breast cancer	Los Angeles County (Asian American women) ($n = 4{,}568$)	Med. diet score (range 0–10) with six beneficial dietary components (vegetables, legumes, fruit/nuts, cereal, fish and seafood, and high ratio of MUFA to SFA) and four detrimental components (meat, milk/dairy products, carbohydrate and alcohol) Value of 0–1 (0 with minimal adherence and 1 indicates maximal adherence)	[a]Adherence to Mediterranean diet was inversely associated with risk (OR 0.65; 95 % CI 0.44–0.95) in women with highest scores (≥8; most adherent) compared to those with lower scores of 0–3 ($P = 0.009$ for trend)
Nkondjock (2007)	Case-control	BRCA-associated breast cancer	89 carriers of *BRCA* genes affected by breast cancer; 48 non-effected carriers	French-Canadian women ($n = 183$)	Alternate Mediterranean diet score (aMED) based on Trichopoulou et al.[a]	[a]No association between aMED and breast cancer risk (ORs comparing highest and lowest tertiles of this diet quality scores were 0.59; 95 % CI: 0.20–1.77; $P = 0.244$ for trend)
Demetriou (2012)	Population-based case-control	Breast cancer	935 incident cases of breast cancer	Women enrolled in the MASTOS case-control study in Cyprus ($n = 1{,}752$)	Two food-based Mediterranean diet scores used: (1) 0–55 point score based on weekly consumption of nine food groups: cereals, potatoes, fruit, vegetables, legumes, fish, red meat and products, poultry, full fat dairy products olive oil, and alcoholic	[a]No association highest *vs* lowest scores or for trend for either score [a]Postmenopausal breast cancer risk decreased across quartile levels of increased adherence to a dietary pattern extrapolated higher in fruits, vegetables, fish and legumes (OR Q4 *vs* Q1

(continued)

Table 8.4 (continued)

Authors last name (year)	Study type	Outcome	# of cases	Population	Scoring criteria characteristics	Major results
					beverages (0 consumption received highest score) (2) 0–9 point scale based on olive oil, fruit, vegetables, starches, alcohol (0 consumption received highest score), fish, legumes	0.67, 95 % CI 0.49–0.92, *P* for trend < 0.0001)
Fung (2006)	Cohort	ER + and ER-breast cancer	3,580 cases of breast cancer (2,367 ER+; 575 ER−)	Nurse's Health Study cohort (n = 71,058)	Alternate Mediterranean diet score (aMED) – based on Trichopoulou et al.[a] with some modifications: excluding potatoes from vegetable group, separating fruit and nuts into two groups, eliminating dairy group, including only whole-grain products, including only red and processed meats from the meat group	[a]No association between diet quality indices and total ER+ breast cancer risk [a]Found that women who scored high in the aMED score had a lower risk of ER – breast cancer (RR 0.79; 95 % CI = 0.60–1.03, *P* for trend = 0.03)
Buckland 2012	Cohort	Breast cancer	9,009 post-menopausal, 1,216 pre-menopausal cases	EPIC (335,062 women, 10 European countries)	Adapted relative Mediterranean Diet Score, high intake of fruits, vegetables, legumes, fish, olive oil, cereals; low intake of meat, dairy; each item worth 0-2 points, score range 0-16	Decreased risk for postmenopausal cancer (RR comparing 10+ points vs <6 points = 0.93, 95% CI = 0.87-0.99, *P* trend = 0.037), and stronger for ER-/PR-tumors (RR for same comparison = 0.80, 95% CI = 0.65-0.99, *P* trend = 0.04)

Couto 2013	Cohort	Breast cancer	1,278 cases	Sweden, the Swedish Women's Lifestyle and Health cohort (49,258 women, age 30-49 at baseline)	Mediterranean diet score, 9 items: high intake of fish, vegetables, fruits, legumes cereals, unsaturated to saturated fat ratio, low intake of dairy, meat; alcohol. Score range 0-9	Adherence to the score showed no association with overall (RR for 8-9 points vs <3 points = 1.42, 95% CI = 0.99-2.05), pre- or post-menopausal, ER- or ER+ breast cancer
Bessaoud 2012	Case-control	Breast cancer	437 cases	France, mean age 58	Mediterranean pattern derived from principal component analysis	High pattern score showed no association with overall breast cancer (RR for 1 standard error increment of score = 10.8, 95% CI = 0.93-1.25)
Fung (2010)	Prospective cohort	Colorectal cancer	1,432 cases of incident colorectal CA in women; 1,032 cases in men	87, 256 women (age 30–55 years) and 45, 490 men (age 40–75 years)	Alternate Mediterranean diet (aMED) score	[a]Higher aMED score was associated with reduced risk of colorectal cancer in both men and women, but not statistically significant (RR = 0.89; 95 % CI 0.77–1.01; *P* for trend = 0.06)
Reedy (2008)	Prospective cohort (NIH-AARP members)	Colorectal cancer	3,110 incident cases	Men and women in the United States (n = 492, 382)	Mediterranean diet score (assessment of nine dietary components – one point for intakes higher than sex-specific median for whole grains, vegetables, fruit, fish, legumes, and nuts; one point for intake less than median for meat/ meat products and MUFA:SFA)	[a]Decreased risk of colorectal cancer for men comparing highest to lowest scores (Med. diet score: RR 0.72 95 % CI 0.62–0.83)

(continued)

Table 8.4 (continued)

Authors last name (year)	Study type	Outcome	# of cases	Population	Scoring criteria characteristics	Major results
Dixon (2007)	Case-control	Colorectal adenoma	3,592 cases with adenomas	US men and women ages 55–74 years old ($n = 37, 563$)	Mediterranean diet score (maximal score of nine – pattern based on Trichopoulou et al.[a])	[a]Men who had intakes most similar to the Mediterranean diet pattern were at a reduced risk for colorectal adenoma's (OR = 0.74, 95 % CI 0.64–0.85; *P*-trend <0.001)
Cottet (2005)	Cohort	Colorectal adenoma	65 men and 27 women with colorectal adenomas	277 men and 165 women	Three dietary patterns ('Mediterranean', 'sweets and snacks,' and 'high fat and protein' patterns) "Mediterranean" dietary pattern (characterized by high consumption of olive oil, vegetables, fruit, fish and lean meats)	[a]Men: no dietary pattern significantly associated with overall reoccurrence of colorectal adenomas [a]Women: 'Mediterranean' dietary pattern significantly reduced adenoma reoccurrence (2nd tertile: adjusted OR = 0.50, 95 % CI = 0.18–1.42; 3rd tertile: adjusted OR = 0.30, 95 % CI = 0.09–0.98; *P* for linear trend = 0.04)
Kontou 2012	Case-control	Colorectal cancer	250 cases	Greece, 259 men, 241 women	MedDietScore, 11 items: high intakes of non-refined cereals, fruits, vegetables, legumes, fish, poultry, potatoes, olive oil; low intakes of red meat, full fat dairy, alcohol. Each item worth 5 points, score range 0-55	Decreased odds for colorectal cancer (RR for every 1 point increase in score = 0.87, 95% CI = 0.82-0.92)

Agnoli 2013	Cohort	Colorectal cancer	435 total CRC, 326 colon	Italian (part of the EPIC cohort), 14,195 men, 31,080 women	Italian Mediterranean Index, 11 items: 1 point for higher (3rd tertile) intake of pasta, typical Mediterranean vegetables, fruits, legumes, olive oil, fish; 1 point for low (1st tertile) intake of soft drinks, butter, red meat, potatoes; alcohol (1 point for up to 12 g/d, 0 for >12 g or abstainers). Score range 0–11	Decreased risk for total colorectal cancer (RR comparing 6-11 points with 0-1 points = 0.50, 95% CI = 0.35-0.71, *P* trend = 0.04)
Samoli (2010)	Hospital-based case-control	Upper aero-digestive tract (UADT) cancer	239 incident UADT cases	Athens, Greece ($n = 433$)	Med. diet score ranges from 0 (minimal adherence) and 1 (maximal adherence) and increases with high intake of plant foods and olive oil and low intake of meat, dairy products and saturated lipids	[a]Significant decrease in UADT cancer risk for stricter adherence to Med. diet (30 % for a two-unit increase in score) [a]Higher consumption of vegetables (OR = 0.62, 95 % CI 0.39–1.00) and legumes (OR = 0.61, 95 % CI 0.39–0.95) was significantly associated with reduced UADT cancer risk, but after adjusted among all MDS components, no association remained significant, concluding that not one dietary component was associated with this risk

(continued)

Table 8.4 (continued)

Authors last name (year)	Study type	Outcome	# of cases	Population	Scoring criteria characteristics	Major results
Bosetti (2003)	Three case-control studies	Upper aero-digestive tract cancers (oral and pharyngeal, esophageal and laryngeal)	598 cases with oral/pharyngeal; 304 cases with esophageal; 460 cases with laryngeal	Men and women from Italian provinces (n = 4,684)	Defined a priori on the basis of eight characteristics of the traditional Mediterranean diet as suggested by Trichopoulou et al.[a]	[a]All cancers: a reduced risk was found for increasing levels of the Mediterranean diet score of 6 or more Med-diet characteristics compared to less than three characteristics (OR = 0.40; 95 % CI, 0.26–0.62 for oral and pharyngeal, OR = 0.26; 95 % CI 0.13–0.51 for esophageal, OR = 0.23; 95 % CI 0.13–0.40 for laryngeal cancer)
Buckland (2010)	Cohort	Gastric adeno-carcinoma (GC)	449 incidences of GC	485,044 total (144,577 men) age 35–70 years from 10 European countries	Relative Mediterranean diet score (rMED) 18-unit rMED score incorporating nine key components of the Mediterranean diet	[a]High compared to low rMED adherence was associated with a significant reduction in GC risk (HR = 0.67; 95 % CI: 0.47–0.94) [a]One-point increase in rMED score associated with decreased GC of 5 % (95 % CI: 0.91–0.99)

Dalvi (2007)	Case-control	Endometrial cancer	500 cases	White, African American and Latina women age 35–79 in San Francisco Bay Area, US ($n = 970$)	Med diet score (based on Trichopoulou et al. criteria[a])	[a]Adherence to the Mediterranean diet (extent to which is consumed by this population) was not associated with endometrial cancer risk (OR for modified scoring method = 0.92, 95 % CI: 0.59–1.4 for the highest *vs* lowest quintile; OR = 0.99, 95 % CI: 0.97–1.0 for continuous score)

[a]Trichopoulou Mediterranean diet score: Conformity to the traditional Mediterranean diet was assessed through a score ranging from 0 to 9. Score relies on 9 dietary components. Frequent consumption of traditional components (vegetables, legumes, fruits and nuts, cereals, fish and seafood, high ratio of MUFA:SFA) received a value of 1, but consumption below the median were assigned a score of 0; for components less frequently consumed (dairy and meat and meat products) consumption below the median was assigned a 1 and otherwise 0. Moderate alcohol consumption also received a score of 1 and 0 otherwise. Total score ranged from 0 (minimal adherence to the traditional Mediterranean diet) and 9 (maximal adherence)

For colorectal cancer, data is quite consistent to suggest that adherence to the Mediterranean diet is associated with a reduced risk. More studies are needed to confirm and refine the relationship. Future research should explore the differences related to tumor site (e.g. colon *vs* rectal) which may have different etiologies.

8.3.4 Mediterranean Diet and Cancer of the Upper Gastrointestinal Tract

Two European case-control studies have examined the association between the Mediterranean diet and cancer of the oral cavity, larynx/pharynx, and esophagus (Bosetti et al. 2003; Samoli et al. 2010). Both studies used the Trichopolou score and found that a high score was strongly associated with reduced odds of cancer of these sites. In the study of middle to elderly Italians, each one point increase in the Mediterranean diet score was associated with 23 % lower odds for oropharyngeal cancer, 28 % lower odds in esophageal cancer, and 29 % lower odds for laryngeal cancer (Bosetti et al. 2003). In a Greek study that was comprised of mostly men, a two unit increase of the Mediterranean diet score was associated with 30 % lower odds for upper aerodigestive tract cancer (Samoli et al. 2010). Case-control studies are retrospective in nature and dietary information is collected after the diagnosis is made; therefore, food intake information may not best represent usual diet prior to diagnosis. These results need to be confirmed by more robust studies such as prospective cohort studies.

Data from the EPIC study was also used to study gastric cancer (Buckland et al. 2010). The Trichopolou score was modified into an 18-point Relative Mediterranean Diet Score. After almost 9 years of follow-up, every one point increase in the score was associated with a significant 7 % reduction of gastric adenocarcinoma risk. Individuals classified in the top third of the score were 33 % ($p < 0.05$) less likely to develop gastric cancer compared with those classified in the bottom third.

Evidence for upper gastrointestinal cancers is quite consistent and suggests that the Mediterranean diet may be associated with a reduction of risk. However, due to the small number of studies, more data, especially from non-European countries, is needed.

8.3.5 Interpretation and Conclusions

Overall, the existing literature points to a possible association between the Mediterranean diet and a lower cancer risk. Because tumors at different sites have different etiologies, it is likely that the Mediterranean diet has a stronger inverse association with some sites and no association with others. Although there is a substantial body of literature examining the relationship between diet and

development of cancer overall, the number of studies for each cancer site is much fewer which diminishes power to detect individual relationships. Almost all of the studies published to date were done in Caucasian populations, and therefore generalizability to other populations may be limited. One difficulty in studying the Mediterranean diet in other populations is that the common diet in many regions is far different from the traditional Mediterranean diet, making a true study only possible within a randomized trial design. Even if conducted, trial duration would limit it to studies of cancer biomarkers and not cancer incidence.

Another limitation of research into the effect of a Mediterranean diet on cancer development has to do with limitations of available tools for assessment of adherence to the Mediterranean diet. While investigators who have developed different versions of the Mediterranean diet score have take care to include the appropriate food groups and nutrients, the expected biological effect can be very different depending on several factors. Different geographical regions differ greatly on the foods within each food group. For example, types of fruits and vegetables consumed vary greatly from country to country. For fruits and vegetables in particular, nutrient composition also varies depending on where they are grown, and is likely further modified by cooking and preservation methods.

Other limitations are based on the assignment of points within the diet score. Because points for Mediterranean diet adherence scores are awarded for consumption above the study sample median for each food or nutrient group, each study set its own cutoff based on its own population. Therefore, the level of intake that would be sufficient to award one point differs across countries. Even a low Mediterranean diet score in Greece could be at a level of intake that would have resulted in a high score in another country. The EPIC study, a multi-country study that provided a lot of data for this review, scored each country separately with country-specific cutoffs. This is the appropriate method so that high *vs* low intake of relevant food groups within each country can be distinguished. If all countries were to use the same cutoffs based on the entire sample, then comparing high score with low score would essentially be comparing Greece with other countries.

General methodological issues also need to be considered when interpreting results. All of the data were obtained from observational studies. Diet is measured with some degree of error, and most studies only measured diet at baseline and, therefore, cannot account for changes during follow-up. Confounding cannot be completely eliminated even with statistical control of potential confounders. Many studies had long follow-up durations, which allowed for a better chance to capture the time period during which diet may have had an effect on cancer development, but the latency period between cancer initiation and diagnosis may be longer than is accounted for even with relatively long follow-up periods.

Nevertheless, existing data does point to a potential for the Mediterranean diet to be able to reduce cancer risk. Future studies should focus on specific cancer sites and since subtypes exist with each cancer site, it is important to obtain sufficient endpoints to separately study each subtype.

8.4 Potential Mechanisms of the Mediterranean Diet for Lower Cancer Risk

The biological mechanisms that modulate the relationship between the Mediterranean diet and cancer development have yet to be determined, although several possibilities have been explored. Suggested mechanisms through which the Mediterranean diet may impact cancer initiation and proliferation include anti-inflammatory and antioxidant effects, high fiber content (particularly in colorectal cancer), increased insulin sensitivity and reduction of excess insulin production, and an overall association with reduced risk of excess weight gain and obesity.

8.4.1 Antioxidant Effects and Anti-inflammatory Effects of the Mediterranean Diet

Several components of the Mediterranean dietary pattern have been associated with both anti-inflammatory and antioxidant activity. Foods associated with lower inflammation include fish and wild game (high in omega-3 fatty acids), legumes, whole grains, fruits, vegetables, and olive oil (Galland 2010). Inflammation may promote conditions favorable to cell damage that may lead to cancer initiation. The Mediterranean diet is also thought to potentially reduce the likelihood of cancer initiation and proliferation through high antioxidant activity. Several individual components within the dietary pattern have been associated with antioxidant activity and the diet as a whole is associated with high antioxidant activity. In addition to plentiful amounts of whole grains, legumes, fruit and vegetables, extra virgin olive oil and red wine are thought to contribute to the total antioxidant load of the diet.

8.4.1.1 Olive Oil

A recent large meta-analysis found that regular consumption of extra virgin olive oil is strongly associated with lower risk of cancer development (Psaltopoulou et al. 2011). The meta-analysis included 19 case-control studies providing a sample of 13,800 cancer patients and 23,340 controls. Classification in the highest category of olive oil consumption was associated with a lower odds of having any type of cancer compared to those in the lowest category (OR $= -0.41$). When specific cancers were considered, the highest intake of olive oil was associated with lower odds of developing breast cancer (OR $= -0.45$), and cancer of the digestive system (OR $= -0.36$) compared to the lowest. The investigators concluded that the association was strong, but that it was unclear whether olive oil's monounsaturated fatty acid content or its antioxidant components are responsible for its beneficial effects.

A good deal of research supports the assertion that extra virgin olive oil acts as an antioxidant, leading to the hypothesis that olive oil may protect against mutations associated with cancer development and proliferation. The antioxidant activity

appears to come from components found in olive oil that may not be present in other good sources of monounsaturated fat such as nuts (López-Miranda et al. 2010).

One clinical intervention trial that investigated the antioxidant effects of olive oil in human subjects examined the impact of three different dietary interventions on total plasma antioxidant capacity (Razquin et al. 2009). Researchers found that after 3 years on the experimental diets, subjects randomized to a Mediterranean style diet high in extra virgin olive oil had significantly higher levels of plasma antioxidant concentrations, compared with a group on a similar Mediterranean style diet that was high in nuts instead of olive oil, and a control group following a standard low fat diet.

Phenolic compounds have been hypothesized to be the primary antioxidant source in olive oil (Fito et al. 2007). The ability of phenolic compounds to affect cancer development has been observed on a cellular level. One tissue-based study examined the impact of two particular phenolic compounds found in olive oil on breast tissue with and without tumors (Warleta et al. 2011). Researchers found that while the phenolic compounds did not stop cancer cell proliferation, it appeared to impede the original mutation process.

While tissue-based research has been positive, it has been questioned whether phenolic compounds have sufficient antioxidant activity to affect cancer development *in vivo*. A human study investigated whether the phenolic content of olive oil impacted biomarkers of oxidative DNA stress. Three groups of subjects were given olive oil with differing amounts of phenolic compounds. The researchers found an overall effect of olive oil supplementation in the reduction of biomarkers of oxidative stress, but did not find that the phenolic content of the olive oil was significantly related to the reduction of stress (Machowetz et al. 2007). This would suggest that it is not the antioxidant activity of the phenols, but either some other component acting as an antioxidant or the reduction is of stress is actually due to another effect of the olive oil. It has been suggested that the anti-inflammatory effect of both olive oil and the diet as a whole may actually impact cancer development more distinctly than the antioxidant activity of the diet.

8.4.1.2 Red Wine

Moderate consumption of alcohol has been strongly linked to decreased incidence cardiovascular diseases, but impact on risk for cancer development has been inconsistent (Nova et al. 2012). It has been suggested that red wine may impact cancer development differently than other types of alcohol (Rizos et al. 2010). Several studies within Mediterranean countries have linked wine consumption to lower incidence of cancer, leading some to hypothesize that there is a unique component within red wine that may reduce cancer risk. One suggestion is that the antioxidant activity of grape-derived phytochemicals may protect against cancer initiation and promulgation (Pauwels 2011).

The most researched phytochemical within wine is resveratrol. Animal and human tissue studies have found this compound to be chemoprotective based on three mechanisms of action: it promotes apoptosis, works as an antiproliferation agent,

and has anti-inflammatory effects (Aluyen et al. 2012). Despite good results with animal and tissue-based models, wine consumption has not consistently been related to decreased risk of cancer development in human studies. Some have suggested that this may be due to limited bioavailability of the resveratrol (Guerrero et al. 2009).

One possibility for why red wine may be more strongly associated with decreased risk of cancer in Mediterranean populations is that the chemoprotective compounds found in wine may act synergistically with other compounds commonly found in the Mediterranean diet. While this hypothesis is still in its very early stages, the concept that nutrients and phytochemicals found in food may have synergistic or antagonistic effects on other nutrients and phytochemicals consumed, is one of the most important rationales for the use of dietary pattern analysis rather than a focus on a single food or nutrient.

8.4.2 The Mediterranean Diet and Insulin Sensitivity and Cancer

Insulin resistance and diabetes have been associated with increased risk of cancer development (Boyd 2003). It has been hypothesized that chronic hyperinsulinemia may cause increased concentrations of insulin-like growth factor which may contribute to cancer cell proliferation (Renehan et al. 2006). Several studies have examined the association between consumption of a Mediterranean diet and markers of insulin sensitivity to determine if the decreased risk of cancer incidence associated with consumption of a Mediterranean dietary pattern may be due to a direct effect of the diet on insulin sensitivity.

While randomized controlled trials have found little to no evidence that following a Mediterranean diet as a short-term clinical intervention is associated with improved insulin sensitivity (Bos et al. 2010; Díez-Espino et al. 2011), analyses of prospective cohort studies have observed greater apparent insulin sensitivity in those who habitually follow a diet more consistent with the Mediterranean dietary pattern. One study examined factors associated with the Metabolic Syndrome in the Framingham Offspring and Spouse Study and found that individuals with a higher score on the Mediterranean dietary pattern had statistically significantly lower homeostasis model assessment-insulin resistance (HOMA-IR), although clinical significance was minimal (3.16 *vs* 3.38) (Rumawas et al. 2009). They also found that individuals with a higher Mediterranean dietary pattern score had significantly lower waist circumferences even after adjustment for both BMI and change in BMI from baseline. This may indicate that the better insulin sensitivity was due to the lower waist circumference rather than that the diet independently influencing insulin sensitivity. It should be noted that no group in this study had high adherence with a Mediterranean dietary pattern, the mean score for the highest quartile was 31.9 out of 100.

Another prospective cohort study based in Greece also found an association between greater adherences to a Mediterranean dietary pattern and significantly lower mean HOMA-IR in individuals scoring in the highest diet score tertile compared with the lowest (Tzima et al. 2007). This study specifically examined

the relationship in participants classified as overweight or obese. When the entire cohort was included in the analysis, participants with the greatest adherence to the traditional diet were less likely to be obese and more likely to be classified as normal weight. While the results show a modest independent relationship between insulin sensitivity and adherence to a Mediterranean dietary pattern, a larger impact appeared to come from the relationship of excess body fat on insulin sensitivity.

8.4.3 Mediterranean Diet and Body Weight

Obesity has been demonstrated to have a direct relationship with increased risk for several types of cancer including colon, postmenopausal breast, pancreatic, kidney, gallbladder, endometrial, liver, uterine, prostate, kidney, and esophageal (Fontham et al. 2009; Simard et al. 2012). Conversely, the Mediterranean diet is correlated in several large studies with lower risk of becoming overweight or obese, suggesting that the strongest mechanism modulating the relationship between the Mediterranean diet and cancer development may be reduced risk of excess weight gain (Esposito et al. 2011).

Obesity has been associated with several mechanisms that may increase susceptibility to cancer initiation and promotion. Adipose cells are known to act as an endocrine organ, excreting hormones that cause low grade systemic inflammation and inducing insulin resistance (Galic et al. 2010). Excess adipose also appears to increase the amount of circulating sex hormones such as estrogen in the body. Both increased circulation of estrogen and insulin resistance have been linked to an increased cancer risk. Chronic inflammation has also been proposed as a mechanism that promotes cancer development (Kanterman et al. 2012).

Several non-nutrient related features of the Mediterranean diet may protect against development of obesity including the emphasis on smaller portions and regular physical activity. Nutrient-related components that have been associated with lower risk of excess weight gain including regular consumption of moderate levels of alcohol, oleic fatty acids in olive oil, the generally high fiber content of the diet, and emphasis on nutrient dense foods.

As a total dietary and lifestyle pattern, the Mediterranean diet has been associated with lower risk of weight gain in prospective cohort investigations. One prospective cohort study of 10,376 men and women in Spain found that over a 5-year period the group most likely to have either gained more than 5 kg or to have moved from a lower weight category into either the overweight or obese range were those who scored the lowest on the Mediterranean dietary score (Beunza et al. 2010). This group was found to have the highest average yearly weight gain, whereas participants with the highest MDS scores had the lowest weight gain.

Another analysis of the relationship between adherence to a Mediterranean dietary pattern and lower risk of weight gain over time, examined weight changes in 373,803 men and women enrolled in the EPIC-PANACEA study (the European Prospective Investigation into Cancer and Nutrition-Physical Activity, Nutrition,

Alcohol Consumption, Cessation of Smoking, Eating out of Home, and Obesity project). After 5 years of follow up, those with greater adherence to the Mediterranean Diet Score had a mean weight change of −0.16 kg and were 10 % less likely to develop overweight or obesity compared to individuals with a low adherence score. When the investigators looked at whether any individual components appeared to explain the difference they found that the low meat content of the Mediterranean diet seemed to account for most of its positive effect against weight gain (Romaguera et al. 2010).

8.4.3.1 Moderate Alcohol Consumption and Weight Regulation

Several large studies have documented a strong and consistent relationship between low to moderate alcohol consumption and lower risk of excess body weight. It has been hypothesized that ethanol acts as a hormone disruptor promoting better insulin sensitivity that may lead to lower risk of obesity among those who drink moderately (Pravdova and Fickova 2006).

One recent analysis of an American prospective cohort examined the relationship between moderate alcohol consumption in men and women and risk of becoming overweight or obese over approximately 13 years of follow up. The trends in both men and women for intake of up to 30 g of alcohol per day were consistently and significantly associated with lower risk of moving from a normal weight category to either the overweight or obese category (Wang et al. 2010).

A systematic review that examined the relationship between amount and type of alcohol consumed with risk of weight gain, found that those who drank moderately were less likely to become overweight or obese, while those who drank more heavily were more likely to become overweight compared to non-drinkers. They further found an apparent association between type of alcohol and risk of overweight/obesity, with those who drank spirits more likely to become overweight and those who drank wine less likely (Sayon-Orea et al. 2011). As the Mediterranean diet is associated with moderate wine consumption, it is possible that the protective effect of the diet may be due to reduced risk of obesity partially based on moderate wine consumption.

Whatever the cause, it appears that the Mediterranean dietary and lifestyle pattern is favorably associated with lower risk of weight gain over time and through this protective effect against excess weight gain, may exert the most protective impact on cancer development.

8.5 Conclusion

The Mediterranean dietary and lifestyle pattern appears to be protective against overall cancer incidence and mortality and is positively associated with lower risk of several types of cancer including colorectal cancers and cancers of the upper digestive system. Research to date suggests that the effect of the diet is small to

moderate depending on the cancer site and that the strongest effect is for those who habitually consume the diet. The mechanism most strongly related to its chemoprotective influence appears to be its relationship with lower likelihood to gain excess body weight. The diet's synergistic antioxidant and anti-inflammatory impact also likely contributes to lower cancer risk associated with higher adherence to a traditional Mediterranean diet.

8.5.1 Future Directions

Although a wide variety of cancers have been studied, at best there were only a handful of studies for each cancer site and further studies in each cancer site is necessary. Furthermore, the Mediterranean diet may influence subtypes within the same cancer site differently, such as pre- *vs* post-menopausal breast cancer. Larger studies that have sufficient cases for meaningful statistical analysis would be helpful in this area. From a clinical perspective, a scoring algorithm for adherence to the Mediterranean diet that is not dependent on specific population intake level and simple enough to be self-administered would be helpful in assisting individuals interested to follow this diet.

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Chapter 9
Modulation of Proteasome Pathways by Nutraceuticals

Sahdeo Prasad, Subash C. Gupta, Bokyung Sung, and Bharat B. Aggarwal

Abstract The proteasome is a multicatalytic proteinase complex, the inhibition of which has been associated with induction of apoptosis, anti-tumorigenesis, and chemosensitization of tumor cells to the conventional chemotherapeutics agents and radiation. Therefore, inhibition of the proteasome pathway could be a novel approach for the prevention and treatment of cancer. Proteasome inhibitors mediate the antitumor effect through modulation of transcription factors, cell cycle regulatory proteins, and pro- and anti-apoptotic proteins. Although numerous proteasome inhibitors have been rationally designed, most of them not only are enormously expensive but also produce serious side effects. Currently, numerous nutraceuticals such as curcumin, sesamin, quercetin, silybinin, sulforaphane, resveratrol, tubocapsenolide A, CDDO-Me, γ-tocotrienol, apigenin, ferulic acid, betulinic acid, anacardic acid, genistein, withaferin A, emodin, withanolide, and gambogic acid derived from fruits, vegetables, spices, nuts, and legumes have shown promise as proteasome inhibitors, which may contribute to their anticancer activities. Although the mechanism of proteasome inhibition by nutraceuticals is different, it plays a crucial role against cancer. In this chapter, we discuss the targets of these nutraceuticals in the proteasome pathway. How inhibition of the proteasome pathway by these natural agents contributes to their anticancer activities is also discussed.

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9.1 Introduction

Protein metabolism involves both synthesis and degradation of proteins (Goldberg and Dice 1974). Synthesis of proteins occurs through central dogma, which include transcription (making of an RNA molecule off of a DNA template) and translation (construction of polypeptide from an RNA molecule). Degradation or elimination of proteins is mediated by two major intracellular devices, lysosomes and proteasomes. Lysosomes deal primarily with extracellular proteins, whereas proteasomes deal with endogenous proteins. The proteasome degradation pathway is essential for many cellular processes, including cellular differentiation (in which transcription factors and metabolic enzymes are degraded), cell cycling (in which cyclins are degraded to prepare for the next step in the cell cycle), regulation of gene expression, responses to oxidative stress, antigen processing for appropriate immune responses, inflammatory responses, and apoptosis (Mitch and Goldberg 1996; Cuervo and Dice 1998; Hershko and Ciechanover 1998; Ciechanover 2005). It also degrades proteins encoded by viruses and other intracellular pathogens and proteins that are folded incorrectly because of translation errors or encoded by faulty genes (Homma et al. 1994). Proteasomes play an important role in the immune system by generating antigenic peptides that are presented by the major histocompatibility complex (MHC) class I molecules (Groettrup et al. 2010).

9.2 Proteasomes

Proteasomes are subcellular organelles found throughout the cytosol, nucleus, endoplasmic reticulum (ER), and lysosomes of eukaryotic cells (Adams 2002). Structurally, proteasomes are cylindrical and composed of four rings stacked on top of each other. Each ring is composed of seven subunits. The two outer rings contain α subunits and do not have enzymatic activity, while the two inner rings comprising β subunits have the proteolytic activities. There are three major proteolytic activities in the β subunits: a chymotrypsin-like (CTL), a trypsin-like (TL) and a caspase-like activity. The most common form of the proteasome, 26S proteasome, contains one 20S core particle structure and two 19S regulatory caps. The core is hollow and provides an enclosed cavity in which proteins are degraded. The 19S components regulate the entry of proteins into the 20S proteasome (Coux et al. 1996). Openings at the two ends of the core allow the target protein to enter. Each end of the core particle associates with a 19S regulatory subunit that contains multiple ATPase active sites and ubiquitin binding sites. This subunit recognizes polyubiquitinated proteins and transfers them to the catalytic core (Wang and Maldonado 2006).

More than 80 % of a cell's unassembled, damaged, or misfolded proteins are processed by proteasomes (Coux et al. 1996). A cascade of enzymes are involved in this process, including ubiquitin-activating E1 enzymes, ubiquitin-carrier protein

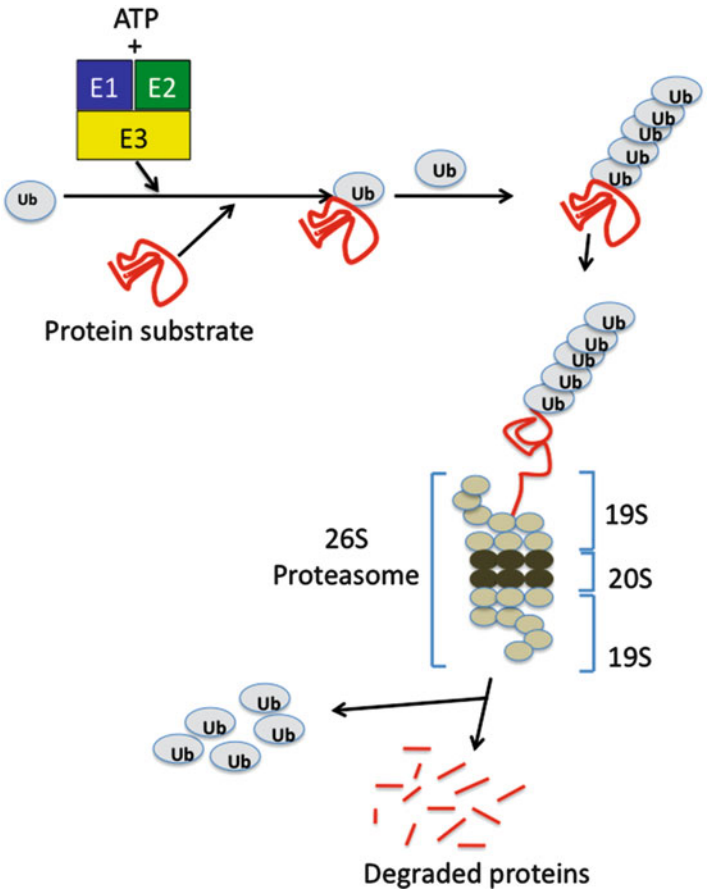

Fig. 9.1 The ubiquitin-proteasome pathway

E2 enzymes, and the ubiquitin-protein E3 ligases, which conjugate the ubiquitin residues to the target protein substrate for degradation. Proteasomes act on target proteins by attaching to another protein called ubiquitin. Degradation signals are generally hidden in a properly folded protein, but become accessible when the protein is misfolded or denatured. When these signals are exposed, enzymes add another small ubiquitin protein to the target called polyubiquitin-tagged proteins, which are then recognized by a receptor protein on the proteasome and the target protein is taken into the proteasome and digested by threonine proteases. The fragmented protein is then released from the proteasome (Rape and Jentsch 2002) (Fig. 9.1). These fragments are processed by intracellular peptidases to yield amino acids, which are then recycled into new proteins (Bochtler et al. 1999). Although the proteasome normally produces very short peptide fragments, in some cases these products are themselves biologically active and functional proteins, such as nuclear factor-κB (NF-κB) (Rape and Jentsch 2004).

9.3 Proteasome, Proteasome Inhibitors, and Cancer

Proteasomes control the half-life of several cell-signaling regulatory proteins. It is responsible for the degradation of all short-lived proteins and 70–90 % of all long-lived proteins, thereby regulating processes such as cell cycle progression and cell cycle arrest, transcription, DNA repair, angiogenesis, apoptosis, survival, growth and development, inflammation, and immunity. Since imbalances in proteasome-mediated protein degradation contribute to various human cancers, the proteasome might be a novel target for anticancer therapy (Pajonk and McBride 2001; Naujokat and Hoffmann 2002). However, inhibition of proteasome function leads to the accumulation of polyubiquitin-tagged proteins and the withdrawal of the cell from the cell cycle, followed by the induction of apoptosis in susceptible cells (Sterz et al. 2008; Driscoll and Dechowdhury 2010).

Several pharmacological inhibitors of the proteasome have been developed, including synthetic peptidyl aldehydes MG132, PSI, ALLN, ALLnV, ALLnM, CEP1612, and Z-LLF; bacterial compound lactacystin; dipeptidyl boronic acid PS-341 (also known as bortezomib); and several natural products (Lee and Goldberg 1998). Proteasome inhibitors have shown to induce apoptosis in some cells and seem to prevent it in others. In proliferating cells, proteasome inhibitors appear to induce apoptosis but they appear to protect quiescent or terminally differentiated cells from apoptosis (Drexler 1997). Lactacystin induced apoptosis of U937 human leukemia and Jurkat cells (Imajoh-Ohmi et al. 1995; Naujokat et al. 2003), MG132 showed cell death in MOLT-4 human leukemia (Shinohara et al. 1996), and ALLN induced apoptosis of human prostate carcinoma (Herrmann et al. 1998). Bortezomib has been shown to have anti-tumor activity in myeloma and lymphoma (Tobinai 2007). In addition, numerous other proteasome inhibitors act as anticancer agents.

How proteasome inhibitors induce apoptosis of cancer cells is not fully understood. Various reports have suggested that these inhibitors act through increasing p53 activity, fostering the accumulation of p27 and p21 molecules, enhancing pro-apoptotic proteins, activating stress-activated protein kinases (Meriin et al. 1998), inducing caspases (Almond et al. 2001), and decreasing NF-κB activity (Russo et al. 2001). In addition, proteasome inhibitors increase unfolded proteins, resulting in activation of ER stress-induced signaling pathways and induction of apoptosis (Bratton and Cohen 2001). Although proteasome inhibitors have been shown to induce apoptosis in cancer cells, only limited reports have suggested the selectivity of proteasome inhibitors to tumor cells. The susceptibility of tumor cells to proteasome inhibitors may be because of their high proliferation rate. Another report suggested that the tumor-specific killing by proteasome inhibitors might be due to the expression of oncogenes such as c-myc that deregulate cell proliferation and also induce apoptosis (Soengas et al. 1999). Alternatively, in some cancer cells, there may be either deficiencies in, or excessive proteasome degradation of, proteins that inhibit cell growth or induce apoptosis, such as p27KIP1, p53, and B-cell lymphoma-2 (Bcl-2)-associated X protein (Bax) (Lloyd et al. 1999; Li and Dou 2000).

Recently, natural compounds have been shown to act as proteasome inhibitors, which may lead to killing and increased sensitivity of cancer cells to conventional agents and thus inhibition of tumor growth. To date, over 60 natural products that inhibit the ubiquitin-proteasome pathway have been identified. Several of these natural products have been developed into anticancer drug candidates, and one proteasome inhibitor has already been approved for the treatment of multiple myeloma (Schneekloth and Crews 2011).

9.4 Natural Compounds as a Regulator of Proteasome Degradation Pathway

Numerous natural compounds derived from spices, fruits and vegetables, legumes, nuts, and microbial metabolites have been shown to be potent proteasome inhibitors (Fig. 9.2). These proteasome inhibitors either downregulate the expression of cell-proliferative, survival, invasion, metastatic and oncogenic proteins (Table 9.1) or upregulate apoptotic and tumor-suppressor proteins (Table 9.2). Thus, these natural proteasome inhibitors potentially can be used not only as chemopreventive and chemotherapeutic agents, but also as tumor sensitizers to conventional radiotherapy and chemotherapy. In this section, we summarize the proteasome inhibitory activity of several natural compounds and will discuss the potential use of these compounds for the prevention and treatment of human cancers.

9.4.1 Spices

In addition to spices' long history of uses as traditional medicine, experimental and clinical studies have proven their efficacy against several human diseases through modulating several cell signaling pathways, including inhibition of the ubiquitin proteasome pathway. Among spices, curcumin (an active component of turmeric) is one of the most often described and best-known natural proteasome inhibitors, with cell growth arrest and cell death inducing as observed in several tumor cell lines and animal models. It mediates degradation as well as restoration of several proteins in different *in vitro* and *in vivo* models (Table 9.1). A study using three breast cancer cell lines (MDA-MB-231, MDA-MB-436, and Hs578T) showed that curcumin mediates its cell cycle inhibitory activities by blocking the CTL activity of the proteasome *in vitro*. It is suggested that curcumin might mediate G1 arrest and possible cytostasis and apoptosis by blocking the proteasome activity and upregulating the p21 protein in breast cancer cells (Efuet and Keyomarsi 2006). Proteasome-mediated downregulation of cyclin E and upregulation of cyclin-dependent kinase (CDK) inhibitors may also contribute to the antiproliferative effects of curcumin by arresting the cells at G1 phase of cell cycle as observed in

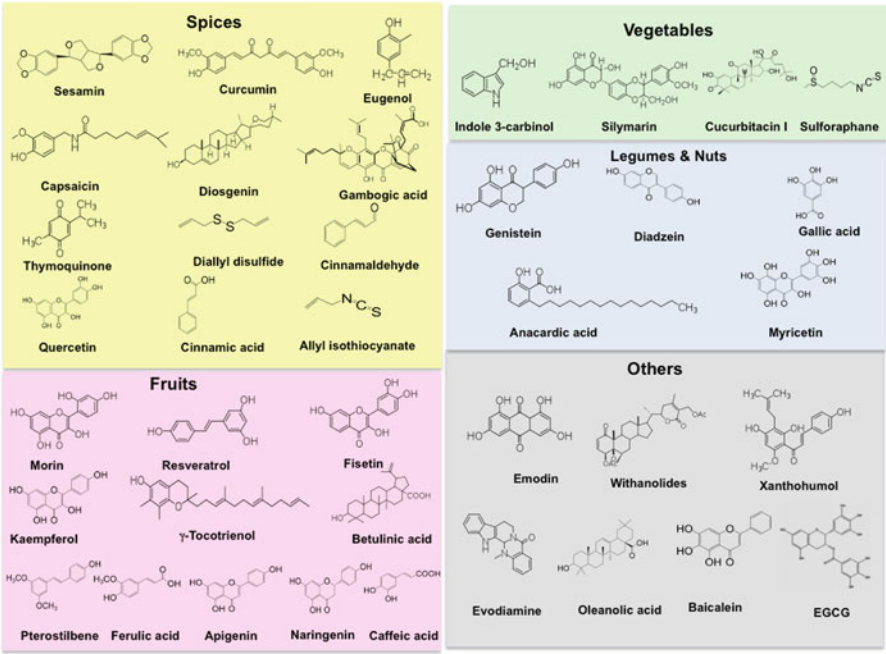

Fig. 9.2 Structure of natural compounds involved in regulation of the ubiquitin proteasome degradation pathway

various tumors (Aggarwal et al. 2007). Curcumin-responsive cells were also found to accumulate poly-ubiquitinated proteins and cyclin B, which results in disturbance of the ubiquitin-proteasome system and cell cycle arrest (O'Sullivan-Coyne et al. 2009).

Further mechanistic study showed that curcumin behaves like proteasome. The nucleophilic susceptibility and in silico docking studies have revealed that both carbonyl carbons of the curcumin molecule are susceptible to a nucleophilic attack by the hydroxyl group of the NH2-terminal threonine of the proteasome CTL subunit. It was confirmed in a biological system that curcumin potently inhibits the CTL activity of purified rabbit 20S and cellular 26S proteasomes and inhibits proteasome activity in human colon cancer cell lines, leading to accumulation of ubiquitinated proteins and several proteasome target proteins, and subsequent induction of apoptosis. Furthermore, in a xenograft colorectal imprinting control region severe combined immunodeficiency (ICR SCID) mice model, curcumin resulted in decreased tumor growth, associated with proteasome inhibition, proliferation suppression, and apoptosis induction in tumor tissues, indicating the mechanisms by which curcumin plays its chemopreventive and therapeutic roles (Milacic et al. 2008).

Other studies have shown that the apoptosis induction by spices was associated with disruption of ubiquitin proteasome system function by directly inhibiting the

Table 9.1 Cell signaling targets downregulated by natural compounds through modulation of proteasome pathway

Targets	References
Spices	
Curcumin	
ARNT	Choi (2006)
Bcl-2	Chanvorachote (2009)
COP9 signalosome	Henke (1999)
COX-2	Neuss (2007)
Cyclin D1	Srivastava (2007)
Cyclin E	Srivastava (2007)
hTERT	Lee (2010)
Id1 and Id3	Berse (2004)
iNOS	Ben (2011)
p38	Poylin (2008)
p300	Marcu (2006)
Sp1	Hsin (2010)
Sesamin	
Cyclin D1	Yokota (2007)
Quercetin	
FLIP	Kim (2008)
HER2/neu	Jeong (2008)
Mcl-1	Spagnuolo (2011)
Ras	Psahoulia (2007)
Survivin	Siegelin (2009)
Fruits and vegetables	
Silybinin	
FLIP	Son (2007)
Survivin	Son (2007)
AR	Deep (2008)
Sulforaphane	
AR	Gibbs (2009)
ER	Ramirez (2009)
Bim-1, H3K27me3, Ezh2	Balasubramanian (2011)
Nrf2	McMahon (2003)
Resveratrol	
Aβ	Marambaud (2005)
Cyclin D1	Joe (2002)
ER	Pozo-Guisado (2004)
HIF-1α	Zhang (2005)
Id1 and Id3	Berse (2004)
Tubocapsenolide A	
Akt, Cdk4	Chen (2008)
CDDO-Me	
FLIP	Zou (2007)
γ-Tocotrienol	
Apolipoprotein B	Wang (1998)
Apigenin	
HER2/neu	Way (2004)

(continued)

Table 9.1 (continued)

Targets	References
Ferulic acid	
MMP-2/MMP-9	Staniforth (2012)
Betulinic acid	
Sp proteins	Chintharlapalli (2007)
Legumes and nuts	
Anacardic acid	
Histone acetylation	Song (2010)
CDDO	
c-FLIP	Zou (2007)
Genistein	
GR	Kinyamu (2003)
Top2β	Azarova (2010)
AR	Basak (2008)
HER2/neu	Magnifico (1998)
c-FLIP	Siegelin (2009)
Others	
Withaferin A	
AR	Yang (2007)
NF-κB	Mohan (2004)
EGCG	
Bad, p27, IκBα	Smith (2002), Nam (2001)
bFGF	Sukhthankar (2008)
VEGF	Konta (2010)
PcG proteins	Choudhury (2011)
P38 MAPK	Kazi (2002)
Emodin	
Id1 and Id3	Berse (2004)
AR	Cha (2005)
ERα	Zhang (2011)
Withanolide	
ER	Zhang (2011)
Gambogic acid	
Mutant p53	Wang (2011)
ABCB1	Wang (2012)
MDM2	Rong (2009)
P53	Wang (2012)

ABCB1 ATP-binding cassette transporter B1, *ABCB1* ATP-binding cassette transporter B1, *Aβ* amyloid-beta, *AR* androgen receptor, *ARNT* aryl hydrocarbon receptor nuclear translocator, *Bcl-2* B-cell lymphoma 2, *bFGF* basic fibroblast growth factor, *CDDO-Me* methyl-2-cyano-3 12-dioxooleana-1 9-dien-28-oate, *Cdk4* cyclin-dependent kinase 4, *COP9* signalosome photomorphogenic 9 signalosome, *COX-2* cyclooxygenase-2, *EGCG* epigallocatechin gallate, *ER* estrogen receptor, *Ezh2* enhancer of zeste homolog 2, *FLIP* Fas-associated death domain-like interleukin-1beta-converting enzyme (FLICE)-like inhibitory protein, *GR* glucocorticoid receptor, *H3K27me3* trimethylated lysine 27 of histone 3, *HIF-1α* hypoxia inducible factor-1alpha, *hTERT* human telomerase reverse transcriptase, *Id1* inhibitor of DNA binding 1, *iNOS* inducible NO synthase, *MAPK* mitogen-activated protein kinase, *Mcl-1* myeloid cell leukemia sequence 1, *MDM2* murine double minute 2, *MMP-2* matrix metalloproteinase-2, *Nrf2* nuclear factor-erythroid 2-related factor 2, *PcG* polycomb group, *Sp1* specificity protein 1, *Top2* topoisomerase II , *VEGF* vascular endothelial growth factor

Table 9.2 Cell signaling targets upregulated by natural compounds through modulation of proteasome pathway

Targets	Natural compounds	References
Spices		
Bax	Capsaicin, thymoquinone	Maity (2010), Cecarini (2010)
HDAC2	Curcumin	Meja (2008)
HIF-1α	Quercetin	Lee (2008)
HIF-2α	Quercetin	Park (2008)
IκBα	Curcumin, capsaicin	Dikshit (2006a, b), Mori (2006)
Nrf2	Quercetin	Tanigawa (2007)
NS2	Curcumin	Franck (2005)
P21	Curcumin	Aggarwal (2007)
P27	Capsaicin	Maity (2010)
P53	Curcumin, capsaicin, thymoquinone	Bech-Otschir (2001), Maity (2010), Cecarini (2010)
XBP1	Quercetin	Klappan (2012)
Fruits and vegetables		
IκBα	Cucurbitacin D, resveratrol, genistein, withanolide D	Ding (2011), Shakibaei (2008), Kazi (2003), Bargagna-Mohan (2006)
P27	Genistein	Kazi (2003)
Legumes and nuts		
p21(Kip1), p53, CHOP	CDDO, GA	Lapillonne (2003), Rong et al. (2009)
Others		
p27(Kip1), p53	Tocopherol	Munteanu (2007)
IκBα, Bax, p27	Withaferin A	Yang (2007)

CHOP CAAT/enhancer binding protein, *GA* gambogic acid, *HDAC2* human histone deacetylase 2, *HIF-1α* hypoxia inducible factor-1alpha, *HO-1* heme oxygenase-1, *Nrf2* nuclear factor-erythroid 2-related factor 2, *NS2* nonstructural 2, *XBP1* X-box binding protein 1

enzyme activity of the proteasome's 20S core catalytic component. Like other proteasome inhibitors, curcumin exposure induces neurite outgrowth and the stress response, as evidenced by the induction of various cytosolic and ER chaperones as well as induction of transcription factor CHOP/GADD153 (Dikshit et al. 2006a). Curcumin has also been shown to inhibit the ubiquitin isopeptidase activity and cause cell death independent of p53 in isogenic pairs of RKO and HCT116 cells (Mullally and Fitzpatrick 2002). Inhibition of isopeptidase activity by curcumin leads to the accumulation of ubiquitin-protein conjugates and polyubiquitin because of the lack of ubiquitin recycling. Excessive accumulation of ubiquitin-protein conjugates could conceivably create proteasome dysfunction. It has been reported that curcumin exhibits anticancer activity in cell lines and the hepatoma xenograft model through inhibition of hypoxia-inducible factor (HIF)-1 activity and downregulation of its targeted genes. Moreover, of the two HIF-1 subunits, only aryl hydrocarbon receptor nuclear translocator (ARNT) was found to be repressed

by curcumin in cancer cells. The repression of ARNT by curcumin was stimulated by the proteasome degradation *via* oxidation and ubiquitination processes (Choi et al. 2006). Curcumin also inhibited telomerase activity and accumulated human telomerase reverse transcriptase (hTERT) in the cytoplasmic compartment of the cell. The curcumin-induced cytoplasmic retention of hTERT protein resulted in rapid ubiquitination and degradation by the proteasome (Lee and Chung 2010). Specificity protein 1 (Sp1), one of the more important transcription factors in hTERT expression, is also inhibited through the proteasome pathway in A549 cells (Hsin et al. 2010).

An important regulator of the ubiquitin proteasome system is the COP9 signalosome (CSN), which controls the stability of many proteins. Curcumin significantly induces ubiquitination and proteasome-dependent degradation of transcriptional regulators and substrates of the ubiquitin system inhibitor of DNA binding (Id) 1 and Id3 in HeLa cells. Id2 and Id4 bind to CSN subunit CSN5 and regulate the ubiquitination by specific ubiquitin ligases (Berse et al. 2004). The CSN, a kinase complex, cooperates with the ubiquitin/26S proteasome system in regulating the stability of proteins involved in signal transduction. The components of CSN possess homologies with eight non-ATPase regulatory subunits of the 26S proteasome. Curcumin has been shown to be a potent inhibitor of the CSN kinase activity with a Ki of about 10 μM (Henke et al. 1999). Because of this, curcumin blocks E6-dependent p53 degradation in reticulocyte lysates as CSN targets human p53 to ubiquitin-26S proteasome-dependent degradation (Bech-Otschir et al. 2001). As an inhibitor of CSN-associated kinases, curcumin also showed proteasome-dependent degradation of cyclooxygenase-2 (COX-2) in HeLa cell lysate and in HeLa cells (Neuss et al. 2007).

Curcumin can overcome cisplatin resistance in cancer cells. For instance, co-treatment of the cells with curcumin and cisplatin resulted in increased apoptosis and reversal of Bcl-2-mediated cisplatin resistance. The mechanism by which curcumin downregulates Bcl-2 and sensitizes cells to cisplatin-induced apoptosis involves proteasome degradation of Bcl-2 (Chanvorachote et al. 2009). This ubiquitin-proteasome degradation of Bcl-2 by curcumin also sensitizes non-small cell lung cancer cell anoikis and detachment-induced apoptosis (Pongrakhananon et al. 2010). In the *Xenopus laevis* (frog) model system, curcumin inhibited proteasome activity and induced the accumulation of heat shock proteins (HSPs) at the transcriptional level. It has been found that the treatment of A6 kidney epithelial cells with curcumin enhanced ubiquitinated protein levels and inhibited CTL activity (Khan and Heikkila 2011). Besides these effects, curcumin promoted proteasome-dependent degradation of p300 and the closely related CREB binding protein (CBP). In addition to inducing p300 degradation, curcumin inhibited the acetyltransferase activity of purified p300 as assessed by using either histone H3 or p53 as substrate (Marcu et al. 2006).

The direct inhibition of proteasome activity also causes an increase in the half-life of IκB α that ultimately leads to the downregulation of NF-κB activation (Dikshit et al. 2006b). Another report suggested that curcumin did not affect phosphorylation of IκB α, although it significantly inhibited proteasomal activity in MCF-7 cells.

It has the capability of inhibiting tumor necrosis factor (TNF)-α-induced NF-κB activation of MCF-7 cells by inhibiting the proteasome activities instead of inhibiting IκB kinase (IKK) activation (Yoon and Liu 2007). Another study reported that curcumin at nanomolar concentrations specifically restores cigarette smoke extract- or oxidative stress-impaired histone deacetylase 2 (HDAC2) activity. Biochemical and gene chip analysis indicated that curcumin at concentrations up to 1 μM propagates its effect *via* antioxidant-independent mechanisms associated with the phosphorylation-ubiquitin-proteasome pathway. It reversed HDAC2 protein expression even in the presence of the protein synthesis inhibitor cycloheximide, which indicates that curcumin acts at a post-translational level by maintaining both HDAC2 activity and expression (Meja et al. 2008). Curcumin has also been shown to promote the ubiquitination and degradation of inducible nitric oxide (NO) synthase (iNOS) after lipopolysaccharide (LPS) stimulation. In LPS-stimulated murine macrophage-like RAW 264.7 cells, curcumin induced the degradation of iNOS protein through ubiquitination and proteasome mechanisms (Ben et al. 2011).

As a proteasome inhibitor, curcumin has been shown to be useful for the treatment of muscle wasting in cancer cachexia. In one study, curcumin completely attenuated proteolysis-inducing factor (PIF)-induced total protein degradation in murine myotubes, and also attenuated the PIF-induced increase in the expression of the ubiquitin-proteasome proteolytic pathway, as determined by the CTL enzyme activity, proteasome subunits and $E2_{14k}$ (Wyke et al. 2004). Another study showed that curcumin influenced proteolytic pathways that are activated in septic muscle, including ubiquitin-proteasome-dependent proteolysis. In a rat model, curcumin treatment prevented a sepsis-induced increase in muscle protein breakdown. It has also been observed that the upregulated expression of the ubiquitin ligases atrogin-1 and MuRF1 was not influenced by curcumin. When muscles from septic rats were treated with curcumin *in vitro*, proteasome-dependent protein breakdown rates were reduced, and nuclear NF-κB activity and activated p38 were decreased, which suggests that sepsis-induced muscle proteolysis can be blocked by curcumin (Poylin et al. 2008). Curcumin also regulates the proteasome degradation of short-lived protein hepatitis C virus nonstructural 2, which is described to be involved in apoptosis inhibition and gene transcription modulation. Curcumin mediates inhibition of casein kinase 2 activity, which leads to suppression of nonstructural 2 phosphorylation and proteasome degradation and, subsequently, stabilized expression *in vitro* (Franck et al. 2005). In polyglutamine diseases, curcumin promotes mutant huntingtin-induced cell death by mimicking proteasome dysfunction. The treatment of curcumin increases the polyglutamine-expanded truncated N-terminal huntingtin (mutant huntingtin) aggregation and mutant huntingtin-dependent cell death. Curcumin also causes rapid proteasome malfunction in the mutant huntingtin-expressing cells in comparison with normal glutamine repeat expressing cells (Dikshit et al. 2006b).

Flavonoids have also been shown to exhibit chemopreventive and chemotherapeutic activities by modulating the proteasome pathway. Quercetin, one of the flavonoids present in a variety of fruits, vegetables, nuts, and spices, inhibits the ubiquitination of HIF-1/2α in normoxia by hindering HIF-1/2α proline

hydroxylase through chelating iron ions (Park et al. 2008). Quercetin also enhanced the antioxidant response element binding activity and NF-E2-related factor 2 (Nrf2)-mediated transcriptional activity as studied in human HepG2 cells. Molecular evidence revealed that quercetin not only upregulated the expression of Nrf2 mRNA and protein, but also stabilized Nrf2 protein by inhibiting the ubiquitination and proteasome turnover of Nrf2 (Tanigawa et al. 2007). In addition, quercetin induced ubiquitination of HER2/neu in which the carboxyl terminus of Hsc70-interacting protein, a chaperone-dependent E3 ubiquitin ligase played a crucial role (Jeong et al. 2008). Treatment with quercetin significantly decreased the protein levels of c-FLIP, an inhibitor of caspase-8, through proteasome-mediated degradation and recovers TNF-related apoptosis-inducing ligand (TRAIL), sensitivity in various human hepatocellular carcinoma cells (Kim et al. 2008). Quercetin exposure also resulted in proteasome degradation of survivin and enhanced death-receptor-mediated apoptosis in glioma cells (Siegelin et al. 2009).

Angioprotective properties of quercetin have been also reported, which is mediated by proteasome proteolysis. It has the ability to modulate proteasome activity in a rabbit model of cholesterol-induced atherosclerosis. A single intravenous injection of the water-soluble form of quercetin (Corvitin) significantly decreased proteasome TL activity 1.85-fold in monocytes, and decreased the CTL and peptidyl-glutamyl peptide-hydrolyzing (PGPH) activities more than two-fold in polymorphonuclear leukocytes. Prolonged administration of Corvitin to animals following a cholesterol-rich diet significantly decreased all types of proteolytic proteasome activities both in tissues and in circulating leukocytes and has been shown to be associated with the reduction of atherosclerotic lesions in the aorta (Pashevin et al. 2011). In other study, quercetin and its derivative quercetin caprylate were identified as a proteasome activator with antioxidant properties that consequently influence the cellular lifespan, survival, and viability of HFL-1 primary human fibroblasts. Moreover, when these compounds are supplemented to already senescent fibroblasts, a rejuvenating effect is observed (Chondrogianni et al. 2010). In human HaCaT keratinocytes, quercetin enhanced arsenic-induced apoptosis. A decrease in the p53 protein with increased protein ubiquitination was detected in quercetin/As(+3)-treated HaCaT cells. The decrease in the p53 protein by quercetin/As(+3) was reversed by adding the proteasome inhibitor, MG132 (Shen et al. 2012).

Several other flavonoids (including apigenin-6-hydroxy-7-*O*-beta-*D*-glucoside, rutin, 6-hydroxyapigenin, 5,6,4′-trihydroxy-7,3′-dimethoxyflavone, 5,6,3′,4′-tetrahydroxy-7-methoxyflavone, glycitecin, and 6,7,4′-trihydroxyisoflavone) also inhibit CTL caspase-like or TL activity of 26S proteasome in pig red blood cells (Chang 2009). Another study showed that apigenin and quercetin are much more potent than kaempferol and myricetin in inhibiting CTL activity of purified 20S proteasome and of 26S proteasome in intact leukemia Jurkat T cells as well as in accumulating putative ubiquitinated forms of two proteasome target proteins, Bax and IκBα, in Jurkat T cells (Chen et al. 2005).

Sesamin, a component of sesame seeds, has shown anticancer effects in different human cancer cells mediated through the ubiquitin proteasome pathway. It inhibits

proliferation of cancer cells *via* downregulation of cyclin D1 in various kinds of human tumor cells, including lung cancer, transformed renal cells, immortalized keratinocyte, melanoma, and osteosarcoma by promoting proteasome degradation of the cyclin D1 protein (Yokota et al. 2007). Thymoquinone, another spice nutraceutical present in *Nigella sativa* (black cumin), induces selective proteasome inhibition, both in isolated enzymes and in glioblastoma cells, which leads to the induction of apoptosis in cancer cells. In one study, U87 MG and T98G malignant glioma cells were treated with thymoquinone, an inhibition of 20S and 26S; proteasome activity was observed in both cell lines, accompanied by the accumulation of ubiquitin conjugates. The accumulation of p53 and Bax, two proteasome substrates with proapoptotic activity, was also observed in both cell lines (Cecarini et al. 2010), indicating thymoquinone's proteasome inhibitory properties.

Cinnamate esters, present in the bark of cinnamon, are reported to be an inhibitor of proteasome activity while cinnamic acid amides had no inhibitory activity against proteasomes (Arbiser et al. 2005). Another study on the garlic component diallyl sulfide showed that it prevented the decreased proteasome peptidase activities in ethanol-exposed VL-17A cells (Osna et al. 2007), suggesting its proteasome modulating properties. Capsaicin, a spice component of chili, induced apoptosis of mouse neuro 2a cells *via* the inhibition cellular proteasome function. Exposure of capsaicin in this mouse neuro 2a cells causes increased oxidative stress and the accumulation of ubiquitinated proteins, as well as various target substrates of proteasomes such as p53, Bax and p27 (Maity et al. 2010). However, capsaicin induced the degradation of tumor suppressor p53, which is blocked by the proteasome inhibitor MG132, and enhanced apoptotic cell death in the human breast cell line MCF10A (Lee et al. 2009). Moreover, capsaicin inhibited TNF-α-stimulated degradation of IκBα through inhibiting proteasome activity and preventing the activation of NF-κB in PC-3 cells. This could be the probable mechanism of capsaicin-induced decrease of prostate tumor growth in a xenograft mouse model (Mori et al. 2006).

9.4.2 Fruits and Vegetables

Fruits and vegetables are considered the most important source of nutraceuticals. Indole-3-carbinol and its dimeric product 3,3′-diindolylmethane (DIM), components of cruciferous vegetables, inhibit prostate cancer cell growth and induce apoptosis. The anticancer effects of DIM were linked to the proteasome mechanism. DIM inhibited proteasome activity in the S phase, which led to the inactivation of NF-κB signaling and the induction of apoptosis in LNCaP and C4-2B cells (Chinnakannu et al. 2009). The inactivation of NF-κB was also achieved with cucurbitacin. Treating T cell leukemia cells with cucurbitacin D induced inhibition of proteasome degradation of IκBα, which resulted in its accumulation, and suppression of NF-κB activation (Ding et al. 2011). Caffeic acid, which occurs naturally in many agricultural products

such as fruits, vegetables, wine, olive oil, and coffee, induced the inhibition of NF-κB, which is associated with the proteasome pathway (Thornton et al. 2010).

Isosilybin B, a component of milk thistle, exhibited anticancer effects in human prostate cancer cells. Treatment of isosilybin B in prostate cancer cells enhances the formation of a complex between Akt, Mdm2, and androgen receptor (AR), which promotes phosphorylation-dependent AR ubiquitination and its degradation by proteasome (Deep et al. 2008). Another component of milk thistle, silibinin, downregulated antiapoptotic proteins FLIP(L), FLIP(S), and survivin through proteasome-mediated degradation. This activity of silibinin modulates multiple components in the death receptor-mediated apoptotic pathway and recovered TRAIL sensitivity in TRAIL-resistant glioma cells (Son et al. 2007).

The flavonoids, such as isothiocyanates, which include benzyl isothiocyanate, phenethyl isothiocyanate and sulforaphane, found in cruciferous vegetables, are highly effective in inducing cell cycle arrest and apoptosis in a variety of cancer cells and animal models through inhibition of the proteasome pathway. Dietary isothiocyanate has been shown to suppress cancer progression by reducing the polycomb group (PcG), which is present at elevated levels in cancer cells, *via* a proteasome-dependent mechanism, thereby inhibiting PcG-dependent pro-survival epigenetic events. It also induced proteasome-dependent degradation of both alpha- and beta-tubulins in a variety of human cancer cell lines (Mi et al. 2009). Benzyl isothiocyanate and phenethyl isothiocyanate significantly inhibited both the 26S and 20S proteasomes, presumably through direct binding in a variety of cell types. The potency of isothiocyanate-induced proteasome inhibition correlates with the rapid accumulation of p53 and IκBα, demonstrating that benzyl isothiocyanate and phenethyl isothiocyanate are the strongest proteasome inhibitors causing cell cycle arrest and apoptosis (Mi et al. 2011).

Sulforaphane induced a concentration-dependent proteasome degradation of PcG protein (Bmi-1, Ezh2) in SCC-13 skin cancer cells and also reduced trimethylation of lysine 27 of histone H3. This is associated with accumulation of cells in G2/M phase; reduced levels of cyclin B1, cyclin A, cyclin dependent kinases (CDK) 1 and 2; and increased p21(Cip1) expression (Balasubramanian et al. 2011). It has also been shown to enhance proteasome activities in mammalian cells and to reduce the level of oxidized proteins and amyloid β-induced cytotoxicity in murine neuroblastoma Neuro2A and N1E 115 cells (Park et al. 2009). Further mechanistic studies revealed that sulforaphane enhances the expression of the catalytic subunits of the proteasome, as well as proteasome peptidase activities. Depending on the upregulation of the proteasome system it acts as cytoprotective agent against hydrogen peroxide-mediated cytotoxicity (Kwak et al. 2007). Another study showed that sulforaphane induced expression of HSP27 through the stimulation of proteasome activity (Gan et al. 2010). In prostate cancer cells, sulforaphane induced the hyperacetylation of HSP90, which in turn destabilizes AR and promotes further proteasome degradation (Gibbs et al. 2009). In breast cancer MCF-7 cells, sulforaphane inhibited the expression of estrogen receptor (ER)α protein in part at the transcriptional level as well as increased proteasome-mediated degradation (Ramirez and Singletary 2009).

Sulforaphane has also been shown to inhibit Keap1-dependent ubiquitination of PGAM5 as a novel substrate for Keap1 (Lo and Hannink 2006). Keap1 is a negative regulator of Nrf2, a bZIP transcription factor that mediates adaptation to oxidative stress. In the RL34 non-transformed rat liver cell line, Nrf2 was found to accumulate rapidly in response to oxidative stress caused by treatment with sulforaphane, and the accumulation resulted from inhibition of proteasome-mediated degradation of the bZIP protein (McMahon et al. 2003). Sulforaphane increases the expression of genes through the Nrf2 signaling pathway that directly detoxify exogenous toxins/carcinogens or endogenous reactive oxygen species, as well as genes involved in the recognition and repair/removal of damaged proteins, which possibly provide secondary protection against DNA or protein damage, enhancing cell survival (Hu et al. 2006).

Resveratrol, pterostilbene, and morin hydrate caused significant inhibition in the activities of CTL, TL, and post-acidic (post-glutamase) proteasome sites in RAW 264.7 cells. Consequently, it inhibits NF-κB activation by the proteasome and suppresses activation of pro-inflammatory cytokines and iNOS genes, resulting in decreased secretion of TNF-α, interleukin (IL)-1β, IL-6, and NO levels, in response to inflammatory stimuli (Qureshi et al. 2012). Another study showed that resveratrol, like N-Ac-Leu-Leu-norleucinal (ALLN), suppressed IL-1beta-induced NF-κB activation through the inhibition of proteasome function and the degradation of IκBα without affecting IKK activation, IκBα-phosphorylation or IκBα-ubiquitination. Resveratrol and ALLN also inhibited IL-1β-induced apoptosis, caspase-3 activation, and poly (ADP-ribose) polymerase cleavage in human articular chondrocytes (Shakibaei et al. 2008). The inhibition of NF-κB activation through the ubiquitin-proteasome pathway by resveratrol was also shown to be effective in the preservation of skeletal muscle mass in cachexia to prevent muscle wasting (Tisdale 2005).

Resveratrol has been reported to increase the expression levels of heme oxygenase (HO)-1 protein through the activation of PI3K/Akt pathway. The induction of HO-1 protein was found to be associated with inhibition of the ubiquitination-dependent proteasome pathway, which in turn melatonin (a neurohormone) potentiates the neuroprotective effect of resveratrol against oxidative injury (Kwon et al. 2011). In Alzheimer's disease, resveratrol has shown proteasome-dependent anti-amyloidogenic activity. Although resveratrol does not inhibit Abeta production, it promotes intracellular degradation of Abeta *via* a mechanism that involves the proteasome (Marambaud et al. 2005). It has also been reported that resveratrol triggers ER stress and cell death in dopaminergic N27 cells through caspase activation, p23 cleavage, and inhibition of proteasome activity in dopaminergic N27 cells (Chinta et al. 2009). Resveratrol also has the potential to negate the cytotoxic effects of proteasome inhibitors *via* regulation of forkhead box protein O1 (FOXO1) transcriptional activity and accumulation of p27Kip1 (Niu et al. 2011).

Resveratrol modulates peroxisome proliferator-activated receptor (PPAR) γ protein levels in 3T3-L1 adipocytes *via* inhibition of PPARgamma gene expression coupled with increased ubiquitin-proteasome-dependent degradation (Floyd et al.

2008). It also inhibits hypoxia-induced HIF-1α accumulation by enhanced protein degradation through the 26S proteasome system (Zhang et al. 2005). Proteasome-dependent degradation of the ERα was also observed in breast cancer MCF-7 cells, which caused inhibition of cell proliferation and induction of apoptosis (Pozo-Guisado et al. 2004).

Fisetin blocks mitosis in a proteasome-dependent manner in several human cell lines. The inhibition of mitosis by fisetin was mediated through targeting Aurora B kinase, which contributes its antiproliferative effects (Salmela et al. 2009). However, fisetin has also been shown to promote nerve cell survival by increased proteasome activity (Maher 2008). Other flavonoids such as luteolin, apigenin, chrysin, naringenin, and eriodictyol also inhibit proteasomes. The order of inhibitory potencies of 20S purified proteasome was luteolin > apigenin > chrysin (Chen et al. 2007). It has been reported that methanol extract of the stems of *Spatholobus suberectus*, which contain liquiritigenin, isoliquiritigenin, genistein, daidzein, medicarpin, 7-hydroxyflavanone, and formononetin, inhibit 20S proteasomes (Shim 2011). Apigenin inhibits the proteasome CTL activity and induces apoptosis not only in cultured MDA-MB-231 cells but also in MDA-MB-231 xenografts (Chen et al. 2007). Apigenin dissociated the complex of HER2/neu and GRP94 that preceded the depletion of HER2/neu. Apigenin-induced degradation of mature HER2/neu involves polyubiquitination of HER2/neu and subsequent hydrolysis by the proteasome in breast cancer cells (Way et al. 2004).

Ferulic acid suppresses matrix metalloproteinase (MMP)-2 and MMP-9 mediated *via* the proteasome pathway in ultraviolet B-irradiated mouse skin tissues (Staniforth et al. 2012). Kaempferol treatment resulted in proteasome degradation of survivin, which was involved in kaempferol-mediated TRAIL-induced apoptosis (Siegelin et al. 2008). Betulinic acid (BA) induces proteasome-dependent degradation of transcription factors specificity protein (Sp) 1, Sp3, and Sp4 in prostate cancer cells and induced antiangiogenic and proapoptotic activities (Chintharlapalli et al. 2007). It is also reported as a potent proteasome activator that preferentially activates the CTL activity of the proteasome. Chemical modifications at the C-3 position of BA (dimethylsuccinyl BA) converted it to proteasome inhibitors with various biological activities against the human 20S proteasome (Huang et al. 2007).

Beta-carotene, which is extensively present in carrots, dark-green leafy vegetables, including spinach and green leaf lettuce, sweet potatoes and broccoli, exhibits anticancer and anti-inflammatory properties through modulation of proteasome pathway. The carotene breakdown products modify tau and ferritin proteins and such modifications enhanced their proteasomal degradation through recognition of 20S proteasome subunit (Sommerburg et al. 2009). Omega-3 fatty acid, another dietary compound appeared to accelerate AR protein degradation, which was blocked by proteasome inhibitor MG132 indicating its degradation is mediated through proteasome pathway (Wang et al. 2012). Knockdown of AR significantly slowed down prostate cancer cell proliferation in the absence of androgens.

Vitamins are fond be major regulator of gene expression in higher organisms. Vitamin D and its metabolite 1α,25-dihydroxyvitamin D(3), has shown to modulate the function of the ubiquitin-proteasome system (Alvarez-Diaz et al. 2010).

Another study showed that proteasome inhibitor enhanced 1,25(OH)(2)D(3)-induced CYP24A1 expression and nuclear vitamin D receptor (VDR) expression indicating 1,25(OH)(2)D(3) induced their expression through ubiquitin-proteasome pathway (Amano et al. 2009). However, numerous studies have shown that ascorbic acid (vitamin C) inhibits bortezomib-induced cytotoxicity against endometrial carcinoma cells (Llobet et al. 2008), multiple myeloma (Perrone et al. 2009) and several other cancer cells (Zou et al. 2006) *in vitro*. It has been shown *in vitro* multiple myeloma cells that vitamin c blocks bortezomib-induced inhibitory effect on 20S proteasome activity. Even *in vivo* study on xenograft model of human multiple myeloma in SCID (severe combined immune-deficient) mice evident that oral intake of vitamin C (40 mg/kg/day) significantly blocks bortezomib-induced inhibition of tumor growth (Perrone et al. 2009).

9.4.3 Legumes and Nuts

Legumes and nuts contain a variety of phytochemicals. These phytochemicals act as chemopreventive and therapeutic agents in several ways, including the proteasome-signaling pathway. Genistein is one of the natural phytochemicals, induced proteasome degradation of topoisomerase II (Top2)β in 32Dc13 cells. It efficiently induced both Top2α and Top2β cleavage complexes in the purified system and in cultured mouse cells, which play a major role in infant leukemia (Azarova et al. 2010). The anacardic acid has been shown to attenuate histone acetylation, as pesticide dieldrin induces acetylation of core histones because of proteasome dysfunction and this hyperacetylation plays a key role in dopaminergic neuronal degeneration (Song et al. 2010).

Treatment of genistein to LNCaP cells exhibited increased ubiquitination of AR, suggesting that AR protein is downregulated *via* a proteasome-mediated pathway in prostate cancer cells. The increased ubiquitination of AR after genistein treatment was associated with a decrease in Hsp90 chaperone activity (Basak et al. 2008). Genistein also enhanced proteasome degradation of the short isoform of c-FLIP and thus facilitated TRAIL-mediated apoptosis in malignant glioma cells (Siegelin et al. 2009). CDDO-Me, an analogue of oleanoic acid, induced ubiquitin/proteasome-dependent c-FLIP degradation. This c-FLIP downregulation contributes to CDDO-Me-initiated apoptosis and enhancement of TRAIL-induced apoptosis (Zou et al. 2007). Another analogue of oleanoic acid, CDDO-Im, either alone or in combination with bortezomib, has been shown to overcome drug resistance in multiple myeloma patients (Chauhan et al. 2004). CDDO also upregulates proteins involved in the ubiquitin-proteasome pathway and p21 (Waf1/CIP1), GADD153, and CAAT/enhancer binding protein transcription factor family members in breast cancer MCF-7 and MDA-MB-435 cells (Lapillonne et al. 2003).

9.4.4 Others

Other natural compounds such as tocotrienol, found in palm oil, rice, and barley, inhibits inflammatory responses by inhibiting activation and nuclear translocation NF-κB through inhibition of proteasome-mediated IκBα degradation (Qureshi et al. 2011). Further mechanistic study showed that α-, γ-, or δ-tocotrienol inhibited the CTL activity of 20S as shown in rabbit muscle proteasomes (Qureshi et al. 2010). Tocotrienol has been shown to modulate differential interaction of mitogen-activated protein (MAP) kinases with caveolin 1/3 in conjuncture with proteasome stabilization, which plays a unique role in tocotrienol-mediated cardioprotection (Das et al. 2008). Other than α-tocotrienol, γ-tocopherol and α-tocopheryl phosphate also inhibited cellular proteasome activity and increased the level of p27 (Kip1) and p53 in THP-1 monocytes (Munteanu et al. 2007).

Emodin (from *Rheum emodi*, a Himalayan rhubarb, buckthorn, and Japanese knotweed) inhibits CSN-associated kinases and significantly induces ubiquitination and proteasome-dependent degradation of transiently expressed Id3 in HeLa cells. Proteasome-dependent degradation of endogenous Id1 in HeLa cells was also stimulated by emodin (Berse et al. 2004). In T cell lymphomas, emodin accelerates c-myc protein turnover in a proteasome-dependent manner (Channavajhala and Seldin 2002). Emodin decreased the association of AR and HSP90 and increased the association of AR and MDM2, which in turn induces AR degradation through the proteasome-mediated pathway in prostate cancer cells and thus exhibits anti-cancer effect (Cha et al. 2005).

In breast cancer cells, withaferin A, a natural compound from the ayurvedic plant (Chitrak), downregulates the ERα protein levels by proteasome-dependent ERα degradation (Zhang et al. 2011). Withaferin A also inhibits inflammation in various cancer cells by inhibiting NF-κB activation through the proteasome pathway. The inhibition of NF-κB in HUVECs by withaferin A occurs by interference with the ubiquitin-mediated proteasome pathway as it increases the levels of poly-ubiquitinated proteins (Mohan et al. 2004). In prostate tumor cells, it accumulates ubiquitinated proteins and the IκBα, Bax, and p27 proteasome targets which results in degradation of the AR protein in androgen-dependent LNCaP cells and apoptosis induction. Furthermore, withaferin A potently inhibits the CTL activity of purified rabbit 20S and 26S proteasomes in human prostate cancer cultures and xenografts. Further computational modeling studies predict that C1 and C24 of withaferin A are highly susceptible for a nucleophilic attack by the hydroxyl group of N-terminal threonine of the proteasome chymotrypsin subunit β5 (Yang et al. 2007). In addition, docking studies carried out with herbal ligand withaferin A in the structures of bovine and human proteasomes substantiate that it has the ability to inhibit activity of mammalian 20S proteasomes by blocking the nucleophilic function of N-terminal Thr1 (Grover et al. 2010).

Xanthohumol, another natural compound from hops, showed proteasome inhibitory activity and induced apoptosis of chronic lymphocytic leukemia cells (Lust et al. 2009). Evodiamine induces A375-S2 cell death through the PI3K/Akt/caspase and

Fas-L/NF-κB signaling pathways, and these signals have been shown to be augmented by the ubiquitin-proteasome pathway (Wang et al. 2010). Another natural compound, baicalein, exhibits cytoprotective activity against 6-hydroxydopamine-induced oxidative injury, which was attenuated by the proteasome inhibitor MG132 (Jiang et al. 2012), which indicates that the cytoprotective effect of baicalein was mediated through the proteasome pathway.

Black tea extract enriched in theaflavins inhibited the CTL activity of the proteasome and the proliferation of human multiple myeloma cells. An isolated theaflavin can bind to and inhibit the purified 20S proteasome, accompanied by suppression of tumor cell proliferation (Mujtaba and Dou 2012). Epigallocatechin gallate (EGCG), a component of green tea, also has been shown to be potent and specifically inhibits the CTL activity of purified 20S proteasome and the 26S proteasome in tumor cell lysates. Treatment of leukemic Jurkat T or prostate cancer LNCaP cells with EGCG accumulated p27 and IκBα proteins, which further resulted in cell cycle arrest and apoptosis by accumulation of the Bad protein (Nam et al. 2001; Smith et al. 2002). Sukhthankar et al. (2008) showed that EGCG increases the ubiquitination of basic fibroblast growth factor (bFGF) and TL activity of the 20S proteasome, resulting in the degradation of the bFGF protein. Combining tea polyphenols with gallic acid inhibited 20S proteasomes with gradual inhibition of CTL, TL, PGPH and BrAAP (Amici et al. 2008). It has been shown that analogues of (−)-EGCG containing a para-amino group on the D-ring in place of the hydroxyl groups have proteasome inhibitory activities (Osanai et al. 2007). However, its methylation decreases proteasome-inhibitory activity, contributing to decreased cancer-preventive effects of tea consumption (Landis-Piwowar et al. 2007).

Both tyrosinase and vascular endothelial growth factor (VEGF) protein levels have been shown to be remarkably reduced during EGCG treatment. Further, the hindrance of tyrosinase and VEGF protein synthesis could be prevented by the use of the proteasome inhibitor lactacystine, indicating that EGCG-induced degradation of these proteins was mediated by the proteasome pathway (Konta et al. 2010). Another study showed that the EGCG-induced reduction in PcG protein level is associated with increased ubiquitination and is reversed by proteasome inhibitors, suggesting proteasome-associated degradation in skin cancer cells (Choudhury 2011). It also inhibits p38 MAPK and the proteasome activities, leading to inhibition of B cell lymphoma-extra large (Bcl-xL) phosphorylation and induction of prostate cancer cell death (Kazi et al. 2002). In patients with cancer of the head and neck or the pelvic region, topical treatment with green or black tea extracts suppressed cytokine release and inhibited the 26S proteasome function, which further resulted in NF-κB inhibition (Pajonk et al. 2006). EGCG also exhibited neuroprotective effects, inhibiting neuronal cell death by suppressing rapid PKC-mediated degradation of the Bad protein by the proteasome pathway (Kalfon et al. 2007).

Mutant p53 proteins are largely accumulated in cancer cells due to their increased stability, therefore targeting mutant form of p53 have shown to be an attractive approach for cancer therapy. In MDA-MB-435 cells, gambogic acid (GA)

(an active component of kokum) downregulates mutant p53 at the posttranscription level. The downregulation of mutant p53 by GA was mediated through the chaperones-assisted ubiquitin/proteasome degradation pathway in cancer cells (Wang et al. 2011). It has been also observed that at the posttranslational level, GA promoted the autoubiquitination of MDM2, followed by proteasome-mediated degradation. Additionally, GA increased p21(Waf1/CIP1) expression in p53-null cancer cells, which was associated with GA-mediated impairing of the interaction between MDM2 and p21(Waf1/CIP1). Furthermore, the apoptosis, cytotoxicity, and G(2)/M cell cycle arrest induced by GA was detected in both p53 wild-type and p53-null cancer cells (Rong et al. 2009). Another study showed that the combination of natural product GA and the proteasome inhibitor MG132 or MG262 resulted in a synergistic inhibitory effect on growth of malignant cells and tumors in allograft animal models (Huang et al. 2011). GA also inhibits the multidrug-resistant protein ATP-binding cassette transporter B1 (ABCB1). GA functions as a non-competitive inhibitor of ABCB1 by reducing its expression levels by promoting protein degradation through the post-translational proteasome pathway. Thus, GA can act as an anti-multidrug-resistance agent (Wang et al. 2013).

9.5 Role of Nutraceuticals in the Prevention and Treatment of Cancer

Because of their multi-targeting nature, cost-effectiveness, efficacy, safety, and immediate availability, nutraceuticals derived from dietary sources have attracted the attention of clinicians and researchers for cancer therapy (Gupta et al. 2011b, 2012). These dietary agents have been shown to modulate every facet of tumor development, including survival, proliferation, invasion, angiogenesis, and metastasis. In this section, we discuss some of the most promising nutraceuticals, including γ-tocotrienol, 6-gingerol, allicin, allyl isothiocyanate, apigenin, betulinic acid, caffeic acid, capsaicin, curcumin, diallyl disulfide, EGCG, emodin, evodiamine, fisetin, GA, genistein, indole-3-carbinol, myricetin, plumbagin, quercetin, resveratrol, sulforaphane, thymoquinone, ursolic acid, and xanthohumol in the context of five specific processes of tumorigenesis: survival, proliferation, invasion, angiogenesis, and metastasis.

One of the characteristic features of cancer cells is that they are able to grow in a rapid and uncontrolled manner and evade apoptosis. Cancer cells acquire this property through upregulation in cell survival proteins and downregulation in pro-apoptotic proteins (Ambrosini et al. 1997; Wang et al. 2003). Therefore, selective downregulation of cell survival proteins and upregulation of pro-apoptotic proteins in cancer cells represent promising therapeutic interventions for cancer therapy. Several nutraceuticals can affect tumor cell survival by inducing apoptosis through the modulation of numerous cell-signaling molecules. Depending upon the cancer types and inducers, nutraceuticals can modulate numerous targets. The most

common targets of nutraceuticals related with tumor survival are apoptotic protease activating factor-1 (Apaf-1), Bcl-2 homologous antagonist/killer (Bak), Bax, Bcl-2, Bcl-xL, Bcl-2–interacting mediator of cell death (Bim), Bcl-2-related gene expressed in fetal liver protein A1 (Bfl-1/A1), caspases, death receptors, FLIP, inhibitor of apoptosis protein (IAP), myeloid cell leukemia-1 (Mcl-1), NF-κB, p53 upregulated modulator of apoptosis (PUMA), signal transducers and activators of transcription protein-3 (STAT-3), and X-chromosome-linked IAP (XIAP) (Prasad et al. 2012a).

Curcumin, a component of golden spice, is a potent inducer of apoptosis in cancer cells. Curcumin induces apoptosis in cancer cells by upregulation of pro-apoptotic proteins (Bax, Bim, Bak, PUMA) and through downregulation of the cell survival proteins (Bcl-2, Bcl-xL) (Shankar and Srivastava 2007; Shankar et al. 2008). GA-induced apoptosis in MCF-7 cancer cells through upregulation of p53 and downregulation of Bcl-2 (Gu et al. 2009). The induction of apoptosis in human malignant melanoma A375 cells by GA was associated with an increase in Bax expression and a decrease in Bcl-2 expression (Xu et al. 2009). Resveratrol, a component of red wine-induced apoptosis in human multidrug-resistant SPC-A-1/CDDP cells through downregulation in survivin expression (Zhao et al. 2010). Some nutraceuticals have been shown to enhance the apoptosis induced by TRAIL in cancer cells. For instance, xanthohumol, a chalcone, enhanced TRAIL-induced apoptosis in prostate cancer cells (Szliszka et al. 2009).

Capsaicin was shown to sensitize malignant glioma cells to TRAIL-mediated apoptosis *via* death receptor 5 upregulation and survivin downregulation (Kim et al. 2010). Most nutraceuticals induce apoptosis by inhibiting NF-κB activation and expression of NF-κB regulated cell survival proteins. For instance, the induction of apoptosis in breast cancer cells was mediated through inactivation of Bcl-2 and the DNA binding activity of NF-κB (Ahmad et al. 2008). Fisetin was found to induce apoptosis in chemoresistant human pancreatic AsPC-1 cells through suppression of NF-κB activation (Murtaza et al. 2009). Sulforaphane inhibited the survival of orthotopically implanted PC-3 tumors through inhibition of NF-κB activation pathways (Shankar et al. 2008). Nutraceuticals have also been shown to inhibit the survival of tumor cells through the modulation of STAT-3 pathway. For instance, capsaicin has been reported to induce apoptosis in multiple myeloid cells through downregulation of STAT-3-regulated expression of Bcl-2, Bcl-xL, and survivin (Bhutani et al. 2007).

An ability to generate growth signals and insensitivity to antigrowth signals is another characteristic of cancer cells (Hanahan and Weinberg 2000). The most common way by which cancer cells acquire these features is through modulation of signaling molecules, including cdc25c, CDK, check point kinase (Chk), c-Myc, COX-2, p21-activated kinase 1 (PAK1), and retinoblastoma protein (Rb). The pro-inflammatory transcription factors NF-κB and STAT-3 are the important mediators of tumor proliferation. Nutraceuticals have been shown to suppress tumor growth by modulating one or more of these signaling molecules (Gupta et al. 2011a).

Most nutraceuticals prevent the transition of cancer cells from the G1 phase to the S phase. Some of these nutraceuticals act through p53 and some through Rb.

For instance, emodin showed anti-proliferative activity through a p53- and p21-dependent pathway and arrested liver cancer HepG2 cells in the G1 phase (Kuo et al. 2002). Fisetin was shown to arrest prostate cancer LNCaP cells at the G1 phase, which was associated with a decrease in cyclin-D1, -D2, and -E and their activating partners CDK-2, -4, and -6 and with the induction of p21 and p27 (Khan et al. 2008). The suppression in proliferation of epithelial ovarian cancer cells by sulforaphane was mediated through G1 cell cycle arrest, reduction in pRb, and in free E2F-1, and an increase in Rb (Bryant et al. 2010). Some nutraceuticals prevent tumor cell proliferation by preventing transitions from the G2 phase to the M phase. Evodiamine exhibited anti-proliferative activity by arresting human thyroid ARO cancer cells at the G2/M phase, which was associated with decreased expression of cdc2-p15 (Chen et al. 2010). GA suppressed proliferation of HepG2 and A549 cells by inducing p53/p21 activation and G2/M arrest (Rong et al. 2009). Betulinic acid evoked an increase in the G2/M phase population and a decrease in the S phase population in human gastric adenocarcinoma cells (Yang et al. 2010).

NF-κB has been shown to bind to the promoter of genes involved in cellular proliferation. A few nutraceuticals target one or more steps in NF-κB activation to regulate tumor cell proliferation. For instance, inhibition of ovarian cancer growth by curcumin was correlated with inhibition in NF-κB and a STAT-3 activation pathway (Lin et al. 2007). Curcumin also exhibited anti-proliferative activity in breast cancer cells in association with decreased expression of cyclin-D1 and CDK-4 (Liu et al. 2009). Over the past two decades, we have identified a number of nutraceuticals that suppress tumor proliferation through modulation of the NF-κB signaling pathway.

Tumor cell invasion is a process that involves cell growth, cell adhesion, cell migration, and proteolytic degradation of tissue barriers such as the extracellular matrix and basement membrane. Several proteolytic enzymes, including MMPs and the intercellular adhesion molecule, participate in the degradation of these barriers. A number of studies in lung, colon, breast, and pancreatic carcinomas have demonstrated overexpression of MMPs in malignant tissues compared to adjacent normal tissues. MMP-9 is upregulated in angiogenic dysplasia and invasive cancers and has been attributed to infiltrating inflammatory cells. Various nutraceuticals have been shown to inhibit the expression of MMP-9 in tumor cells, which leads to the inhibition of invasion and metastasis. These include curcumin (Anto et al. 2002; Shishodia et al. 2003; Kamat et al. 2007), resveratrol (Banerjee et al. 2002), disogenin (Shishodia and Aggarwal 2006), plumbagin (Sandur et al. 2006), indole-3-carbinol (Takada et al. 2005a), thymoquinone (Sethi et al. 2008), GA (Pandey et al. 2007), and betulinic acid (Takada and Aggarwal 2003). We have shown that curcumin inhibits the expression of MMP-9 in orthotopically implanted pancreatic tumors (Kunnumakkara et al. 2007) and ovarian tumors in nude mice (Lin et al. 2007).

Angiogenesis, driven by VEGF, is known to be a crucial process for tumor development. Since the role of angiogenesis in tumor development was first revealed (Folkman et al. 1971), a number of anti-angiogenic compounds have been developed, including bevacizumab (Avastin), sunitinib (SUTENT), sorafenib

(Nexavar), cediranib maleate (Recentin), and pazopanib (Gordon et al. 2010). We have identified several nutraceuticals that can suppress the expression of VEGF, including curcumin (Kunnumakkara et al. 2007, 2008), resveratrol (Aggarwal et al. 2004; Harikumar and Aggarwal 2008), ursolic acid (Prasad et al. 2012b), thymoquinone (Sethi et al. 2008; Yi et al. 2008), γ-tocotrienol (Ahn et al. 2007), and GA (Pandey et al. 2007). Our group also showed that curcumin has the ability to inhibit the expression of VEGF in pancreatic cancer patients (Dhillon et al. 2008).

Metastasis, a highly complex process, occurs in as many as 90 % of cancer-associated deaths and involves interactions between the cancer cells and the host. Numerous molecules have been linked with cancer metastasis including MMPs, VEGF, TNF, platelet-derived growth factor, transforming growth factor (TGF)-β, and twist transcription factor. Several studies have suggested that cancer cells express chemokine receptors that mediate metastasis to target organs expressing their cognate chemokines. One of the well-studied chemokines in tumor cell migration and metastasis is a stromal cell-derived factor 1α (SDF-1α, also known as CXC chemokine ligand 12 (CXCL12)) and its receptor, CXCR4. Different cancers preferentially metastasize to different organs, and production of SDF-1α by an organ is responsible for the migration of cancer cells to that organ. CXCR4 has been linked with tumor metastasis in a wide variety of cancers. Because CXCR4 binds to its ligand CXCL12, leading to tumor migration, agents that can interrupt the CXCR4-CXCL12 cell-signaling pathway have the potential to suppress tumor metastasis. We have identified several compounds that can suppress chemokine signaling and thus have potential for suppression of cancer metastasis. The most common nutraceuticals known to modulate tumor metastasis include curcumin (Lin et al. 2007; Kunnumakkara et al. 2009), demethoxycurcumin (Yodkeeree et al. 2010), allyl isothiocyanate (Yodkeeree et al. 2010), apigenin (Way et al. 2004), caffeic acid (Park et al. 2005), capsaicin (Shin et al. 2008), diallyl disulfide (Thejass and Kuttan 2007), evodiamine (Takada et al. 2005b), fisetin (Liao et al. 2009), genistein (Valachovicova et al. 2004), [6]-gingerol (Lee et al. 2008), indole-3-carbinol (Meng et al. 2000), quercetin (Vijayababu et al. 2006), resveratrol (Liu et al. 2010b), sulforaphane (Shankar et al. 2008), γ-tocotrienol (Liu et al. 2010a), and ursolic acid (Prasad et al. 2012b).

9.6 Conclusion

In the past several years, numerous studies have advanced our understanding of the mechanisms by which the nutraceutical acts as an anticancer agent. Nutraceuticals induce apoptosis and tumor regression in a broad spectrum of tumor cell lines, and in *in vivo* xenograft models. These nutraceuticals have been shown to have the ability to overcome drug resistance and to synergize with a number of conventional therapies through modulation of inflammatory transcription factors and other signaling molecules. Nutraceuticals have also been shown to inhibit the proteasome

pathway that might contribute to their anticancer activities. However, studies are required to demonstrate whether nutraceuticals can inhibit the proteasome pathway in cancer patients.

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Chapter 10
Attenuation of Multifocal Cell Survival Signaling by Bioactive Phytochemicals in the Prevention and Therapy of Cancer

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Abstract Extensive researches within the last decade have revealed the presence of a complex network of signaling pathways overtly active in cancer cells augmenting proliferation and suppressing apoptosis. In parallel, epidemiological literature summarized a casual association between consumption of well balanced diet comprising adequate fruits and vegetables with reduced risk of developing cancer. This was projected to be associated with the presence of well characterized bioactive compounds, e.g. flavonoids, isothiocyanates, catechins, phenolic acid and organic sulphur present in diet that lead to the development of preventive and therapeutic strategies. Emerging evidence narrate mechanistic insight relating to the pleiotropic role of these bioactive compounds in modulating proliferation linked signaling at multiple levels including Akt, nuclear transcription factor-κB, c-Myc, cycloxygenase-2, MAPK, STAT and other signaling pathways that are active during embryogenesis, such as hedgehog and Wnt signalings. Preclinical studies including genetically modified mouse models revealed that dietary bioactive compounds hitherto classed as 'chemopreventive agents' induce apoptosis in cancer cells and inhibit development and/or progression of tumor. This chapter presents a comprehensive overview of deregulated signaling mechanisms prevailing within tumor cells along with broad knowledge-based evidence intertwining biological and molecular basis of action of chemopreventive agents citing representative examples. Additionally, an important aspect relating to the noteworthy rationale of using bioactive chemopreventive agents for sensitizing tumors to improve efficacy of chemotherapy in clinics has been discussed briefly. Finally, to bring forward the potential of natural chemopreventive agents to full extent, the development of safe and tolerable pharmaceutical grade analogs with improved bioavailability is encouraged to reduce the incidence and mortality of cancer.

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10.1 Introduction

During ontogeny of development and adulthood in multicellular organisms, a complex well-coordinated circuitry of signaling pathways maintains a cohort of differentiated and committed cells which responds to external factors such as: growth factors, hormones, chemokines, cytokines, etc. Extensive research during the past decade has unraveled this complex network of signaling communication becomes aberrant in transformed cells and malignant tumors making them behave in disorderly conduct and loose tissue integrity. This reinforces the view that upregulation or constitutive activation of multiple oncogenic mitogenic signaling pathways due either to altered proteins produced from mutation, defects or amplification of genes stimulate tumor initiating cells to proliferate, evade apoptosis and invade into surrounding tissues and metastasize. It is predicted that proper restraining of abnormal mitogenic signal communication in cancer cells may restrict or attenuate the disorderly proliferative stimulus; the later believed to be paramount in the initiation of tumor formation and its progression thru distinct stages of malignancy under paradigm of functional tumor suppressor genes. Accumulating preclinical data indicates that tumor cells use a large number of clearly defined signaling pathways to regulate their activity and remain viable (Cho 2012). Arguably, this is the principal reason why single inhibitors that target only one pathway have often failed to show expected results in the clinics. Time has shown that even when a drug has a single target and great efficacy, like androgen antagonists in early prostate cancer, it is not uncommon for the regressing tumor to acquire addition mutations making them resistant to the drug on tumor recurrence (McCarty 2004). Current cell signaling targeted therapies include inhibition of survival signals and augmenting apoptosis by death ligands or proapoptotic proteins emphasizing features of new prevention-trial design (Wang et al. 2013).

Over the past decade, there has been a growing consensus to explore the major survival signaling pathways prevailing within tumor cells that are susceptible to modulation by chemopreventive agents. It has been known and widely accepted that several non-nutritive chemopreventive agents derived from fruits, vegetables or culinary spices are effective in inhibiting carcinogenesis. The molecular basis of their pleiotropic action is now emerging through examination of discreet regulatory pathways including Akt, NF-κB, mitogen-activated protein kinase (MAPK), *p53*, cyclooxygenase (COX)-2, Ras, and many other molecules that are known to regulate apoptosis without unacceptable side effects (Souza et al. 2012; Vinod et al. 2013). Moreover, clinicians often encounter challenge of treatment failure resulting from the induction of drug resistance in cancer cells. Interestingly, data from our laboratory and elsewhere have demonstrated that multidrug resistance (MDR), NF-κB, Akt and other complex molecular operations within the apoptosis signaling axis are involved in the development of drug resistance. Importantly, chemopreventive agents can also potentially synergize in sensitizing cancer cells to cytotoxic chemotherapeutics- or radiation-based therapy. Current state of knowledge has revealed some pivotal mechanisms regulating core signaling molecules and

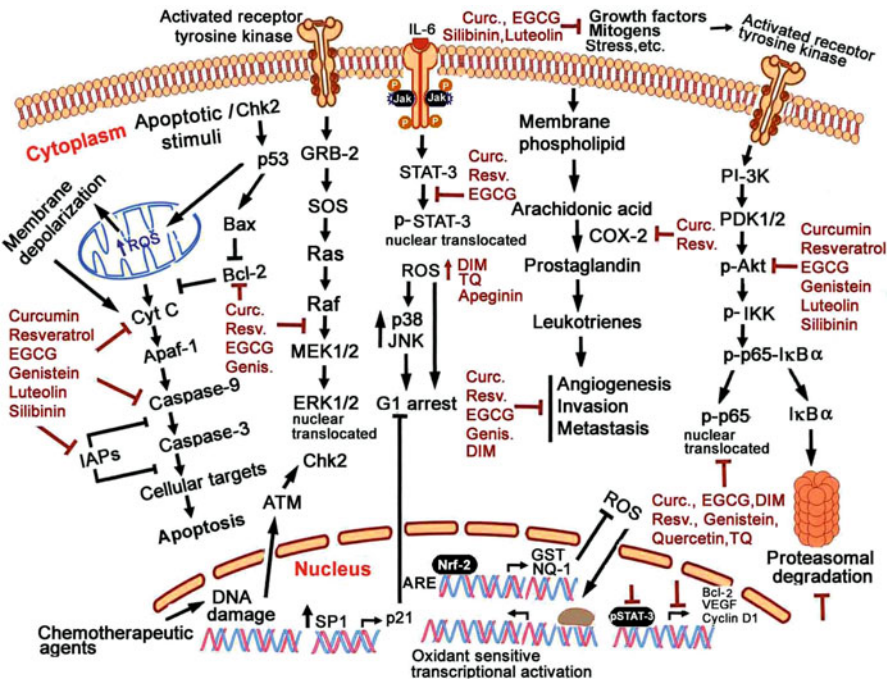

Fig. 10.1 A schematic summary of the molecular targets and cell signaling pathways altered by chemopreventive phytochemicals

apoptotic pathways. We summarize herein, a succinct overview of signaling mechanisms prevalent in most tumor types and their modulation citing representative examples of bioactive compounds that owe their origin to common food components (Fig. 10.1) and currently under clinical trials (www.clinicaltrials.gov). Conceptually, this justifies a strong rationale towards development of novel multitargeted pharmaceutical grade analogs primarily intended to abrogate key mitogenic signaling pathways within acceptable therapeutic window. Finally, continued focus could translate this aspect into novel therapeutic adjunct for treatment of different site specific cancers and attainment of long term disease free survival.

10.2 Regulation of the Akt Pathway

Akt is an evolutionarily conserved serine/threonine kinase and one of the most frequently hyperactivated signaling pathways in human cancers. Akt signaling and targeting the Akt protein kinase for cancer chemoprevention has been extensively reviewed (Vivanco and Sawyers 2002; Altomare and Testa 2005; Crowell et al. 2007). The principle role of Akt is to facilitate growth factor mediated cell survival, being negatively regulated by the tumor suppressor phosphatase and tensin

homolog on chromosome 10 (PTEN). Akt pathway does not operate in isolation but instead numerous studies point to Akt as a central molecule that tangentially integrates and cross-talk with other signaling entities including among others: receptor tyrosine kinase signaling, NF-κB signaling and extra cellular regulated kinase (ERK) signaling cascade. Additionally, it antagonizes the action of proapoptotic protein BAD (Hayakawa et al. 2000). Akt is essentially an inactive cytosolic protein which is recruited to the plasma membrane through binding by growth factor and becomes active through phosphorylation at two key sites: threonine 308 and serine 473 resulting in its full activation. Once activated, it dissociates from the plasma membrane and translocates to phosphorylate both cytoplasmic and nuclear target proteins, most notably glycogen synthase kinase (GSK)-3β, $p27^{Kip}$, mammalian target of rapamycin (mTOR) and forkhead transcription factor (Scott et al. 1998; Brunet et al. 1999; Testa and Bellacosa 2001; Shin et al. 2002); several of these are related to regulation of proliferation, progression, invasion and evasion of apoptosis. In different cell types it has been documented that diminution of phosphorylated Akt leads to induction of apoptosis and therefore there has been immense interest in developing novel Akt inhibitors for the treatment of cancer. Akt also counteracts the action of chemotherapeutics and radiation therapy leading to the development of chemo- and radio-resistance. In breast and pancreatic cancer cells with constitutively active Akt, the ability of standard chemotherapeutics and radiation to induce apoptosis is compromised (Sun et al. 2001; Arlt et al. 2003; Luo et al. 2003; Kucab et al. 2005). In contrast, PI3K/Akt inhibitors sensitize cancer cells to chemotherapy suggesting the importance of inhibition of phosphorylated Akt signaling as an important therapeutic target for cancer treatment.

Many chemopreventive agents have been documented to inhibit cancer cell growth and induce apoptosis through the inhibition of the Akt pathway (Crowell et al. 2007; Ko and Auyeung 2013). A few examples include curcumin, selenium, the flavonoids quercetin, kaempferol genistein, apigenin, and silibinin (Chaudhary and Hruska 2003; Gong et al. 2003; Spencer et al. 2003; Mallikarjuna et al. 2004; Way et al. 2004; Wu et al. 2006; Jeong et al. 2009). Kaempferol found in tea, propolis and grapefruit inhibits UV-B induced phosphorylation of Akt signaling suggesting kaempferol as a putative antitumor promoting agent (Lee et al. 2010b). Epigallocatechin-3-gallate (EGCG), a polyphenol constituent present in green tea, promotes apoptosis in T24 human bladder cancer cells by inhibiting PI3K/Akt activation that in turn, results in modulation of Bcl-2 family proteins, leading to enhanced apoptosis (Qin et al. 2007). Thymoquinone, derived from *Nigella sativa*, has been found to inhibit tumor growth and angiogenesis through downregulation of PI3/Akt pathway (Yi et al. 2008). In $Apc^{Min/+}$ mice, oral administration of the green tea component EGCG, or cruciferous vegetable component sulforaphane exhibited marked chemopreventive effects in association with inhibition of Akt signaling (Ju et al. 2005; Hu et al. 2006). Furthermore, other chemo protective bioactive derivatives from cruciferous vegetables: Phenethyl isothiocyanate (PEITC) and 3,3′-diindolylmethane (DIM) exhibited inhibition of the angiogenic features of human umbilical vein endothelial cells *in vitro* associated with the inactivation of Akt suppressing vascular endothelial growth factor (VEGF) secretion and down regulating VEGF receptor 2 protein levels (Xiao and Singh 2007; Kong et al. 2008). The growth inhibitory effect of SR13668, a synthetic

analog of DIM, in breast, ovarian and prostate xenografts *in vivo* and cell lines *in vitro* correlate with decreased pAkt and p-GSK-3β expression (Chao et al. 2007). In a study involving sulforaphane treated ovarian cancer cells both total Akt protein and active phosphorylated Akt (Ser473) were significantly decreased, signifying the inhibitory effect of sulforaphane on the Akt pathway (Chaudhuri et al. 2007). Deguelin, a member of the rotenoid family with chemopreventive activities has been found to decrease tumor incidence in animal models for lung, colon, mammary, and skin carcinogenesis through Akt inhibition (Udeani et al. 1997; Murillo et al. 2003; Gills et al. 2005; Lee et al. 2005a; Nair et al. 2006). Deguelin is also effective in reducing pAkt levels in the lung of Akt-inducible transgenic mice stimulating apoptosis and suppressing proliferation of premalignant and malignant human bronchial epithelial cells at doses in which only minimal effects were observed in normal bronchial cells (Lee et al. 2005a; Yan et al. 2005). We and other investigators have found that isoflavone genistein inhibit cancer cell growth and induce apoptosis through the downregulation of Akt in breast, lung, ovarian, prostate and pancreatic cancer cells (Li and Sarkar 2002; Gong et al. 2003; Banerjee et al. 2007). Collectively, these results reflect that Akt is an important target for action of chemopreventive agents in cancer prevention and towards therapeutic approach.

As mentioned previously evidence has also shown that activated Akt is critical for acquiring drug resistance in multiple cancers types (Kim et al. 2005a; Han et al. 2006; McCubrey et al. 2007; Tazzari et al. 2007; Huang and Hung 2009). This leads one to anticipate downregulation of Akt by chemopreventive agents would sensitize cancer cells to chemo- or radio-therapy. We and other investigators reported enhancement of chemotherapeutic and radiation effects by isoflavone genistein being partially mediated by the inhibition of Akt signaling (Akimoto et al. 2001; Banerjee et al. 2005; Yashar et al. 2005). It has been found that genistein also enhanced necrotic-like cell death with the significant inhibition of Akt activity in breast cancer cells treated with genistein and Adriamycin, suggesting that the enhanced growth inhibition by combination treatment is through the inactivation of the Akt pathway (Satoh et al. 2003). Phenoxodiol, one of the synthetic derivatives of genistein, inhibits Akt signaling pathway and subsequently activates the caspase system inhibiting X-linked inhibitor of apoptosis protein (XIAP), which in effect lead to increased chemosensitization (Kamsteeg et al. 2003). Curcumin, the polyphenol from the plant *Curcuma longa*, downregulates taxol-induced phosphorylation of Akt along with its interaction with NF-κB, revealing insight into improved antitumor effect of curcumin being mediated through the inactivation of the Akt and NF-κB pathways (Bava et al. 2005). In light of these prospective reporting's, it is believed that in future, Akt inhibition is likely to emerge as a prognostic marker for patient risk stratification.

10.3 mTOR Signaling

mTOR signaling is one of the major downstream signaling targets of PI3K/Akt and plays a critical role among other functions in promoting cellular proliferation and inhibiting apoptosis (Alayev and Holz 2013). mTOR exists as two functionally

distinct complexes: mTORC1 and mTORC2, differing in subunit compositions and biological functions. Dysregulated mTORC1 signaling is often observed in human tumors. Initially, rapamycin and its derivatives, CCI-779 and RAD001, showed much promise in clinical settings but later due to drug mediated hyperactivation of the Akt and ERK-MAPK pathways, increased tumor cell viability and drug resistance became prominent. Due to such unfavorable clinical activity in a limited number of tumor types, it has now been rationalized to combine drugs which inhibit both signaling network in therapeutic front. Recently, it has been reported that the combined use of resveratrol and rapamycin resulted in modest additive inhibitory effects on the growth of breast cancer cells, mainly through suppressing rapamycin-induced AKT activation (He et al. 2011). Similar findings have been reported in human glioma cells wherein rapamycin further enhanced resveratrol induced apoptosis (Jiang et al. 2009).

One study by our group reported using a newly recognized platelet-derived growth factor (PDGF)-D overexpressing PC3 cells (PC3 cells stably transfected with PDGF-D cDNA and referred to as PC3 PDGF-D) exhibited rapid growth rates and enhanced invasion associated with the activation of mTOR and reduced Akt activity (Kong et al. 2008). Interestingly, Bio-response DIM® (B-DIM, a formulated DIM with improved bioavailability) significantly inhibited both mTOR and Akt in these cells which correlated with decreased cell proliferation and invasion and elicited other beneficial therapeutic effects by inhibiting both mTOR and Akt activity (Kong et al. 2008).

PEITC inhibits mTORC1 activity and along with inhibition of mTORC1 contribute to optimal growth inhibition including the angiogenesis regulator HIF-1α RNA translation in MCF7 breast cancer cells (Cavell et al. 2012).

Beevers et al. (2009) reported that curcumin inhibited phosphorylation of the mTOR and its downstream effector molecules by dissociating raptor, a protein component of mTORC1 complex, from mTOR. Silibinin, a milk thistle plant derivate, inhibits translation initiation by inhibiting the mammalian target of rapamycin signaling pathway (Lin et al. 2009).

10.4 MAPK Signaling

The ubiquitous MAPK cascade pathway integrates various external stress signals into intracellular responses that dictate decision on cell fate for death or to survive and proliferate, and therefore MAPK signaling has received increasing attention as a target molecule (Sebolt-Leopold 2000; Wagner and Nebreda 2009; Santarpia et al. 2012). Accumulating evidence indicate constitutive and inappropriate activation of MAPK, due to amplified or over expressed growth factor receptors, along with oncogenic *Ras* as a critical component in a number of solid malignancies such as breast, prostate and gastric cancers (Dhillon et al. 2007; Kim and Choi 2010). Physiological interventions of the MAPK cascade by bioactive phytochemicals have been acknowledged as a promising approach to cancer therapy by several investigators. There are three distinct but parallel MAPK cascades identified in

mammalian cells: ERK, c-Jun N terminal kinase (JNK) and p38 (Cano and Mahadevan 1995). ERKs can be activated by mitogens and growth factors while JNK and p38 signaling can be activated by many environmental stress stimuli such as UV and γ-irradiation, as well as many anticancer drugs e.g. cisplatin or etoposide (Chen et al. 1996; Wada and Penninger 2004). Some chemopreventive agents exhibit selectivity, but ultimately the relative level of activation of ERK, p38 and JNK influences the cells decision to proliferate or undergo apoptosis or exit cell cycle (Xia et al. 1995). Constitutive activation of the ERK and p38 MAPK pathway have also been implicated in chemoresistance and therefore pharmacological inhibition of MAPKs hold additional promise in stimulating drug resistant cancer cells to undergo apoptosis upon treatment with chemotherapeutic drugs (Zhao et al. 2006; Guo et al. 2008). MEK1 is an important downstream component of MAPK signaling possess unique binding pocket adjacent to its adenosine triphosphate (ATP)-binding site. Computer modeling indicate that several phytochemicals including flavones quercetin, myricetin and equol can dock with this allosteric pocket strongly inhibiting MEK1 kinase activity (Ohren et al. 2004; Kang et al. 2007a; Lee et al. 2007, 2008).

Kong et al. (2000) reviewed flavonoids (EGCG, ECG) and isothiocyanate class of chemopreventive compounds on MAPK signaling and concluded high concentrations of chemopreventive compounds lead to activation of the caspase pathway of apoptosis and potentiate cytotoxicity. Similar observations have been reported by other investigators revealing inhibition of UVB-induced phosphorylation of ERK1/2, JNK, and p38 proteins by a variety of chemopreventive phytochemicals including silibinin, proanthocyanidins, apigenin, etc. EGCG inhibited the expression of ERK1/2 phosphorylation up to 93 % in the dorsolateral prostate of transgenic TRAMP mice (Harper et al. 2007). DNA microarray analysis of PC3 prostate cancer cells exposed to indole-3-carbinol or DIM rich in cruciferous vegetables showed parallel down regulation in the expression of upstream kinases MAP2K3, MAP2K4, MAP4K3, and MAPK3 (Li et al. 2003). Curcumin inhibits the activation of MAPK and shown to inhibit JNK activation induced by various agonists including PMA, ionomycin, anisomycin, UV, gamma radiation, tumor necrosis factor (TNF) and sodium orthovanadate (Chen and Tan 1998; Kim et al. 2005b). Furthermore, curcumin attenuates experimental colitis through a reduction in the activity of p38 MAPK and reduce drug resistance by its inhibitory effect on MAPK signaling (Salh et al. 2003; Sreekanth et al. 2011; Tsai et al. 2011). Resveratrol treatment on the skin of ICR mice resulted in a decrease in the ERK, as well as a decrease in catalytic activity of p38 MAPK (Kundu et al. 2004). Another study revealed topical application of resveratrol prevented UV-B light induced cutaneous damage including skin cancer by diminishing UVB-mediated upregulation of upstream kinase-MAPK kinase and MAPK signaling (Reagan-Shaw et al. 2004).

Gingerol, the principle active constituent of ginger, inhibits COX-2 expression by blocking the activation of p38 MAPK and NF-κB in phorbol ester induced mouse epidermis (Kim et al. 2005c). Transcriptome and proteome profiling of colon mucosa of quercetin fed rats point to tumor preventive mechanism through downregulation of potentially oncogenic MAPK *in vivo* (Dihal et al. 2008).

Xue et al. (2005) showed that DIM can upregulate the expression and stimulate secretion of interferon-gamma (IFN-γ) in human MCF-7 breast cancer cell line which was shown to be mediated by activation of both JNK and p38 pathways. This novel observation offer an important clue that explains the anticancer effects of DIM, because it is well known that IFN-γ plays an important role in preventing the development of primary and transplanted tumors. Patten and DeLong (1999) reported increased activation of JNK in colon cancer cells upon treatment with benzyl isothiocyanate, and suggest that most likely such activation is involved in the induction of cytoprotective enzyme – NAD(P)H: quinine oxidoreductase. In breast cancer cells, trans-resveratrol induced apoptosis by activating the MAPK pathway (Filomeni et al. 2007). Guggulsterone, found in the resin of the plant *Commiphora wightii* induced prostate cancer cell undergo apoptosis instigated by ROS dependent JNK activation (Singh et al. 2007). These pieces of evidence clearly show that depending on the context of simulus and cell system, the modulation of MAPK signaling by bioactive natural products can be exploited to formulate targeted cancer therapy.

10.5 Regulation of the Growth Factor Signaling Pathway

Over the past decade evidence has emerged revealing the functional relationship and molecular characterization between growth factor specific receptors (GFR), and their ligands that drives cell proliferation and tumor growth. Typically, in a normal tissue, cell proliferation is initiated by the binding of extracellular growth factors with GFR that reside either at the cell surface or in the cytoplasm. This interaction then triggers a signaling cascade mediated by the assembly of signaling complexes *via* specific protein-protein interactions, or in case of cell surface GFR, the activation of multiple protein kinases. These process are held tightly in check by counter balancing signals in normal tissues but are activated by a variety of genetic defects in tumor cells that either lead to a constitutive activation or the loss of negative regulatory signals resulting in activating cellular proliferation along with suppression of apoptotic pathway and stimulating invasion and metastasis. Some of the growth factors implicated in carcinogenesis are epidermal growth factor (EGF), PDGF, fibroblast growth factors (FGFs), transforming growth factors (TGF-α and -β), erythropoietin (Epo), insulin-like growth factor (IGF), interleukin (IL)-1, 2, 6, 8, TNF, INF-α and colony-stimulating factors (CSF). The signaling initiated through their respective receptors has significant impact on tumorigenesis and amenable by many chemopreventive and chemotherapeutic agents. Several chemopreventive phytochemicals including curcumin, genistein, resveratrol and catechins have been shown to be potent inhibitors of several growth factor signaling pathways.

Curcumin inhibits ligand-stimulated activation of EGFR through its inhibitory effect on EGFR phosphorylation indicating that it has the potential to break the autocrine loops that are established in several advanced cancers. Seminal findings also indicate that curcumin enhances the growth inhibitory effects of 5-flurauracil

(5-FU) and oxaliplatin through EGFR and IGF receptor pathways (Korutla et al. 1995; Dorai et al. 2000; Patel et al. 2008). Blocking EGF receptor directs the cancer cells to enter apoptosis and also abrogate the invasive potential of the cancer cells. The molecular mechanism by which EGCG and other catechins exert their protective effects towards dysregulated receptor tyrosine kinases (RTKs) in cancer cells have recently been summarized by Larsen et al. (2010). It has been concluded from ligand binding assays that EGCG blocks the binding of EGF to its receptor to cause inhibition of EGFR phosphorylation (Fu and Chen 2006). In addition, it has been found that tea catechins inhibits receptor expression through a complex circuitry by inhibiting the activity of ERK which regulates the transcription factor Egr-1; it has been found that Egr-1 controls the expression of EGFR (Fu and Chen 2006). Inhibition of EGFR signaling has also been shown to decrease the production of VEGF in cancer cells (Masuda et al. 2002). Adachi et al. demonstrated that EGCG disrupts lipid order and membrane organization to cause internalization of EGFR such that EGF could no longer bind (Adachi et al. 2008). EGCG has also been shown to bind directly to EGF, VEGF and PDGF ligands (Kondo et al. 2002; Suzuki et al. 2004; Shimizu et al. 2008). Furthermore, EGCG treatment potentiated the effects of the tyrosine kinase inhibitor erlotinib in head and neck tumors (Masuda et al. 2001; Zhang et al. 2008). In pancreatic cancer, growth inhibition and apoptosis were associated with inhibition of EGFR tyrosine kinase activity by flavonoid luteolin, present abundantly in several green vegetables (Lee et al. 2002). Luteolin also inhibits VEGF induced angiogenesis and tumor growth in a murine xenograft model (Bagli et al. 2004). Quercetin is a potent inhibitor of EGFR tyrosine kinase activity. However, it does not directly inhibit EGFR, but interferes with different signaling pathways downstream of EGFR that regulate cell proliferation and survival (Jung et al. 2010a, b; Lee et al. 2004). The growth inhibitory and apoptotic effects of silibinin in prostate cancer cells could be achieved by targeting EGFR signaling. Silymarin and silibinin are also effective in inhibiting TGF-α and EGF-mediated tyrosine phosphorylation of EGFR along with its adapter protein Shc in androgen independent human prostate cancer cells harboring constitutively active EGFR (Zi et al. 1998; Sharma et al. 2001). IGF's are mitogenic ligands, their activity being firmly controlled by the presence of IGFBPs. Similar to EGFR, IGF receptor (IGFR) signaling also promotes growth and survival of cancer cells. It has been shown that silibinin, apigenin, EGCG and inositol hexaphosphate downregulates IGF-IR signaling and significantly increases the levels of IGF binding protein-3 (IGFBP-3) in prostate cancer inhibiting cell growth both *in vitro* and *in vivo* (Singh et al. 2002, 2004; Shukla et al. 2005).

10.6 Regulation of the Cyclin D/Cyclin-Dependent Kinase (CDK) Pathway

Pivotal to growth and hyperproliferation of tumor cells is deregulation of cell cycle check points and overexpression of growth-promoting cell cycle factors, such as cyclin D1 and CDKs, along with the suppression of associated CDK inhibitors.

Tumor cells progresses like normal cells through the four phases of cell cycle, G1, SG2 and M. Cyclin D1 along with component subunit of CDK-4 and CDK-6, controls the proliferative transition from G1 to S phase in the cell cycle. Cyclin D1 expression is regulated by NF-κB and the suppression of NF-κB activity by diet derived bioactive constituents have shown the potential to down regulate cyclin D1 in multiple studies. Studies from several laboratories have revealed that cyclin D1 is overexpressed in a wide variety of tumors including those derived from breast, esophagus, lung, head and neck, hepatocellular and pancreatic cancers and this can be targeted therapeutically for the treatment of cancer (Bartkova et al. 1994; Nishida et al. 1994; Adelaide et al. 1995; Gansauge et al. 1997; Caputi et al. 1999; Kim and Diehl 2009). To this end, not only inhibitors of cyclin D are being investigated, but also other component subunits that affects the complex signaling networks that could be of major clinical use as potentiators of standard chemotherapeutic drugs and/or radiation therapy. Curcumin, resveratrol, diosgenin, sulphorophane, thymoquinone, lupeol, DIM, genistein, acetyl-11-keto-boswellic acid, and betulinic acid have all been reported to down regulate cyclin D1 expression or its component subunits in different site specific cancers (Aggarwal and Shishodia 2006).

We earlier reported that curcumin can completely downregulate cyclin D1 expression through both transcriptional and post transcriptional mechanism(s) and showed a decrease in the formation of cyclin D1/Cdk4 enzyme complex resulting in suppression of proliferation and induction of apoptosis (Mukhopadhyay et al. 2002; Bharti et al. 2003b). Corollary to our findings, another independent study reported curcumin upregulated Cdk inhibitors (such as p21/Cip1/Waf1 and p27/Kip1) and downregulated cyclin B1 and Cdc2 (Park et al. 2002b). CDK inhibitors (such as the p21/Cip1/waf1 and p27Kip1 proteins) attenuate formation of these complexes and block cell cycle progression. 3′,4′,7-trihydroxyisoflavone, a metabolite of the soybean isoflavone daidzein, is a direct inhibitor of CDK2 and CDK4 (Lee et al. 2010a).

Citing other examples relating to modulation of cyclin D1 by chemopreventive agents, Benitez et al. (2007, 2009) found that treatment of prostate cancer LNCaP (androgen receptor positive) and PC-3 (androgen receptor negative) cells with resveratrol caused a significant reduction not only in the levels of expression of cyclins D1, E and CDK4, but also a reduction in cyclin D1/CDK4 kinase activity compromising cells capability to proliferate and also causing an increase in apoptosis in time- and dose-dependent manner. The predominating tea polyphenol-EGCG treatment to LNCaP and DU145 prostate cancer cells resulted in significant dose- and time-dependent downregulation of cyclin D1, cyclin E, cdk2, cdk4, and cdk6 (Gupta et al. 2003). Furthermore, in an *in vivo* model of intestinal tumorigenesis in $Apc^{Min/+}$ mice, feeding grape seed extract reduced the total number of intestinal polyps compared to control mice (Velmurugan et al. 2010). The findings paralleled with decreased cyclin D1 and c-Myc protein levels in small intestine along with downregulation in expression of other important molecules such as: COX-2, iNOS, β-catenin along with increased expression Cip1/p21 with reduced cell proliferation and increased apoptosis (Velmurugan et al. 2010). Thus, the suppression of cyclin D1 by natural agents could be an effective strategy in abating the proliferation of tumor cells *in vivo*.

We and other investigators have also shown that the overexpression of cyclin D1 contribute to the chemoresistance of pancreatic cancer cells because of the dual role of cyclin D1 in promoting cell proliferation and inhibiting drug induced apoptosis (Gansauge et al. 1997; Biliran et al. 2005). Therefore, we speculate that chemopreventive agents which downregulates c-Myc, cyclin D and CDK could be used in combination with chemotherapeutic agents to improve the treatment outcome in cancer therapy.

Kornmann et al. (1998) reported suppressing cyclin D1 expression in human pancreatic cancer cells not only inhibited pancreatic cell growth but also increased the cytotoxic actions of cisplatinum. The findings imply the dual role of cyclin D1 in promoting cell proliferation and further imply that cyclin D1 is critical in the maintenance of chemoresistance in these cells (Kornmann et al. 1998, 1999). In similar context, we also reported that cyclin D1-overexpressing Ela-myc pancreatic tumor cells exhibit significantly reduced chemosensitivity and a higher survival rate upon cisplatin treatment (Biliran et al. 2007).

10.7 Regulation of the c-Myc Pathway

c-Myc is an oncogene overexpressed in many types of human cancers leading to deregulated proliferation and survival of cancer cells through autonomous growth factor signaling. Intriguingly, c-Myc is also a strong inducer of cell death *via* induction of several proapoptotic signal transduction mechanisms; paradoxically, the proapoptotic signals generated by c-Myc become suppressed due to parallel activation of survival signals by the same growth factor signaling pathway. According to currently accepted dogma, c-Myc induced apoptosis involves first the activation of p19/ARF which sequesters Mdm2 into the nucleolus, this leads to a reduced ubiquitination of p53 by Mdm2 which causes subsequent accumulation of p53. In turn, p53 upregulates Bax and downregulates Bcl-2, this directly activates the apoptotic machinery. However, in many forms of human cancer both a mutation of *p53* and *Bax* or a hypermethylation of *p19/ARF* or amplification of *Mdm2* makes the apoptotic pathway redundant and the proliferative pathway overrides. Almost all types of human cancers show high frequencies of *c-Myc* amplification or overexpression of its protein product c-Myc. c-Myc can induce cyclin D1 which interacts with CDK4 and CDK6 to promote cell cycle progression (Daksis et al. 1994; Mateyak et al. 1999). As reported previously, we found that ectopic overexpression of *c-Myc* in human and murine pancreatic cancer cells resulted in increased sensitivity to cisplatin along with other chemotherapeutic drugs (Biliran et al. 2007). It has been reported that curcumin inhibits the expression of *c-Myc* and tumorigenesis (Kakar and Roy 1994; Han et al. 1999; Lin 2004). Other chemopreventive agents, including EGCG, sulforaphane and quercetin also show their ability to downregulate *c-Myc* (Csokay et al. 1997; Lin 2002; Tao et al. 2002; Bertl et al. 2006; Manna et al. 2006). Downregulation of *c-Myc* has been shown to be a critical molecular event of resveratrol mediated anti-medulloblastoma activity

causing growth suppression, cell cycle arrest and apoptosis of medulloblastoma cells (Zhang et al. 2006).

It has been reported that some chemotherapeutics including cisplatin, doxorubicin, paclitaxel and 5-FU can induce c-Myc expression (Park et al. 2002a). Interestingly, tumor cells surviving from cisplatin treatment display significant elevation in *c-Myc* expression (Walker et al. 1996). Thus, c-Myc could be an interesting potential chemoresistance and growth proliferative factor in cancer.

10.8 Regulation of the NF-κB Pathway

The NF-κB signaling plays critical roles in the control of cell proliferation, survival, inflammation, tumor invasion, metastasis, drug resistance and stress response. A large number of cancer cells, especially poorly differentiated cancer cells, show activated NF-κB in the nucleus, wherein it induces the expression of more than 200 genes that have been shown to suppress apoptosis, cause neoplastic transformation and promote cancer cell growth. So, it seems logical to target NF-κB for the prevention and/or treatment of cancer. Briefly, under non-stimulating conditions, NF-κB is sequestered in the cytoplasm through tight association with NF-κB inhibitory protein IκBα and following stimulation by factors such as cytokine binding to its receptor, free radicals, inflammatory stimuli, endotoxins, tumor promoters, UV light and X-rays, activation of IκB kinase (IKK) complex occurs. This leads to phosphorylation with subsequent degradation of the inhibitory protein IκB, allowing NF-κB to translocate into the nucleus and bind to specific DNA sequences in target genes designated as κB-elements. In addition, the expression of NF-κB target genes is also regulated through the recruitment of coactivators together with corepressors (Aggarwal 2004). Many of the target genes that are activated in cancer are critical to the establishment of the early and late stages of aggressive cancers. Examples include expression of cyclin D1, apoptosis suppressor proteins (such as Bcl-2 and Bcl-xL), and those required for metastasis and angiogenesis (such as matrix metalloproteases (MMP) and VEGF) (Aggarwal and Shishodia 2006). Akt pathway is also known to activate NF-κB (Aggarwal 2004).

Accumulating evidence from several laboratories including ours have concluded that several bioactive agents originating from dietary sources (like curcumin, resveratrol, guggulsterone, ursolic acid, betulinic acid, emodin, gingerol, flavopiridol, zerumbone, evodiamine, indole-3-carbinol, ellagic acid, anethole, green tea catechins, S-allyl cysteine, lycopene, diosgenin, garcinol, plumbagin, silibinin, thymoquinone, sulforaphane) have been found to be potent inhibitors of NF-κB (Aggarwal and Shishodia 2006; Banerjee et al. 2009a; Ahmad et al. 2010; Shanmugam et al. 2011). These inhibitors block one or more steps in the NF-κB signaling pathway such as the signals that activate the NF-κB signaling cascade, its translocation into the nucleus, DNA binding of the dimers, or interactions with the basal transcriptional machinery. Sulforaphane inhibits NF-κB DNA binding without affecting its translocation to the nucleus or phosphorylation of IκB (Heiss et al. 2001). In an *ex vivo* study reported by

our group, human volunteers received 50 mg of soy isoflavone supplements Novasoy™ (Archer Daniels Midland Company, Decatur, IL, USA, containing genistein, daidzein, and glycitein at a 1.3:1:0.3 ratio) twice daily for 3 weeks. TNF-α failed to activate NF-κB activity in lymphocytes harvested from these volunteers, while lymphocytes from these volunteers collected prior to soy isoflavone intervention showed activation of NF-κB DNA binding activity upon TNF-α treatment *in vitro* (Davis et al. 2001). These results demonstrated that soy isoflavone supplementation had a protective effect against TNF-α induced NF-κB activation in humans. DIM or the formulated B-DIM treatment could inactivate NF-κB DNA binding activity in prostate, breast, head and neck, and pancreatic cancer cells resulting in inhibition of NF-κB downstream genes-VEGF, urokinase-plasminogen activator and MMP-9 limiting cell growth concurrent with induction of apoptosis and inhibition of angiogenesis, invasion, as well as metastasis of tumor cells (Banerjee et al. 2011b).

5-FU, cisplatin, carboplatin, taxol, and gemcitabine have been reported to induce NF-κB activation in different cells. Our own findings along with other investigators have reported the inhibition of NF-κB by chemopreventive agents increases the sensitivity of cancer cells to the apoptotic action of chemotherapeutic agents and radiation exposure (Li and Sethi 2010; Nambiar et al. 2011). Inhibition of chemotherapy induced NF-κB activation by super repressor IκBα protein sensitized non-small cell lung cancer and pancreatic cancer cells to gemcitabine induced apoptosis in *in vitro* and *in vivo* (Jones et al. 2000; Fujioka et al. 2003). Therefore, targeting NF-κB by chemopreventive agents appear to be a promising strategy to enhance the antitumor activity of chemotherapeutics.

10.9 Regulation of the COX Pathway

COX enzymes play an important pivotal role in an organ specific manner in the conversion of free arachidonic acid released from membrane phospholipid to prostaglandins with prostanoids *via* an intermediary reaction catalyzed by the enzyme-phospholipase A2. Two isoforms of COX enzymes have been identified: COX-1 being constitutively expressed in almost every cell type that is involved in maintaining homeostasis of normal physiological functioning such as cytoprotection of gastric mucosa, regulation of renal blood flow along with control of platelet aggregation, while COX-2- the inducible isoform which is barely detectable under normal physiological conditions accounts for the elevated production of prostaglandins in response to various inflammatory stimuli, hormones, and growth factors. Mounting evidence reveals that COX-2 is overtly overexpressed in many premalignant, malignant and metastatic human cancers, including cancer of the colon, breast, lung, stomach, head, neck, pancreas, uterine cervix, urinary and gall bladder and the skin. The level of its overexpression significantly correlates with invasiveness, angiogenesis, prognosis, and survival (Subbaramaiah and Dannenberg 2003). Further studies have shown that in many cell types, NF-κB is a positive regulator of COX-2. Besides NF-κB, AP1 is also implicated in COX-2 transcriptional activation (Scheckel

et al. 2008). Since the initial observation that COX-2 inhibitors and non-steroidal anti-inflammatory drugs (NSAIDs) decreases the risk of various cancers (including colon and lung cancers), several evidences deem that the down regulation of COX-2 could be one of the molecular mechanisms by which tumor growth can be prevented and inhibited. Hence the notion that chemopreventive agents that can block COX-2 expression without affecting COX-1 got prioritized as novel target for chemoprevention. Moreover, it has been reported that forced expression of COX-2 caused enhancement in multiple drug resistance MDR1 expression and functional activity, suggesting the existence of a causal link between COX-2 activity and MDR1 expression (Sorokin 2004). Therefore, the use of COX-2 inhibitors to decrease MDR1 function may enhance the accumulation of chemotherapy agents, and decrease the resistance of tumors to chemotherapeutic drugs. Furthermore, laboratory findings have revealed that mice deficient in COX-1 or COX-2 enzyme develop few tumors when subjected to standard DMBA/TPA protocol of tumor development.

Several dietary source derived bioactive compounds have shown the potential to suppress COX-2. Curcumin was one of the first chemopreventive phytochemical shown to possess significant COX-2 inhibiting activity through the suppression of NF-κB. We reported earlier that resveratrol suppressed mammary carcinogenesis in female Sprague Dawley rats and this suppression was in part associated with the inhibition of COX-2 and NF-κB activation. Other reports indicate that resveratrol inhibits COX-2 enzyme activity in phorbol ester treated human mammary epithelial cells through suppression of protein kinase C and AP-1 mediated gene expression abridging logical conclusion that both NF-κB and AP-1 can bind the COX-2 promoter and upregulate transcription of COX-2 gene (Newton et al. 1997; von Knethen et al. 1999; Allport et al. 2000). Thus, bioactive compounds that can suppress these transcription factors may have the putative potential to inhibit COX-2 expression. Furthermore, the effect of resveratrol pretreatment on 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced COX-2 expression in the skin of mice revealed an inhibitory effect in a dose-dependent manner (Kundu et al. 2004). Resveratrol also suppresses *N*-nitrosomethylbenzylamine-induced rat esophageal tumorigenesis with evidence of COX-2 overexpression at tumor site (Li et al. 2002). According to Zykova et al. (2008), resveratrol directly targets COX-2 to inhibit carcinogenesis. Ginger, an extensively consumed spice material and its constituents have shown to inhibit COX-2 and inducing apoptosis (Kim et al. 2005c). Gene expression analysis of sulforaphane treated HeLa cells reveals significant downregulation of COX-2 (Sharma et al. 2011). In LPS-activated murine macrophage cell line RAW264.7, the monoterpene D-limonene from orange peel oil, decreased the expression of COX-2 proteins along with the principle COX-2 product prostaglandin-E2 (PGE-2) production (Yoon et al. 2010). In COX-2 overexpressing pancreatic cancer cells, we reported thymoquinone and its analogs downregulated COX-2 protein along with reduction in PGE-2 (Banerjee et al. 2009a, 2010). Many other dietary source derived components, including galangin, luteolin, apigenin, kaempferol, sasanquol, genistein, wogonin, green tea catechins, have been shown to suppress COX-2 (Aggarwal et al. 2006; Koeberle et al. 2009; Lee et al. 2010c).

Green tea polyphenols have been reported to reduce COX-2 expression (24 %) in the TRAMP mice model (Harper et al. 2007). A reduction in invasive potential of highly metastatic human melanoma by EGCG has been recorded by targeting the endogenous expression of COX-2 and prostaglandin receptors (Singh and Katiyar 2011). However, the mechanism of EGCG-induced COX-2 inhibition appears to be through the suppression of transcription factor NF-κB compared to the protein binding/inhibition of COX-2 inhibitors (Smith et al. 2000). Other experimental studies have shown that curcumin and EGCG could down regulate COX-2 expression without any change in the expression of COX-1 at both the mRNA and protein levels in prostate cancer cells (Hussain et al. 2005). EGCG downregulates COX-2 in TPA-stimulated human mammary cells (MCF-10A) in culture (Kundu et al. 2003). Furthermore, green tea catechins (EGCG and EGC) as well as theaflavins from black tea inhibited COX-dependent arachidonic acid metabolism in microsomes from tumors and normal colon mucosa, indicative of tea polyphenols affecting arachidonic acid metabolism in human colon mucosa and also colon tumors reducing the risk for developing colon cancer in humans (Hong et al. 2001), a premise currently under clinical trial (NCT01360320, Table 10.1).

The synergistic growth inhibitory effect of curcumin and celecoxib has been demonstrated in colorectal cancer cells through the inhibition of COX-2 pathway (Lev-Ari et al. 2005). Genistein downregulates COX-2 promoter activity in colon cancer cells transfected with COX-2 reporter gene (Mutoh et al. 2000). Combination of 5-FU and isoflavone genistein revealed greater therapeutic effects than single agents in colon cancer through the COX-2 pathway (Hwang et al. 2005). Inhibition of tumor growth independent of the COX-2 pathway cannot be ruled out; further indepth mechanistic studies are needed to fully elucidate the mechanism of action of COX-2 inhibitors toward cancer prevention and therapy.

10.10 Regulation of the STAT Pathway

The STAT family of cytoplasmic latent transcription factors came to light while examining the transcriptional activation in response to interferon and other extracellular signal proteins (Darnell et al. 1994). Of the seven STAT family members identified till date with all sharing common structural elements, aberrant constitutive activation of STAT-3 have been identified in a number of human cancers including breast, lung, ovarian, pancreatic, skin, prostate and in multiple myeloma, leukemia and lymphomas. STAT-3 is tyrosine phosphorylated by three types of kinases: receptor tyrosine kinases (such as EGFR, FGF receptor, or PDGF receptor), Janus kinase family members which are constitutively bound to the cytoplasmic tails of cytokine receptors or non receptor associated tyrosine kinases (including Ret, Src, or the Bcl-Abl fusion proteins) (Brantley and Benveniste 2008). Active STAT-3 dimers bind to consensus sequences in the promoters of genes regulating cell proliferation and antiapoptotic behavior in cooperation with other transcription factors to regulate expression of genes, such as *Bcl-2* and *Bcl-xL, Mcl-1*, $p21^{WAF1/CIP1}$ along with cyclin D1 (Bowman et al. 2000; Reich and Liu 2006; Germain and Frank 2007).

Table 10.1 Current ongoing clinical trials

Genistein/isoflavone	
NCT01028001	Phase 2 trial of preoperative soy isoflavone supplementation and molecular markers in the prevention of head and neck squamous carcinoma
NCT01036321	Phase 2 clinical trial of purified isoflavones in prostate cancer: comparing safety, effectiveness and mechanism of action between African American and Caucasian men
NCT01174953	High risk prostate cancer prevention study
NCT01126879	Phase 2 trial of genistein in men with circulating prostate cancer cells
NCT00499408	Phase 2 trial of vitamin D and soy supplementation for biochemically recurrent prostate cancer following definitive local therapy
NCT01489813	Phase 2 randomized placebo-controlled clinical trial of genistein in reducing the toxicity and improving the efficacy of intravesical therapy
NCT01538316	Clinical trial on the effectiveness of the flavonoids genistein and quercetin in men with rising prostate-specific antigen
NCT01325311	Phase 2a, randomized placebo-controlled trial of single high dose cholecalciferol and daily genistein (G-2535) *vs* placebo in men with early stage prostate cancer undergoing prostatectomy
NCT01182246	Safety, pharmacokinetics and efficacy of AXP107-11 in combination with standard gemcitabine (Gemzar) treatment in patients with locally advanced or metastatic, unresectable, adenocarcinoma of the pancreas, stage III-IV: a prospective, open label, multicentre, sequential phase 1b /2a study (the drug substance, AXP107-11 is a crystalline form of genistein)
3,3′ diindolylmethane	
NCT00888654	Phase 2 trial of Bio-response 3,3′-diindolylmethane on intermediate endpoint biomarkers in patients with prostate cancer who are undergoing prostatectomy
NCT01391689	Evaluation of diindolylmethane supplementation to modulate tamoxifen efficacy in breast cancer, the diindolylmethane efficacy study
Curcumin	
NCT01160302	Exploratory biomarker trial of the food substances curcumin C3 complex in subjects with newly diagnosed head and neck squamous cell carcinoma
NCT01294072	Phase 2 clinical trial investigating the ability of plant exosomes to deliver curcumin to normal and malignant colon tissue
NCT00927485	Use of curcumin for treatment of intestinal adenomas in familial adenomatous polyposis
NCT00641147	Curcumin for treatment of intestinal adenomas in familial adenomatous polyposis
NCT00689195	Evaluation of curcumin formulation and ashwagandha root powder extract in the management of advanced high grade osteosarcoma
[a]NCT00969085	Phase 2 trial of curcumin in cutaneous T cell lymphoma patients
[a]NCT01490996	Phase 1/2a study combining curcumin (curcumin C3-complex, Sabinsa) with standard care FOLFOX chemotherapy in patients with inoperable colorectal cancer
[a]NCT01238198	Oral curcumin for radiation dermatitis in breast cancer patients
[a]NCT01608139	Pilot study of curcumin, vorinostat, and sorafenib in patients with advanced solid tumors
[a]NCT01219673	Study of reducing the symptom burden produced by chemoradiation treatment for head and neck cancer
[a]NCT01269203	Phase 2 randomized study of the efficacy of curcumin for reducing symptoms during maintenance therapy in multiple myeloma patients

(continued)

Table 10.1 (continued)

Genistein/isoflavone	
Green tea/EGCG	
NCT01317953	Phase study of oral green tea extract as maintenance therapy for extensive-stage small cell lung cancer
[a]NCT01589887	Clinical and biologics evaluation of polyphenon E, an extract of green tea containing EGCG, in plasma cell dyscrasias-pilot study
[a]NCT00942422	Clinical and biologics evaluation of polyphenon E, an extract of green tea containing EGCG, in plasma cell dyscrasias-pilot study
[a]NCT00262743	Phase 1/2 study of daily oral polyphenon E in asymptomatic, Rai stage 0-II patients with chronic lymphocytic leukemia
NCT00917735	Phase 2, randomized, double-blind, placebo-controlled, study of the efficacy of Green tea extract on biomarkers of breast cancer risk in high risk women with differing catechol-*O*-methyl transferase genotypes
NCT01060345	Pilot study of chemoprevention of green tea in women with ductal carcinoma *in situ*
[a]NCT00676793	Phase 2 clinical trial to determine if polyphenon E inhibits c-Met signaling and activation of pathways contributing to breast cancer progression
[a]NCT00516243	Phase 1B randomized, double-blinded, placebo-controlled, dose escalation study of polyphenon E in women with a history of hormone receptor-negative breast cancer
NCT01360320	Minimizing the risk of metachronous adenomas of the colorectum with green tea extract
NCT00596011	Phase 2, randomized, double-blind, multi-centered study of polyphenon E in men with high-grade prostatic intraepithelial neoplasia or atypical small acinar proliferation
[a]NCT00233935	Phase 1B randomized, double-blinded, placebo-controlled, dose escalation study of polyphenon E in patients with Barrett's esophagus
[a]NCT01606124	Randomized phase 2 trial of polyphenon E *vs* placebo in patients at high risk of recurrent colonic neoplasia
NCT01116336	Phase 1 chemoprevention study with green tea and erlotinib in patients with premalignant lesions of the head and neck
NCT01032031	The effect of dietary bioactive compounds on skin health in humans *in vivo*
Resveratrol	
NCT01476592	A biological study of resveratrol's effects on Notch-1 signaling in subjects with low grade gastrointestinal tumors
NCT01489319	Evaluation of the ovarian dynamic response and the inflammatory response to oral lipid challenge in relation to body composition in polycystic ovary syndrome
Lycopene	
NCT00844792	Randomized, double-blind study of combination vitamin E, selenium and lycopene *vs* placebo in men undergoing radical prostatectomy for prostate cancer
NCT00669656	Phase 2 trial of a combination herbal therapy for men with biochemical recurrence of prostate cancer after initial local therapy

[a]Study is not yet open for participant recruitment

Under normal conditions, multiple STAT3 endogenous negative regulators such as suppressors of cytokine signaling proteins, protein inhibitors of activated STATs and protein tyrosine phosphatases (such as SHP-1 and SHP-2 that dephosphorylates

active STAT-3 complexes) abrogate STAT-3 signaling (Chung et al. 1997; Starr et al. 1997; Rakesh and Agrawal 2005).

Natural chemopreventive agents such as green tea, resveratrol and curcumin have shown to modulate STAT activation in tumor cells. In transgenic TRAMP mouse prostate cancer model, green tea polyphenol inhibited STAT3 expression precluding tumor growth and promoting apoptosis (Siddiqui et al. 2008). Zyflamend, a herbal preparation and non-selective inhibitor of cyclooxygenase activity inhibit STAT3 phosphorylation in LNCaP cells (Bemis et al. 2005). Polyphenon E, a standardized mixture of green tea polyphenols suppresses STAT-3 activation in breast cancer cells concurrent with inhibition of markers of angiogenesis (Leong et al. 2009). Resveratrol inhibits Src tyrosine kinase activity blocking STAT3 activation (Kotha et al. 2006; Bhardwaj et al. 2007). Bharti et al. (2003a) demonstrated that curcumin inhibited IL-6-induced STAT3 phosphorylation abrogating nuclear translocation of activated STAT. Administration of curcumin to athymic nude mice bearing ovarian HeyA8 tumors resulted in significant inhibition of STAT3 phosphorylation (Lin et al. 2007). Luteolin, a flavonoid abundant in green vegetables such as broccoli, cabbage celery green pepper and spinach inhibits phosphorylation of STAT3 and targets it for proteasomal degradation, in this manner inhibits the expression of cyclin D1, survivin, Bcl-xL and VEGF (Selvendiran et al. 2006). Capsaicin, a constituent of green and red peppers, suppresses both constitutive and inducible STAT3 activation pathway causing inhibition of the growth of multiple myeloma in nude mice (Bhutani et al. 2007). Thus, suppression of the STAT signaling pathway by dietary agents provides opportunities for both prevention and treatment of cancer.

10.11 Regulation of the Wnt and SHH Pathway

Our current knowledge relating to cell survival linked signaling pathways reveals existence of other prospective signaling targets, namely Sonic hedgehog (SHH) and Wnt. These pathways critically predominate during embryonic development. However, in cancer related context, activation of these pathways is linked with biological features related to acquisition of an epithelial to mesenchymal transition and cancer stem cell phenotype – the Holy Grail in cancer, that are intimately associated with cancer invasion and metastasis (Woll et al. 2008; Syn et al. 2009; Varnat et al. 2009; Takahashi-Yanaga and Kahn 2010). Available literature points to Wnt and hedgehog signaling as a vicious cycle for the maintenance for cancer cells towards aggressiveness and contributing to cancer related deaths. Wnt pathway is activated in over 85 % of sporadic colon cancers the end result of which is shortened overall survival. The role of nutraceuticals in the regulation of various components of Wnt and Sonic hedgehog signaling in cancer has been recently reviewed by our group and others (Teiten et al. 2011, 2012; Sarkar et al. 2010; Amado et al. 2011; Tarapore et al. 2012). Apigenin was the first described flavonoid as regulator of the Wnt pathway; it reduced the levels of β-catenin and disheveled

proteins and accelerated the degradation of β-catenin promoting cell cycle arrest in breast cancer cells (Song et al. 2000; Landesman-Bollag et al. 2001). Data accrued from animal study with microarray analysis showed that isoflavone genistein downregulated Wnt signaling in genistein treated animals (Su et al. 2007). Furthermore, curcumin, resveratrol, EGCG, DIM, lycopene, deguelin and the plant flavonoid fisetin have also been found to inhibit the expression of several molecules of Wnt signaling pathway in different tumor types and cells (Teiten et al. 2011, 2012; Pahlke et al. 2006; Hope et al. 2008; Wertz 2009; Syed et al. 2011). In breast cancer cells, Wnt signaling has been found to be inhibited by EGCG in a dose-dependent manner, an effect mediated by mRNA stability of a transcriptional repressor HBP1 which is a suppressor of Wnt signaling (Kim et al. 2006a). EGCG augment Wnt signaling antagonist (Wnt inhibitory factor-1) that binds directly to Wnt molecules in the extracellular space and inhibit Wnt signaling (Mazieres et al. 2004; Yang et al. 2009). In the $Apc^{Min/+}$ mouse, white and green tea extract intake, where the major compounds are catechins, reduced tumor multiplicity by inhibiting the translocation of Wnt mediator β-catenin to the nucleus (Dashwood et al. 2002; Ju et al. 2005; Bose et al. 2007). The chemopreventive effect of resveratrol in colon cancer is due to significant decrease in the amount and proportion of β-catenin in the nucleus along with reduced expression of the two regulators of β-catenin localization (Hope et al. 2008). The flavonoid silibinin is seen effective in colon tumor cell lines only where the Wnt pathway is found to be altered such as SW480 colorectal cancer cell line; silibinin treatment resulted in inhibition of cell growth, induced cell death, and decreased nuclear and cytoplasmic levels of β-catenin (Kaur et al. 2010). Quercetin suppresses the growth of several leukemia and lymphoma cells by inhibiting components of the Wnt signaling pathway (Kawahara et al. 2009).

The *in vitro* and *in vivo* effect of isoflavone genistein, EGCG and resveratrol in the regulation of hedgehog signaling has also been reported (Slusarz et al. 2010). The inhibition of hedgehog signaling correlates with delayed prostate tumor growth *in vivo* in TRAMP mice (Slusarz et al. 2010). An alternative to TRAMP model is the transgenic LADY model (Gipp et al. 2007). In this model, prostate tumors rarely produce metastasis due to lack of increased hedgehog signaling markers during tumor development, evidence that strengthens the importance of hedgehog signaling in the process of metastasis (Gipp et al. 2007).

10.12 Regulation of the Apoptotic Pathway

Apoptosis is an important defense mechanism against tumor development and the major mechanism of anticancer action of chemopreventive along with chemotherapeutic agents. It is now well recognized that there are two major signaling pathways for the induction of apoptosis: the intrinsic (or mitochondrial) pathway and the extrinsic (or receptor mediated) pathway. Apoptosis resulting from activation of either of these pathways usually precede through activation of a class of intracellular cysteine proteases (a family of caspases enzymes) that cleave a variety of

cellular substrates resulting in distinct morphological alterations associated with cell death phenomenon, most notably being the characteristic ladder-type fragmentation of the DNA in agarose gel electrophoresis. Additionally, in humans, the induction of apoptosis is closely related to normal *p53* functioning since lack of its expression or function is associated with increased risk of tumor development. Moreover, it has been well known that caspase inhibitors as well as, survival signaling molecules (such as Akt, c-Myc, NF-κB, and COX-2) intervene and antagonize the process of apoptosis. Accordingly, the premise that novel agent that may reverse the inhibition of apoptosis or induce apoptosis in cancer cells would be therapeutically advantageous. Indeed, many diet derived bioactive phytochemicals are being recognized to affect the cysteine proteases in the apoptotic pathway and shown effectiveness in downregulating survival signaling component at various levels by their interference as well as, disrupting mitochondrial membrane integrity.

Corollary to this concept, a large number of studies have emphasized the potential of chemopreventive agents (such as resveratrol, curcumin, genistein, DIM, EGCG, etc.) exerting anticancer effects by causing cell cycle arrest and inducing apoptosis in numerous different cancer cell types. The induction of apoptosis has repeatedly been portrayed to be accompanied by increased caspase activity, cell cycle arrest in G1 phase or inhibition of cell cycle progression from S to G2 phase, decrease in Bcl-2, Bcl-xL, McL-1 oncoprotein levels and augmenting proapoptotic Bax levels (Bode and Dong 2006). EGCG, the principal flavonoid constituent of green tea polyphenols inhibits Akt and NF-κB signaling which is consistent with the concept that this generally promotes apoptosis. EGCG was found to promote apoptosis in T24 human bladder cancer cells by modulating PI3K/Akt pathway and Bcl-2 family proteins (Qin et al. 2007). EGCG induced the expression of p53 and its target p21 and Bax in prostate cancer cells with wild type p53 but not with inactive p53 (Hastak et al. 2005). EGCG also activates p53 and Bax in breast carcinoma cells (Roy et al. 2005). Our initial studies identified genistein, indole 3-carbinol (I3C), DIM, thymoquinone (TQ), garcinol, plumbagin, etc. as having anticancer effects through apoptosis as their primary molecular targets in a wide range of human cancer models regardless of their p53 status. Additionally, resveratrol together with DIM has been described to extend to another fundamental biological process and shown to interfere with mitochondrial functions by inhibiting mitochondrial ATP synthesis through its binding to F1-ATPase (Gong et al. 2006; Gledhill et al. 2007; Roy et al. 2008). This leads to generation of oxidative stress in cells through stimulation of ROS release from mitochondria perturbing signal transduction pathway and links ROS within mitochondria as playing an important role in augmenting proapoptotic activity. Interestingly, combination of chemopreventive agents demonstrate an additive effect in inducing apoptosis by significantly inhibiting downstream signaling molecules of the Akt-NF-κB pathway. We reported combination of resveratrol and curcumin significantly inhibits tumor growth in SCID mice implanted with HCT-116 colon cancer cells (Majumdar et al. 2009). Analysis of our *in vitro* results suggest that curcumin or resveratrol attenuated the constitutive activation of EGFR family members as well as IGF-1R, and that together, they cause a greater inhibition in

activation of the growth factor receptors than that observed with either agent alone. Further, the inhibition of IGF-1R activation in response to either mono or combinatorial treatment could be attributed to enhanced expression of IGF-binding protein (IGFBP-3) leading to increased sequestration of IGF-1 by IGFBP-3, rendering the growth factor unavailable for binding and activation of IGF-1R (Majumdar et al. 2009). Similarly, a combination of low doses of curcumin and PEITC substantially induced apoptosis by inhibiting NF-κB and EGFR signaling while inhibiting tumor growth in immuno-deficient mice (Khor et al. 2006b; Kim et al. 2006b). Profiling the gene expression in the small intestinal polyps of $Apc^{Min/+}$ mice fed sulforaphane, revealed that several proapoptotic genes (such as *MBD4, TNF-7* and *TNF ligand-11*) were upregulated while pro-survival genes (such as *cyclin D2, integrin-E1, Wnt-9A*) were downregulated (Khor et al. 2006a).

Phenoxodiol, the genistein derivative, under phase 2 clinical trials has been reported to bind to the tumor associated NOX receptor, block its function, and subsequently inhibit the anti-apoptotic proteins XIAP and FADD-like ICE inhibitory protein, eventually inducing apoptotic cell death (Kamsteeg et al. 2003). Additional findings reported from our laboratory and elsewhere have found that isoflavone genistein, DIM, TQ, sulforaphane, EGCG combined with either doxetaxel, cisplatin, oxaliplatin, or gemcitabine significantly inhibit Bcl-2, Bcl-xL, survivin and induced p21 WAF1, supporting that the enhanced antitumor effect in combination treatment is through the regulation of these important molecular entities in the apoptotic pathway axis. Furthermore, it has been found that curcumin combined with either cisplatin or paclitaxel decreased the expression of several apoptosis related genes including: *c-Myc, Bcl-xL, c-IAP-2*, neuronal apoptosis inhibitory protein and XIAP (Aggarwal et al. 2005; Notarbartolo et al. 2005).

The extrinsic pathway regulated by cytokines, mainly CD95L and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), secreted by cytotoxic T cells or natural killer (NK) cells is now being considered as important endogenous anticancer immuno-surveillance agent inducing apoptosis selectively in tumor cells (Mantovani et al. 2008). Mechanistically, it has been shown that the binding of cytokines to members of the superfamily of TNF receptors initiates receptor oligomerization and initiate cascade of signaling events leading to the activation of caspase-8 and effector caspase-3 (Ashkenazi 2008; Thorburn et al. 2008). Intriguingly, some cancer cells are resistant to TRAIL-mediated apoptosis and studies have examined the ability of bioactive chemopreventive agents to induce apoptosis targeting the extrinsic pathway. In this context, resveratrol has been shown to induce cell death in some tumor types expressing high CD95 (Fas, APO-1) by augmenting CD95 (FasL) expression thereby manipulating CD95-CD95L system as 'apoptotic trigger' inducing cell death (Gusman et al. 2001). Resveratrol, EGCG, genistein, and sulforaphane have been shown to sensitize tumor cells, but not normal human fibroblasts, to TRAIL-induced apoptosis (Fulda and Debatin 2004; Shankar et al. 2008c). The combination of curcumin and TRAIL induces the cleavage of procaspase-3, -8, as well as -9, truncation of Bid, along with the release of cytochrome c from the mitochondria, indicate stimulation of apoptotic pathway in these cells (Deeb et al. 2003). Quercetin from onions and apples is a potent

enhancer of TRAIL-induced apoptosis in prostate and hepatocellular carcinoma cells (Jung et al. 2010a, b; Kim et al. 2008). These findings suggest that bioactive chemopreventive agents also regulate the extrinsic apoptotic pathway extending the possibility to sensitize cells to proapoptotic cytokine stimuli during cancer prevention and therapy.

10.13 Regulation of Other Pathways

The forkhead transcription factors of the O class (FOXO) plays a direct role in cellular proliferation and regulate antitumor activities in cancer cells (Singh et al. 2011; Zhang et al. 2011a, b). It has been demonstrated that resveratrol, sulphorophane EGCG cause growth arrest and induce apoptosis through activation of FOXO transcription factors (Shankar et al. 2008a; Davis et al. 2009; Roy et al. 2010, 2011). We have reported that the isoflavone genistein enhances the antitumor and antimetastatic activities of docetaxel through the regulation of osteoprotegerin/receptor activator of NF-κB (RANK)/RANK ligand/MMP-9 signaling in prostate cancer, implying that isoflavone genistein could be a promising non-toxic agent to improve the treatment outcome of metastatic prostate cancer with docetaxel (Li et al. 2006). Another study by us revealed that soy isoflavone enhances prostate cancer radiotherapy through the downregulation of apurinic/apyrimidinic endonuclease 1/redox factor-1 expression (Raffoul et al. 2007). The NSAID activated gene (*NAG-1*) is a pro-apoptotic and anti-tumorigenic gene which is regulated by several transcription factors such as p53, activating transcription factor 3 (ATF3), and early growth response gene-1 (*EGR-1*) (Baek et al. 2004). An insight into modulation of *NAG-1* status in some tumor cells reveals upregulated *NAG-1* expression by ECG and DIM resulting in poly(ADP-ribose) polymerase cleavage and apoptosis (Baek et al. 2004; Lee et al. 2005b). There are evidence suggesting that for maintenance of biological homeostasis, peroxisome proliferators activated receptors (PPARγ) are molecular targets of resveratrol and TQ in the regulation of cell proliferation (Woo et al. 2011; Ulrich et al. 2006). PPARγ are ligand activated transcription factors whose activity induces apoptosis. Further, a combination of genistein and resveratrol downregulated PPARγ resulting in an enhanced effect on apoptosis (Rayalam et al. 2007). Another important aspect relates to the potential of genistein and isoflavone analog (daidzein and glycitein) to decrease the side effects of tamoxifen by inhibiting CYP1A2; this suppresses the formation of α-hydroxy tamoxifen and its sulfate conjugate believed to be responsible for DNA adduct formation (Umemoto et al. 2001; Chen et al. 2004). Likewise, resveratrol, restrains bioactivation of polycyclic aromatic hydrocarbons, a class of ubiquitous environmental chemicals, through reduced expression of the *CYP1A1* and *CYP1B1* genes in human bronchial epithelial cells (Berge et al. 2004); increased CYP1A1 expression and activity are associated with a high risk of lung and colorectal cancer (McLemore et al. 1990; Sivaraman et al. 1994). It has been shown that DIM interferes with regulation of the estrogen-metabolizing CYP enzymes (e.g. CYP3A1/2 activity) associated with cancer susceptibility and provides

an important mechanism for preventing the tumorigenic process in estrogen-responsive sites (Parkin and Malejka-Giganti 2004).

Significant findings emerging from field of experimental tumor immunology points to the absence of functional T cells or T cell derived cytokines such as, IL-6, IL-12 and IFN-γ or NK cells as playing a role in the onset of spontaneous and carcinogen-induced tumor in mice models (Dunn et al. 2004). It is conceivable that activation of functional T cells or production of cytokines (such as INF-γ or IL-12) would mount an antitumoral immune response in some types of human cancers and contribute to cancer prevention. It has been reported that quercetin is able to enhance susceptibility to NK cell-mediated lysis of cancer cells through the induction of NKG2 (NK group-2, member D) ligand (Bae et al. 2010). EGCG enhances $CD8^+$ T cell-mediated antitumor immunity induced by DNA vaccination (Kang et al. 2007b). Genistein modulates immune responses and increase host resistance to B16F10 tumor purportedly by enhancing the activities of cytotoxic T cells and NK cells (Guo et al. 2001). Sulforaphane inhibits prostate carcinogenesis and pulmonary metastasis in TRAMP mice in association with increased cytotoxicity of NK cells (Singh et al. 2009). Based on the preceding narrative, it becomes clear that chemopreventive agents are pleiotropic and thus, could be considered as efficient multitargeted agents for the design of anticancer therapy and likely to revolutionize our approach for the prevention and treatment of cancer.

10.14 Chemosensitization of Conventional Cancer Therapeutics by Chemopreventive Agents

Classical chemotherapy often remains the most used anticancer therapy. Unfortunately, one of the persistent problems with chemotherapy is the emergence of drug resistant clones after prolonged treatment. It remains clinically challenging to effectively target this resistant population of cells exhibiting repopulating capacities, high tumorigenicity and self renewal characteristics during course of disease management. Despite implication of novel and innovative strategies such as tumor-suppressor and suicide gene-based therapy, the tumor relapse rate remains high after these treatments. We and other investigators conceptualized the potential benefit of natural agents harboring pleiotropic effect, including inactivation of survival signaling and simultaneous activation of multiple death pathways as a rationale to sensitize tumor cells to therapy. Thus, rationally designed novel cocktail regimen including conventional cytotoxic chemotherapeutic agent and a chemopreventive agent are speculative to yield beneficial outcome, including potential for reduction in adverse side effect. In this direction of development, ongoing preventive trials demonstrate the beneficial effects of chemopreventive agents, including soy isoflavone, curcumin, tea polyphenols-EGCG, ECG, NSAIDs, resveratrol, quercetin and DIM against multiple cancers. Concurring preclinical study designs using xenograft and orthotopic models have convincingly shown that

isoflavone genistein could potentiate the antitumor effects of chemotherapeutic agents (gemcitabine, docetaxel, cisplatin, oxaliplatin) and targeted drug (erlotinib) resulting in greater apoptotic cell death parallel with the inhibition of tumor growth in pancreatic, osteosarcoma, and prostate cancers (Li et al. 2004; Banerjee et al. 2005, 2007, 2011a; El-Rayes et al. 2006; Zhang et al. 2010; Liang et al. 2012). Additionally, the effect of triple combination including gemcitabine and erlotinib against background of genistein treatment revealed more potent inhibitory effect on pancreatic cancer growth *in vitro* compared to monotherapy (El-Rayes et al. 2006). In a xenograft model, we reported that combination of curcumin and taxol enhanced drug cytotoxicity and inhibited lung metastasis of human breast cancer in nude mice (Aggarwal et al. 2005). Curcumin also sensitizes hormone refractory and TRAIL-resistant xenograft in the prostate suggesting this treatment approach could be useful for the prevention, as well as treatment of prostate cancer (Deeb et al. 2005, 2007; Shankar et al. 2008b). In another study, the chemosensitizing effect of liposomal curcumin in paclitaxel chemotherapy of cervical cancer has been reported (Sreekanth et al. 2011). To improve the drawbacks in bioavailability of curcumin, several novel analogs of curcumin have been developed and reviewed by Anand et al. (2008). Our laboratory reported a synthetic analog of curcumin named curcumin difluorinated (CDF) showing greater tissue bioavailability in pancreas and prostate of mice than natural curcumin, and enhancing the activity of gemcitabine in pancreatic cancer targeting cancer stem like cells (Padhye et al. 2009a, b; Ali et al. 2010; Bao et al. 2011).

The chemosensitizing effect of DIM in orthotropic mouse model of pancreatic cancer rendering significant inhibitory effect on pancreatic tumor growth in combination with erlotinib and third generation platinum drug oxaliplatin, currently in clinical use with major limitation of drug resistance, have been reported by us (Ali et al. 2008; Banerjee et al. 2009b). Similar growth inhibitory effects were also noted in an *in vivo* prostate tumor model treated with DIM and taxotere (Rahman et al. 2009). At the molecular level, this has been attributed to partaking by DIM in down regulating survivin, AR and NF-κB signaling (Rahman et al. 2006, 2009). Other bioactive compounds from cruciferous vegetables, PEITC and sulforaphane have been found to inhibit angiogenesis *in vitro* and *ex vivo* through a broad spectrum of anticancer and antiproliferative activity in multiple cancer types (Asakage et al. 2006; Bertl et al. 2006; Xiao and Singh 2007; Hudson et al. 2012). The prominent tea component, EGCG, enhanced the antitumor effect of tamoxifen in MDA MB-231 human breast cancer cells (Chisholm et al. 2004) and improved the therapeutic efficacy of temozolomide in an orthotopic mouse glioblastoma model (Chen et al. 2011). EGCG have been shown to sensitize xenograft tumors developed with drug resistant KB-A-1 human cervical carcinoma cells to doxorubicin *in vivo* through an increase in the accumulation of doxorubicin, and increased DOX-induced apoptosis in the tumors compared with DOX alone (Zhang et al. 2004). The synergistic action of EGCG and EGFR-tyrosine kinase inhibitor erlotinib on growth inhibition of squamous cell carcinoma of the head and neck (SCCHN) in a mouse xenograft model has also been reported (Zhang et al. 2008). The combined treatment resulted in significantly greater inhibition of tumor growth and delayed tumor progression as

a result of increased apoptosis, decreased cell proliferation and reduced pEGFR and pAKT compared to monotherapy proclaiming promising regimen for future chemoprevention and treatment strategy for SCCHN (Zhang et al. 2008). Another assessment found that PES (a combination of Polyphenon E, a highly characterized green tea extract standardized to EGCG) and siliphos (main component silibinin) inhibited colorectal tumor growth as well as hepatic metastases, but awaiting implementation as a perioperative anticancer therapy (Yan et al. 2012). In murine model of chemoresistant hepatocellular carcinoma (HCC), the tea catechins ECG and EGCG sensitized chemoresistant HCC cells to DOX by decreasing the level of P-glycoprotein while suppressing MDR1 expression, consequently increasing the intracellular accumulation of the drug (Liang et al. 2010).

Resveratrol has been reported to prevent and inhibit the development of tumors and exhibits anticancer properties in a variety of tumor cells including lymphoid, myeloid, skin, breast, prostate, pancreatic and colon cancer cells (Bishayee 2009). We recently reported that the biological effect of resveratrol could be enhanced in lower pH (Shamim et al. 2012); this is a provocative finding because the pH within the tumor is lower than the overall physiological pH *in vivo* and suggests that resveratrol could be effective in cancer in human patients because the pH within the tumor is lower than the overall physiological pH *in vivo*. It has been reported that resveratrol sensitizes human pancreatic cancer cells to gemcitabine therapy and the effect was found to be mediated by downregulation of cell survival molecules, including NF-κB, cyclin-D1, COX-2, MMP-9 and survivin (Harikumar et al. 2010). In human multiple myeloma cells, resveratrol inhibits cells proliferation, induces apoptosis and overcomes chemo resistance through downregulation of STAT-3, Akt, NF-κB, cyclin D1, Bcl-2, Bcl-xL, and XIAP (Bhardwaj et al. 2007). In murine liver cancer, resveratrol has been found to enhance the antitumor effect of 5-FU and markedly antagonizing its toxicity (Wu et al. 2004).

10.15 Clinical Trials for Cancer Therapies by Dietary Agents

Based on information available at http//www.clinicaltrials.gov several clinical trials are under way reflecting the clinical usefulness of natural chemopreventive agents in cancer patients (Table 10.1). It has been found that dietary supplements of pure unconjugated isoflavones (genistein, daidzein, and glycitein) given to humans in single doses exceeding normal dietary intake by many folds resulted in minimal clinical toxicity (Busby et al. 2002). Another phase 1 trial, using multiple-doses of isoflavone in men with prostate cancer also showed similar results (Fischer et al. 2004). Results from a clinical trial showed that supplementing early stage prostate cancer patients with soy isoflavones altered surrogate markers of proliferation such as: serum PSA and free testosterone, in large number of subjects in the isoflavone supplemented group than the group receiving placebo, suggesting the beneficial effects of isoflavone in early stage prostate cancer (Kumar et al. 2004). Several clinical trials are being conducted using isoflavone genistein or formulated

genistein in combination with IL-2 or gemcitabine in the treatment of melanoma, kidney and pancreatic cancers (Table 10.1). Phenoxodiol, is one of the isoflavone analogues that has shown a broad spectrum anticancer effect. Phenoxodiol is currently undergoing clinical trials in phase 2/3 trials to investigate the effects of phenoxodiol combined with carboplatin, docetaxel, cisplatin or paclitaxel in patients diagnosed with ovarian, fallopian tube, or primary peritoneal cavity tumors (Table 10.1).

Phase 1 data support the non-toxic nature of DIM in healthy volunteers (Reed et al. 2008). Other ongoing clinical trials have been recently summarized by us (Banerjee et al. 2011b). Polyphenon-E and other green tea polyphenols received investigational new drug status and are currently undergoing clinical trials. Recently, a phase 3 clinical trial is being conducted using combination of EGCG and erlotinib to chemoprevent head and neck cancer with premalignant lesions (NCT ID: 01116336). A phase 2 trial to evaluate the safety and effectiveness of administering several doses of lycopene to men with clinically localized prostate cancer has been reported showing that serum free testosterone decreased with lycopene supplementation, suggesting that steroid hormones related mechanisms are involved (Kumar et al. 2008). The results from another clinical trial showed that lycopene supplements reduced tumor size and PSA level in localized prostate cancer suggesting its promising effect on prostate cancer prevention and/or treatment (Kucuk et al. 2001, 2002).

10.16 Conclusion and Perspective

Studies reported over the past decade focusing specifically on signal transduction pathways have revealed the existence of complex deregulated cellular signaling networks unilaterally augmenting growth and survival of cancer cells. With increasing knowledge on bioactive food compounds along with aforementioned molecular events, the ability of chemopreventive agents to potentially intervene and slow down the initiation and progression of cancer by its effect on either one or multiple signaling pathways is unquestionable. Unfortunately, a regular diet cannot provide adequate amount of bioactive phytochemicals. Nevertheless, it is convincing to note as a ‘proof of principle’ that such strategy could be exploited rationally towards development of therapeutically relevant novel analogs aimed to establish greater bioavailability and potency to disrupt the cross talk between survival and anti-apoptotic signal transduction network circuitry primarily indicted in the initiation and progression of cancer and development of chemoresistance. This also calls for in-depth molecular characterization profiling of the compounds along with appropriate preclinical animal experimentation using genetically modified animal models of cancer. Finally, it is imperative that clinicians should enthusiastically pursue clinical trials to validate the usefulness of natural bioactive compounds or their analogs in the treatment of cancer either as single agents or combined with conventional existing cancer therapies to win the battle against cancer.

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