Oncology: Biochemists' Perspective

9

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Abstract

In this chapter, the stepwise development of carcinogenesis has been addressed along with biochemical aspects of cancer cells. Different physical, chemical, viral, and other genetic causes of cancer and deregulation of different molecular pathways associated with cancer are also discussed. It was found that alterations of molecular pathways like cell fate, cell survival, and genome maintenance are important for the development of cancer and their key regulatory genes have been identified as the molecular targets to diagnose or understand the treatment procedure for cancer. However, it was found that different natural compounds could prevent the process of carcinogenesis. So precisely, it can be suggested that healthy lifestyle and food habit till date could help an individual to stay away from this dreadful disease. However, there has been reasonable advancement in the molecular targeted treatment procedure to treat the patients at different stages of cancer.

Keywords

Hallmark of cancer · Cancer genes · Carcinogenesis · Molecular pathways

9.1 Introduction

Cancer has now become one of the most dreadful diseases and the main cause of human death after heart diseases. It is hard to find any family without an incidence of cancer. This chapter is concerned with how a normal cell or a normal stem cell is acquiring some properties to behave abnormally and irreversibly to become a cancer

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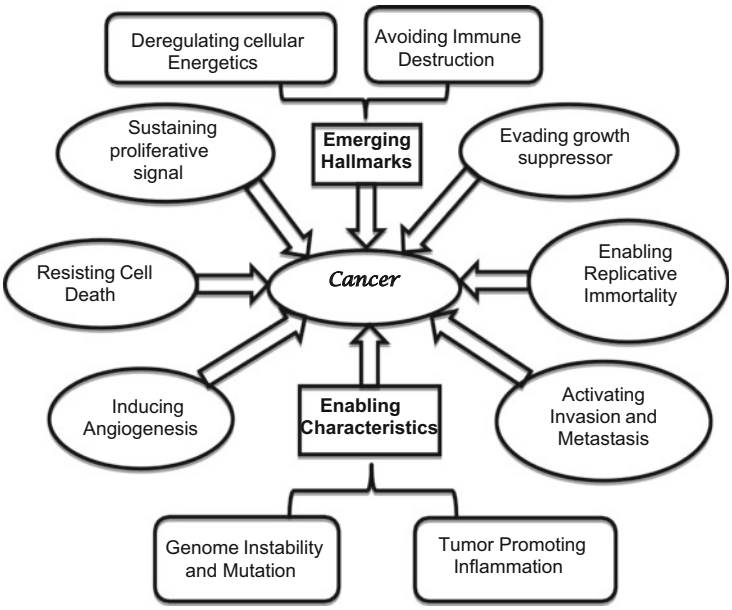

Fig. 9.1 Hallmark features of cancer (adapted and modified from Ref. (Hanahan and Weinberg 2011))

stem cell. The biochemical cause, natural prevention, and molecular therapies of cancer have also been discussed in this chapter.

In general, the characteristic features of human cancer cell are dependent upon the acquisition of the following capabilities: persistent cell division, escape from growth suppression, restriction of cell death, unlimited replicative potential, induction of angiogenesis, alteration of cellular metabolism, and escape from immune destruction (Hanahan and Weinberg 2011) (Fig. 9.1). Most of the above features are either inherited or acquired somatically by stepwise accumulation of alterations in the genes associated with cancer such as oncogenes, tumor suppressor, and stability genes (Table 9.1), each conferring a specific growth advantage to the cell, which leads to gradual conversion of normal cell into cancer cell (Romero-Garcia et al. 2011; Karakosta et al. 2005; Hanahan and Weinberg 2000).

Among these different hallmarks, the deregulation of cellular metabolism is the most important biochemical change during carcinogenesis. The main characteristic features of cancer cells compared with normal cells are to use aerobic glycolysis (Warburg effect), i.e., to use glycolysis pathway even in the abundance of oxygen. Healthy cells use anaerobic glycolysis (Phan et al. 2014). In cancer cells, glucose uptake also increased due to upregulation of glucose transporters mainly Glut1, Glut2, Glut3, and Glut4 (DeBerardinis and Cheng 2010). c-Myc and HIF-1α are important among oncogenes to play critical role in induction of some key glycolytic enzymes like HK2, PFK1, TPI1, and LDHA in tumors due to the presence of consensus Myc and HIF-1α-binding motifs in their promoter region (Vander Heiden

Table 9.1 List of genes associated with cancer

Genes associated with cancer	Definition	Activation/ inactivation	Example	References
Oncogenes	Initially, these genes were identified as genes those were carried by viruses that cause transformation of their target cells. These genes termed as "proto-oncogene" are essential for normal cellular functions like controlling cellular proliferation, differentiation, and apoptosis	Mutation, chromosomal translocation, gene amplification, and viral insertions altered their normal function to gain-of-function phenotype leads to constitutive activation	*Transcription factors* [e.g., MYC, FOS, JUN gene family], *chromatin remodelers* [e.g., SWI/SNF Complex], *growth factors* [e.g., FGF, PDGF, EGF], *growth factor receptors* [e.g., EGFR, PDGFR, VEGFR], *signal transducers* [e.g., ABL, SRC, RAF, RAS gene family, PI3K], and *regulators of cell cycle and cell death* [e.g., cyclin family, MDM2, BCL-2, BCLXL]	Croce (2008), Vogelstein and Kinzler (2004)
Tumor suppressor genes (TSGs)	These genes are required to suppress tumor formation.	Inactivated by (a) loss-of-function mutations; (b) Complete or part deletion of these genes; (c) Reduced expression due to promoter hypermethylation; (d) Deregulation of imprinting; and (e) alternate splicing	TSGs known so far are involved in *all major cellular physiological processes,* e.g., cell cycle, DNA damage repair pathways, cell signaling pathways [TP53, RB, LIMD1, RBSP3, APC, ATM, ATR, MSH2, MLH1, BRCA1, BRCA2, BUB1, SMAD4, PTEN, phospholipase A2, etc.]	Sharp et al. (2004), Kashuba et al. (2009), Berger et al. (2011)

(continued)

Table 9.1 (continued)

Genes associated with cancer	Definition	Activation/ inactivation	Example	References
Cancer susceptibility genes	The extent of susceptibility to cancer is often determined by the degree of penetrance of the genes, i.e., high penetrance and low penetrance. The alterations of the genes can be inherited via germline or can be acquired somatically in sporadic malignancies. High penetrance genes often result in multiple cases of cancer among first- and second-degree relatives, generally at young age. Nonhereditary sporadic cancers can also develop in genetically predisposed individuals resulting from alterations of several low penetrance genes	Inactivated by (a) loss-of-function mutations; (b) Complete or part deletion of these genes; (c) Reduced expression due to promoter hypermethylation	High penetrance genes: BRCA1, BRCA2, etc. Low penetrance genes: PTEN, TWIST1, etc.	Vogelstein and Kinzler (2004)
Replication fidelity genes	There is a definite life span for each type of eukaryotic cells, which is determined by the number of telomeric repeats on end of chromosome. After successive rounds of replication, the	In majority of human tumors, activation of telomerase resulting in acquisition of replicative immortality is an essential step	Telomerase is an example of this class of gene	Hanahan and Weinberg (2011)

(continued)

Table 9.1 (continued)

Genes associated with cancer	Definition	Activation/ inactivation	Example	References
	telomere repeats shorten, ultimately triggering senescence. Thus, telomere controls life span of a cell by replication			

Note: *EGF* epidermal growth factor, *EGFR* epidermal growth factor receptor, *FGF* fibroblast growth factor, *PDGF* platelet-derived growth factor, *PDGFR* platelet-derived growth factor receptor, *VEGFR* vascular endothelial growth factor receptor

et al. 2009; DeBerardinis et al. 2008). HIF-1α and c-Myc are mainly functional in hypoxia and normoxia, respectively (Dang 2007). This synchronization is quite important for continuous energy supply for cell proliferation and biosynthesis for glycolysis.

Moreover, pyruvate, the end product of glycolysis, is converted into lactate in tumor cells instead of acetyl-CoA in normal cells due to overexpressed lactate dehydrogenase. The role of lactate is very important for creating tumor microenvironment (Vander Heiden et al. 2009). Lactate restricts intracellular oxidative stress by reducing reactive oxygen species (ROS) in cancer cells and induces tumors survival. The pH of extracellular microenvironment is reduced by lactate accumulation, and it helps metalloproteases to induce invasion and metastasis by breaking down extracellular matrix (Phan et al. 2014).

Glycolytic metabolites, e.g., glucose-6-phosphate and dihydroxyacetone phosphate, could be used up in different other metabolic pathways like nucleotide and lipid biosynthesis pathway. Thus, glycolysis plays an important role in cell proliferation and tumor growth promotion.

9.2 Causes of Cancer

Multiple factors influence carcinogenesis. Among them, environmental factors, genetic constitution of an individual, diet, and lifestyle all share equal importance for causation of cancer. Broadly, environmental factors can be divided into physical, viral (Carrillo-Infante et al. 2007; Martin and Gutkind 2008), and chemical factors (Parsa 2012). Genetic constitution of an individual also determines the effectiveness of the environmental factors for pathogenesis of cancer. The genetic agents that influence carcinogenesis are shown in Table 9.2.

Table 9.2 List of agents influencing carcinogenesis

Compounds	Main source	Type of cancer
Physical carcinogens		
Ultraviolet (UV) radiation (UVA, UVB, UVC rays)	Sun light, tannin, lamps	Skin
Ionizing radiation	Cosmic ray, radioactive decay	Leukemia, skin
Viral carcinogens		
Epstein–Barr virus	Oral transfer of saliva, genital secretions	Burkitt's lymphoma, Hodgkin's lymphoma, nasopharyngeal carcinoma
Helicobacter pylori	Stomach	Stomach
Hepatitis B, hepatitis C	Blood transfusion, body fluid	Liver
Human immune deficiency virus (HIV-1)	Blood transfusion, body fluid	Cervical cancer, Kaposi sarcoma, non-Hodgkin lymphoma, etc.
Human papillomavirus (HPV 16, HPV 18, HPV 31, HPV 33, etc.)	Sexually transmitted infection	Oral cancer, cervical cancer, etc.
Chemical carcinogens		
Polycyclic aromatic hydrocarbon (PAH)		
7,12-dimethylbenz [a]-anthracene (DMBA)	Environmental pollutant, vehicles exhaust	Skin, lung, stomach
Benzo(a)pyrene (BaP)	Tobacco smoke, grilled meat, coal tar, smoke from the burning of fossil fuels, coal tar	Lung, skin
Benzo(g)chrysene (BgC)	Coal tar	Skin
3-methylcholanthrene (MCA)	Burning organic compounds	Prostate cancer, sarcoma
20-methylcholanthrene (MCA)	Research tool	Sarcoma, transformation of fibroblast
N-Nitroso compounds		
4-(methyl nitroso amino)-1-(3-pyridyl)-1-butanone (NNK)	Cigarette smoke, fried, foodstuffs, meat, beer, fish, latex product	Lung, nasal cavity, liver, oral, and pancreas
N′-nitroso nornicotine (NNN)		Lung, oral, esophagus
4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL)		Lung, nasal cavity, liver, and pancreas
N-Nitrosodimethylamine (NDMA)		Liver, gastric, esophagus
N-Nitrosodiethylamine (NDEA)		Liver, gastric, esophagus
N-methyl-N-nitrosourea (MNU)	Not used	Bone, brain, pancreas, blood

(continued)

Table 9.2 (continued)

Compounds	Main source	Type of cancer
Natural carcinogen		
Aflatoxin B1	Mycotoxin from *Aspergillus flavus* (found in contaminated peanut, grains)	Liver
Asbestos	Thermal insulation	Lung, mesothelioma, gastrointestinal, colorectal
Metals		
Arsenic (As)	Natural ores, alloys, groundwater	Skin, lung, liver
Cadmium (Cd)	Natural ores, batteries, pigment, ceramics	Lung, prostate, kidney
Chromium (Cr) (hexavalent)	Groundwater, tap water	Lung
Lead (Pb)	Battery, smelter, metal products, paint	Lung, bladder
Nickel (Ni)	Natural ores, electrodes	Lung, nasal cavity
Different dyes	Pigment, coloring oil, textiles, paints, printing inks, paper, and pharmaceuticals	Liver, lung, bladder, stomach, kidney, oral, larynx, esophagus, liver, gallbladder, pancreas
Ethanol	Alcoholic beverages	Liver, colon, oral, breast
Others		
Acetaldehyde	Alcoholic beverages	Liver, colorectal
Ethylene oxide	Textile, detergent, industry, cosmetics, sterilant for food	Leukemia, stomach, pancreas
Formaldehyde	Cigarette smoke, air pollution, fungicide, germicide, etc.	Lung, leukemia, brain cancer, etc.
Ortho-toluidine	Synthetic chemical used too	Urinary bladder cancer, liver
Vinyl chloride	Petroleum-derived chemicals	Liver cancer

9.3 Development of Cancer

A series of genetic and epigenetic alterations progressively convert the normal cell to a premalignant state and finally a cancerous state (Yokota and Sugimura 1993). Different proposed models are there to understand the whole process (Fig. 9.2).

Thus, to understand the genetic and molecular mechanism of cancer development, Fould (1957) was the first to propose a progression model stating that cancer was due to phenotypic manifestation of several genetic damages (Fig. 9.2) followed by Nowell's *clonal evolution model* of neoplastic progression, which was continuous appearance of genetically variant cells within a tumor of monoclonal expansion that could compete with each other on the basis of highest cellular growth rate (Fig. 9.2) (Nowell 1976). Then, it was proposed by Ref. (Bodmer 1997; Farber and Cameron 1980; Scrable et al. 1990) that normal cell progressing to fully malignant phenotype might be due to a nested set of aberrations (Fig. 9.2). Then, Ilyas et al.

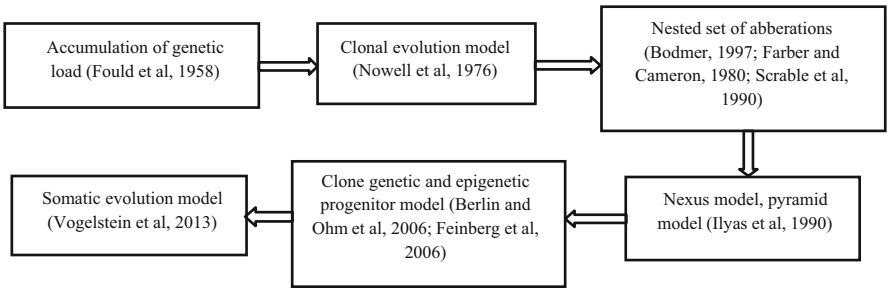

Fig. 9.2 Development of different predicted models explaining stage-wise progression of cancer

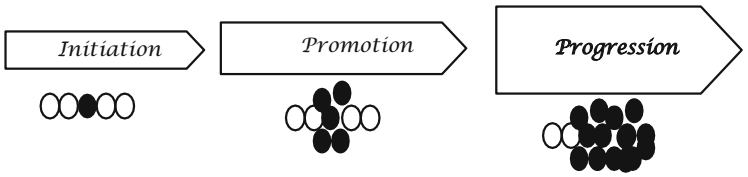

Fig. 9.3 Schematic diagram showing stepwise progression of cancer

(1999) explained tumor progression through *nexus model* (Fig. 9.2), which explained that tumor developed by nexus of interconnecting mutations and selection pressures applies at each point. They also proposed an *inverted pyramid model* (Fig. 9.2), where one mutation could predict the selection of next mutation and their interaction ensure optimal activity of both. Further studies lead to the development of *clonal genetic model and epigenetic progenitor model* of cancer progression (Baylin and Ohm 2006) (Fig. 9.2). Clonal genetic model was supported by induction of oncogenes and inactivation of tumor suppressor. Epigenetic model stated that cancer developed through three steps: an epigenetic alteration of progenitor cells; an initiating mutation along with genetic and epigenetic plasticity; and finally, the advanced *somatic evolution model* was proposed by Vogelstein et al. (2013). This model stated that mutation of certain gene associated for the development of carcinoma from normal cell was due to somatic evolution (Fig. 9.2).

Clinically, human tumors can be divided into three groups: *premalignant lesions*, *primary tumors, and metastases* (Yokota and Sugimura 1993). The process of carcinogenesis passes through three major sequential steps: (a) initiation, (b) promotion, and (c) progression (Fig. 9.3).

9.3.1 Initiation

The first step involves irreversible changes incorporated into the cellular genetic material. If cellular repair mechanism fails to detect damaged DNA, the base

sequence would be modified (insertion/deletion/ modification) in the next round of replication. Ultimately, transcription and translation of this modified template would synthesize a modified protein with altered function. The initiated cell has proliferative stimulus, enough to generate clones of modified cell, though this is unable to generate malignant cell population (Yokota and Sugimura 1993; Feinberg et al. 2006). However, repeated exposure to initiating agent with certain frequency might lead to clonal expansion of mutated cells.

9.3.2 Promotion

The initiated cells have limited proliferative potential, which is not sufficient for continuation of the carcinogenesis process. Initiation followed by promotion provides an impact for further progress of the carcinogenic process. Tumor promotion involves genetic activation or inactivation to stimulate the proliferative potential of initiated cells, leading to the development of multiple *benign tumors* or *hyperplastic lesions*. Promoting activity can be achieved by alteration in normal signal transduction pathway with enhanced rate of transcription and translation of genes responsible for cellular proliferation.

9.3.3 Progression

This final step of carcinogenesis involves conversion of benign tumors to malignant neoplasms that is able to invade adjacent tissues resulting in metastasis (Slaga 1983; DiGiovanni 1992). Specific characteristics of metastatic cells like increasing cellular proliferation, reprogramming cellular metabolism, alteration in hormonal response, and loss of cellular differentiation, decreased antigenicity and acquisition of drug resistance provide selective growth advantage for tumor cell population (Nowell 1986). Multiple host tissue factors, for example, proteolytic enzymes, activators of plasminogen, tumor angiogenic factor, platelet-agglutinating capacity, and different membrane molecules including laminin, fibronectin, and major histocompatibility complex gene products, play important role in tumor progression (Welch et al. 1984).

9.4 Molecular Pathways Associated with Cancer

Stepwise accumulation of genetic and epigenetic changes leads to neoplastic conversion of a cell (Yokota and Sugimura 1993). Collections of cellular pathways are altered during the process of carcinogenesis. A brief description of a few pathways is given in Fig. 9.4. The pathways of the groups seem to have cooperativity to have selective growth advantage at each stage of tumor development.

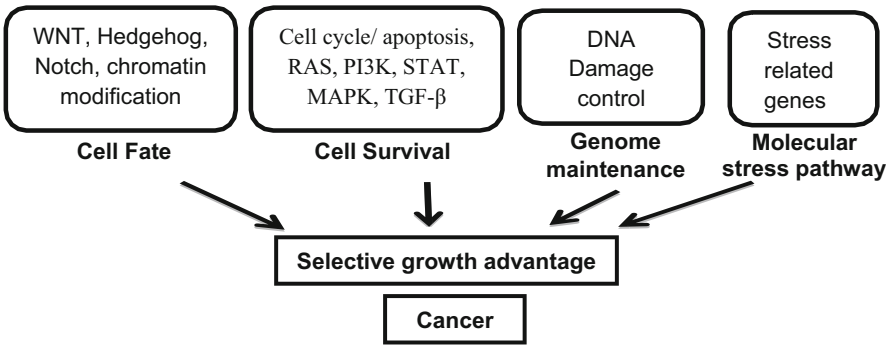

Fig. 9.4 Cellular pathways associated with cancer development (Edited and modified from Ref. (Vogelstein et al. 2013))

9.4.1 Cell Fate

Alteration in cell division and differentiation due to acquired growth advantage leads to tumor progression. These self-renewal signal transduction pathways, namely Wnt, Hedgehog Notch, and Bmi-1, are important for the determination of fate of the stem cells (Lei et al. 2017; Perrimon et al. 2012; Hoffmann 2012). Most of these pathways are frequently deregulated in several cancers and most importantly within the cancers that possess stem-like properties (Curtin and Lorenzi 2010). Also, the key genes involved in chromatin modification and transcription pathways belong to this category (Fig. 9.4).

9.4.1.1 Stem Cell Self-Renewal Pathway

WNT Pathway

This pathway is a well-known self-renewal pathway regulating embryonic development and tissue homeostasis and contributes to control the cell proliferation, differentiation, and epithelial–mesenchymal transition (Sarkar et al. 2010). β-catenin is the main effector molecule of this pathway. In the absence of active Wnt ligands, β-catenin forms complex with scaffold proteins axin and adenomatosis polyposis coli (APC). Then, β-catenin is phosphorylated by casein kinase Iα (CKIα) and glycogen synthase kinase (GSK-3β) at N-terminal serine/threonine residues sequentially followed by ubiquitination and proteasomal degradation. In the presence of Wnt ligand, Wnt binds to frizzled receptor and LRP co-receptor leading to the inhibition of β-catenin–axin–APC degradation complex formation resulting in release of β-catenin from the complex. The cytoplasmic-free β-catenin then either binds to E-cadherin at membrane or phosphorylated at tyrosine-654 residue by activated receptor tyrosine kinases (like EGFR) followed by phosphorylation at serine 675 by protein kinase A and translocate to the nucleus. In the nucleus, β-catenin complexes with T-cell/lymphoid enhancer transcription factors (TCFs/LEF) and activates the transcription of Wnt target genes such as c-Myc, cyclin D1,

CD44, and EGFR. Several secreted or intracellular proteins like secreted frizzled receptor proteins (sFRPs) negatively regulate Wnt signaling by inhibiting Wnt ligands binding to receptor (Sarkar et al. 2010; van Veelen et al. 2011).

Aberrant activation of the Wnt/β-catenin pathway due to mutation of β-catenin gene (*CTNNB1*) at exon 3 and/or inactivation of APC, axin, or WNT antagonists SFRP1 and SFRP2 by mutation/deletion/ promoter hypermethylation could lead to nuclear β-catenin accumulation and transcriptional activation of WNT target genes like c-Myc and cyclin D1 as seen in different cancers (Sarkar et al. 2010).

Hedgehog (Hh) Pathway

The Hedgehog self-renewal pathway is an important regulator of cell proliferation, differentiation, and polarity. Alteration in this pathway leads to numerous human diseases including cancer (Sarkar et al. 2010; Chen and Jiang 2013). The effector molecule of this pathway is Gli. When Hh ligand is absent, PTCH receptor inhibits the transmembrane receptor-like protein smoothened (SMO) and Gli2/3 cytoplasmic form complex with Costal2 (Cos2), Fused (Fu) and Suppressor of fu (Sufu) leading to sequentially phosphorylation by PKA, CK1, and GSK-3β at several serine/ threonine sites of Gli to form truncated repressor (Gli-r) (Sarkar et al. 2010; Chen and Jiang 2013). In the presence of Hh ligand, PTCH receptor activates SMO to engage COS2/Fu complex resulting in accumulation of activated full-length Gli that could enter into nucleus and transcribe several Hh target genes like Cyclin D1, c-Myc, Bcl2, Gli1, and PTCH (Chen and Jiang 2013; Sarkar et al. 2010).

Reduced expression of antagonists of the pathways like PTCH, HHIP, and SUFU due to deletion/mutation/promoter methylation and high expression of SHh, SMO, and Gli1 resulting in increased expression of target genes are reported in several cancers (Moeini et al. 2012; Chen and Wang 2015).

Notch Pathway

This signal is triggered by binding of ligand on the membrane of one cell (delta/ delta-like/jagged/serrate) to a receptor (NOTCH1/2/3/4) on the membrane of the contacting cell leading to proteolytic cleavage of NOTCH receptors to release the cytoplasmic tail of NOTCH, i.e., intracellular domain of NOTCH (NICD). NICD translocates to the nucleus and associates with transcription factors p300, master-mind protein (MAM), and recombination signal-binding protein for κ-immunoglobulin kappa J region (RBPJκ) in mammals to turn on transcription of target genes [hairy/enhancer of split (HES) family of transcription factors] (Sikandar et al. 2010).

Alteration of NOTCH signaling was reported to be associated with several cancers like mutations in NOTCH1 in non-small cell lung cancer/oral cancer and upregulation of NOTCH2 in colorectal cancer (Andersson et al. 2011).

BMI Pathway

Self-renewal of hematopoietic stem cells takes place by this pathway. BMI1 (B-cell-specific Moloney murine leukemia virus integration site 1) is a polycomb ring finger oncogene. It promotes cell proliferation by transcriptional inhibition of cyclin-

dependent kinase inhibitor INK4A (p16) and p19ARF (p14) (Park et al. 2003). Overexpression of BMI-1 has been previously reported in several cancers including gastric, ovarian, breast, head and neck, pancreatic, lung, liver, and endometrial carcinoma (Wang et al. 2015).

9.4.1.2 Chromatin Modification

Chromatin modification takes place mainly by DNA methyltransferases (DNMTs: DNMT1, DNMT3a, DNMT3b), histone acetyltransferase (HAT), histone deacetylases (HDACs), and histone methylase (HMT).

DNMT1 is a predominant methyltransferase for CpG methylation in hemimethylated DNA (Lopez-Serra et al. 2006). Overexpression of DNMT1 was reported in several cancers, for example, pancreatic cancer, pediatric gastric cancer, and retinoblastoma (Li et al. 2011; Ma et al. 2017; Qu et al. 2010). Among HDACs, HDAC 1, HDAC 2, HDAC 5, and HDAC 7 could play important roles in carcinogenesis (Miller et al. 2011; Urbich et al. 2009; Lei et al. 2017). Altered expressions of different HDACs have been reported in various cancers. HDAC 1 and HDAC 2 were found to be upregulated in colon cancer and gastric cancer, respectively (Miller et al. 2011). HDACs and histone acetyltransferases could bind to DNA indirectly through multiprotein complexes like co-repressors and co-activators (Sengupta and Seto 2004). Histone methylases (HMTs) have important role in cancer development (Albert and Helin 2010). HMTs could modify histones at specific Lys and Arg residues to alter their functions (Albert and Helin 2010). In addition, upregulation of histone demethylases has been seen in different cancers and suggested to be associated with chemoresistance (Yang et al. 2017).

9.4.2 Cell Survival

Cell survival is mainly controlled by several signaling proteins like EGFR, HER2, FGFR2, PDGFR, TGFbR2, MET, KIT, RAS, RAF, PIK3CA, and PTEN through cell cycle and apoptosis (Vogelstein et al. 2013) (Fig. 9.4). Progression through the cell cycle can be directly controlled by driver genes that directly regulate the cell cycle or apoptosis, such as P16, MYC, and BCL2, which are very frequently mutated in cancers. Inactivating mutations in VHL gene could enhance cell survival and stimulate angiogenesis through secretion of vascular endothelial growth factor (VEGF).

9.4.2.1 Alteration of Cell Cycle

Cell cycle is a highly ordered series of events, responsible for cellular duplication. Different extracellular signals, for example, growth factor binding, hormonal responses, cytokines, supply of nutrients, and anchorage attachments, stimulate a cell to divide (Michalides 1999). The process of cell cycle is highly regulated by sequential activation and degradation of the cyclins (cyclin D, cyclin E, cyclin A, and cyclin B), the cyclin-dependent kinases (CDKs: serine/threonine kinases; CDK1, CDK2, CDK4, and CDK6), and their inhibitory proteins known as cyclin-dependent kinase inhibitors [CKIs: INK (p16, p15) and KIP (p21, p27, and p57)

family members] (Michalides 1999). Each of these cyclin–CDK complexes, together with CKIs, is responsible for controlling cell cycle progression through checkpoints. Induction of DNA damage results in activation of cell cycle checkpoint proteins to arrest cell cycle and make necessary DNA repair or elimination of damaged cells by apoptosis (Hartwell and Weinert 1989). The eukaryotic cell cycle is secured by 4 checkpoints at G1/S phase; S phase; G2/M phase; and M phase (Tyson and Novak 2008). Deregulations of these checkpoints are important events during carcinogenesis.

9.4.2.2 Alteration of Apoptosis Pathway

Programmed cell death or apoptosis is one of the mostly altered pathways during carcinogenesis. This is an essential cellular event during embryonic development, immune system function, and control of tissue homeostasis (Vaux and Korsmeyer 1999). Programmed cell death follows two alternative pathways depending on death-inducing cellular response: (1) extrinsic pathway and (2) intrinsic pathway (Gupta 2003).

Extrinsic pathway is activated by binding of ligands to death receptors [tumor necrosis factor (TNF) receptor superfamily, including Fas/CD95, TNFR1, DR3, DR4, and DR5] on the cell surface. Upon ligation, this receptor recruits adaptor molecule (FADD, TRADD) by its cytoplasmic death domain (DD). The death effector domain (DED) in adaptor further recruits procaspase-8. Procaspase-8 (cysteinyl-aspartate-specific proteases) cleaves to form active caspase-8, which further activates effector caspase-3 to execute apoptosis process.

Intrinsic pathway is mitochondria-mediated pathway and is initiated by cellular stress (UV radiation, cytotoxic drug application) that alters the mitochondrial membrane potential. Mitochondrial membrane permeability is controlled by Bcl-2 family proteins (Bcl-2, Bax, Bad, Bak, Bcl-xL, Bid) (Walensky 2006). Cellular stress response mediated by p53 or c-Myc activates pro-apoptotic protein bax, which is translocated from cytosol to mitochondrial membrane to form dimer. During apoptosis, Bad is dephosphorylated and translocated to the outer membrane of mitochondria. Otherwise, the phosphorylated form of Bad is sequestered within cytoplasm. On outer membrane, Bad heterodimerizes with Bcl-xL to block its anti-apoptotic function (Walensky 2006). Bak also loosely associate with outer membrane. Bak forms homo-oligomers within mitochondrial membrane resulting in release of cytochrome-c in cytosol and binds with APAF1 (apoptotic protease-activating factor-1) to form apoptosome complex. Apoptosome activates procaspase-9 (initiator caspase), and subsequent caspase cascades to precede apoptosis (Pollack and Leeuwenburgh 2001; Okada and Mak 2004). Several other proteins were also released from mitochondria. For example, Smac (second mitochondria-derived activator of caspases) and DIABLO bind to IAPs (inhibitors of apoptosis proteins) and AIF (apoptosis-inducing factor). Translocation of AIF to nucleus induces chromatin condensation and DNA fragmentation. Deregulation in apoptosis pathway is a hallmark feature of carcinogenesis (Hanahan and Weinberg 2011).

9.4.3 Genome Maintenance

Alterations in the genes that control DNA damage response pathway (Vogelstein et al. 2013) (Fig. 9.4) allow cells to undergo chromosomal alterations like translocation, inversion, and duplication to survive and divide.

Direct reversal: This is the most simple DNA repair pathways in human that directly reverse the O6-methylguanine (O6-mG) (frequently mutated by alkylation) by the product of the MGMT gene (O6-methylguanine DNA methyltransferase) (Margison and Santibanez-Koref 2002). Cellular metabolism also produces low levels of O6-mG lesion in guanine residues of DNA molecule (Sedgwick 1997).

Base excision repair (BER): If the DNA bases are damaged by several cellular processes like oxidation, methylation, and deamination, this multistep pathway plays active role to detect and remove the damaged bases. This pathway is of two types "short patch" and "long patch." The former involves replacement only of the damaged base, whereas the later replaces a stretch of about 2–10 nucleotides including the damaged base (Memisoglu and Samson 2000).

Nucleotide excision repair (NER): This repair system helps to pyrimidine dimers caused by the UV component of sunlight, bulky chemical adducts, DNA intrastrand cross-links, and some forms of oxidative damage (Hess et al. 1997).

Double-strand break repair (DSB): This type of repair pathway is the most important to detect the problem of central dogma of a cell, i.e., replication and transcription (Mehta and Haber 2014). Several factors like ionizing radiation, exposure of genotoxic chemicals, and any mechanical stress on chromosomes can induce DSB. There are two pathways for the repair of DSBs viz homologous recombination (HR) and nonhomologous end joining (NHEJ) (Lieber 2010). Which pathway will be selected by the cell is unpredictable; the cell cycle stage at that time, however, plays important role for this decision (Jackson 2002).

Mismatch repair (MMR): This pathway corrects replication errors such as base–base mismatches and insertion/deletion loops (IDLs) that result from DNA polymerase misincorporation of nucleotides and template slippage, respectively (Fukui 2010). Mispairing generated by the spontaneous deamination of 5-methylcytosine and heteroduplexes formed following genetic recombination is also corrected via MMR. A defective MMR pathway leads to "mutator phenotype" characterized by increased frequencies of spontaneous mutations and microsatellite instability (MSI), which are the hallmarks of cancer (Loeb et al. 2008).

9.4.4 Molecular Stress Pathway

The hypoxic stress in the intratumor microenvironment augments molecular stress and provides the required stimulus for expression of the pro-angiogenic factors (VEGF), which mediates intricate interplay between various extracellular signaling pathways viz Notch and Hedgehog (Foxler et al. 2012). Hypoxia-inducible factor (HIF-1α) is a crucial player of tumor angiogenesis. Under normoxic condition, the oxygen-sensing prolyl hydroxylase (PHD) catalyzes hydroxylation of Pro-564

residue of HIF-1α. The TSG VHL binds and ubiquitinates this hydroxylated HIF-1α, subjecting it to proteasomal degradation, thereby shutting down the expression of hypoxia-specific genes (Fedele et al. 2002). Another candidate TSG, LIMD1, acts as a molecular scaffold to interact with PHDs and VHL to efficiently degrade HIF-1α during normoxia (Foxler et al. 2012). However, the intratumoral hypoxic condition inhibits the aforesaid hydroxylation of HIF-1α, thereby preventing VHL-mediated ubiquitination and degradation of HIF-1α, leading to its stabilization (Fedele et al. 2002). Under these hypoxic circumstances, the oxygen-dependent asparaginyl hydroxylase (FIH) fails to hydroxylate Asn-803 residue of HIF-1α, thereby enabling binding of the co-activators CBP/p300 and HIF-1β to HIF-1α leading to its transcriptional activation and expression of hypoxia-responsive genes viz VEGF and D-ll4 (Diez et al. 2007). VHL is inactivated in various malignancies, especially in kidney cancer, facilitating stabilization of HIF-1α and consequent tumor angiogenesis, even under normoxia (Banks et al. 2006).

9.5 Cancer Biomarkers

In response to an abnormal or disease conditions like cancer, our body produces some biological molecule that present in body fluids or tissues, according to National Cancer Institute (NCI) (Henry and Hayes 2012). According to World Health Organization (WHO), a biomarker can be any substance, structure, or process that can be detected, quantified, and influence or predict the incidence or outcome of disease (Sturgeon et al. 2010); for example, a cancer biomarker measures the risk of cancer development or measures the cancer progression risk or potential response to therapy. The widely used cancer biomarkers are mainly proteins (e.g., an enzyme or receptor) (Table 9.3). In addition, there are also other types of cancer biomarkers, for example, nucleic acid (e.g., microRNA or other noncoding RNA; microsatellite DNA markers), antibodies, and peptides (Sturgeon et al. 2008). A biomarker can be synthesized as a result of alterations of different metabolic and biosynthetic pathways. Biomarkers are usually detected in noninvasive procedures like collection of samples (blood, serum, plasma, stool, urine, sputum). Sometimes, it requires special imaging for evaluation or biopsy sampling for tissue-based analysis. Genetic biomarkers can be detected as DNA base sequence variations in germline DNA isolated from whole blood and sputum. Cancer biomarkers can be classified into the following categories:

Predictive biomarkers: With the help of this marker, a response against specific therapy such as response against certain chemotherapeutic drugs for specific cancers could be assessed (Cramer et al. 2011). Like in colorectal cancer, cetuximab treatment will be in vain if patient has KRAS-activating mutations. So, KRAS mutation status is a predictive biomarker for this case (Diamandis 2010) (Table 9.3).

Prognostic biomarker: With the help of these markers, disease recurrence or disease progression can be predicted; i.e., it helps to detect the clinical outcomes of the disease. An example of a prognostic cancer biomarker is the 21-gene recurrence

Table 9.3 Cancer biomarkers (edited and modified from Ref. (Goossens et al. 2015; Dawood et al. 2014; Clevers 2011))

Class of biomarker	Specific biomarker	Deregulation in cancer	Reported in cancer	Associated drug
Hormone receptor	Estrogen receptor (ER)/progesterone therapy (PR)	Receptor expression related to prognosis of the disease	Breast cancer	Tamoxifen
	Aromatase inhibitors	Decrease amount The estrogen		Anastrozole, letrozole
Growth factor receptor	HER2	Overexpression	Breast cancer, esophagogastric adenocarcinoma	Tucatinib, trastuzumab, pertuzumab, ado-trastuzumab emtansine
	EGFR (HER1)	Mutations in tyrosine kinase domain lead to constitutive activation	Non-small cell cancer (NSCLC) Lung	Receptor kinase erlotinib, Tyrosine inhibitor: Gefitinib, afatanib, lapatinib
	EGFR (HER1)	Resistance to EGFR therapy if KRAS is mutated	Colorectal cancer	Receptor tyrosine kinase inhibitor: cetuximab, panitumumab
	BCR-ABL	BCR-ABL translocation Philadelphia chromosome (9; 22) leads to the formation of a constitutively active tyrosine kinase	Chronic myeloid leukemia (CML)	Imatinib
		Imatinib-resistant CML		Dasatinib, nilotinib
	PML–RARa	t(15;17)(q24;q21) translocation is associated with favorable prognosis	Acute myeloid leukemia (AML)	All-trans-retinoic acid
	Anaplastic lymphoma receptor tyrosine kinase gene (ALK)	Inversion in chromosome 2 leads to EML4-ALK fusion oncogene	Non-small cell lung cancer (NSCLC)	Crizotinib, ceritinib

Other cellular pathway markers	MEK in RAS-RAF-MEK pathway	Resistance to EGFR therapy	Pediatric gliomas, neuroblastoma, AML renal cell carcinoma (RCC), and NSCLC	AZD6244
	mTOR pathway			Ridaforolimus
	MET		Melanoma, colorectal cancer	Tivantinib, everolimus
	B-RAF V600	B-RAF enzyme inhibitor	Skin melanoma	Vemurafenib, dabrafenib, trametinib, binimetinib
	B-RAF	Small molecule B-RAF inhibitor that targets key enzymes in the MAPK signaling pathway	Melanoma	LGX818
	PI3Kα	Potent and selective PI3Kα inhibitor		BYL719
	BRCA1/2		Breast cancer	Olaparib, iniparib
	TOP2A	In subjects with HER2 overexpression		Anthracycline-based neoadjuvant chemotherapy
	RAS	Mutation type	Colorectal	FOLFOXIRI and bevacizumab
	HER	HER inhibitor	Head and neck squamous cell carcinoma; breast cancer	HM781-36B
	GATA-3		Peripheral T-cell lymphomas	MLN9708
Stem cell pathway	CD44	Broadly on many tissues	Breast/liver/head and neck/ pancreas cancer	

(continued)

Table 9.3 (continued)

Class of biomarker	Specific biomarker	Deregulation in cancer	Reported in cancer	Associated drug
markers	CD90	T cells	Neurons Liver cancer	
	CD133	Proliferative cells in multiple organs	Brain/colorectal/lung/liver cancer	
	EpCAM	Panepithelial marker	Colorectal cancer, pancreatic cancer	
	CD19	Broadly on B lymphocytes	B-cell malignancies	
	CD20	Broadly on B lymphocytes	Melanoma	
	CD24	Broadly on B cells	Neuroblasts Pancreas/lung cancer, negative on breast cancer	
	CD34	Hematopoietic and endothelial progenitors	Hematopoietic malignancies	
	CD38	Multiple stages of B and T cells	Negative on AML	
	ABCB5	Keratinocyte progenitors	Melanoma	

score, which was predictive of breast cancer recurrence and overall survival in node-negative, tamoxifen-treated breast cancer (Diamandis 2010; Goossens et al. 2015).

Diagnostic biomarker: These markers help to diagnose the disease, i.e., the condition of a patient in specific disease (Diamandis 2010; Goossens et al. 2015).

Cancer stem cell biomarkers: In our normal system, a particular cell type with self-renewal ability is called stem cells, which give rise to all the cell lineages in the corresponding tissues. These cells undergo either asymmetric division generating one stem cell (S) and another differentiating cell (D) or symmetric division generating two stem cells. These stem cells have highest potential for proliferation and have longer life span so they have much greater tendency to acquire necessary number of transformation-associated genetic/epigenetic changes by exposure of inflammation, radiation, chemicals, or infection to become a *cancer stem cell* (CSC) (Moore and Lyle 2011). In a primary tumor or cancer cell lines, a very small percentage of the cells are CSC. The prevalence of CSCs is not the same in each tumor type and varies from tissue to tissue. Cancer stem cells (CSCs) express different surface markers. Based on the surface markers (Table 9.3 modified and edited from Ref. (Dawood et al. 2014; Clevers 2011)), e.g., CD44, CD90, CD133, and EpCAM, different cancers like head and neck, liver, breast, and lung can be evaluated.

9.6 Molecular Therapy of Cancer

A molecular therapeutic target for cancer can be identified with the following criteria; for example, it should be an important key regulatory protein or pathway without which cellular proliferation will be restricted. It should be upregulated in cancer tissue and not in normal tissue. However, drug should be available for the particular target. MYC, KRAS, and TP53 are the most common driver genes in human cancers but are reported to be resistant to therapeutic intervention (Kessler et al. 2012; Luo et al. 2009). On the other hand, approaches to inhibit kinases are well developed (Zhang et al. 2009).

To date, several studies identified large number of candidate targets and their anticancer therapies are now under development. However, only very few pathway-based targeted therapies have got place in clinical practice (Goossens et al. 2015). Some of these are listed in Table 9.3. One such example is treatment against chronic myeloid leukemia (CML) (Nowell and Hungerford 1960).

In breast cancer, amplification of the HER2 gene defines a subset of disease that is typically highly aggressive. Imatinib and trastuzumab were used as effective targeted therapy for patients with HER2-enriched breast cancer (Slamon et al. 2001). BRCA1/BRCA2 is also targeted for treating ovarian cancer (Table 9.3). Olaparib and iniparib are under clinical trial in patients with BRCA-driven breast cancer after being passed the preclinical test (Fong et al. 2009; O'Shaughnessy et al. 2011). A major difficulty in cancer treatment is the intracellular pathways that are interlinked. Thus, for the development of better molecular targeted therapy of cancer the discrete analysis of cellular pathways associated with tumor development should be analyzed

to find out the key regulatory step(s). Then, respective drugs may be designed accordingly.

9.7 Cancer Chemoprevention

The term chemoprevention was first coined by Michael Sporn (Sporn 1976). Chemoprevention of cancer is to use natural, synthetic, or biological chemical agents to reverse, suppress, or prevent carcinogenic progression to invasive cancer (Sporn 1976). It is one of the important areas of current cancer research. Wattenberg L.W. pioneered the research by chemopreventive approach using animal model systems (Wattenberg 1993). Epidemiological studies and experiments in *in vitro* and *in vivo* models indicated that several dietary items/products viz. vitamins; beverages; and food components have cancer-preventive property (Table 9.4). The

Table 9.4 List of few chemopreventive agents along with their sources (Edited and modified from Ref. (Wang et al. 2012; Pal et al. 2012; Sur et al. 2016)

Food components	Name	Active compound
Beverages	Green tea Black tea	Epigallocatechin-3-gallate Theaflavins
Fruits	Grapes Berries	Resveratrol Resveratrol
Vegetables	Broccoli Cabbage Carrot Chili peppers Soybean Tomato	Sulforaphane Indole-3-carbinol Beta-caroteneCapsaicin Genistein Lycopene
Spices	Bay leaves Cardamom Cinamon Clove Coriander Cumin Garlic Ginger Mustard Parsley Pepper Sesame seed Turmeric	Eugenol Do Do Do Apigenin Thymoquinone Diallyl disulfide Gingerol Ferulic acid Apigenin Piperin Ferulic acid Curcumin
Others	Honey Peanut Mushroom Sunflower oil	Caffeic acid phenethyl ester Resveratrol Vitamin D2 Vitamin E
Medicinal plant	Chirata Karanja	Amarogentin Pongapin and Karanjin

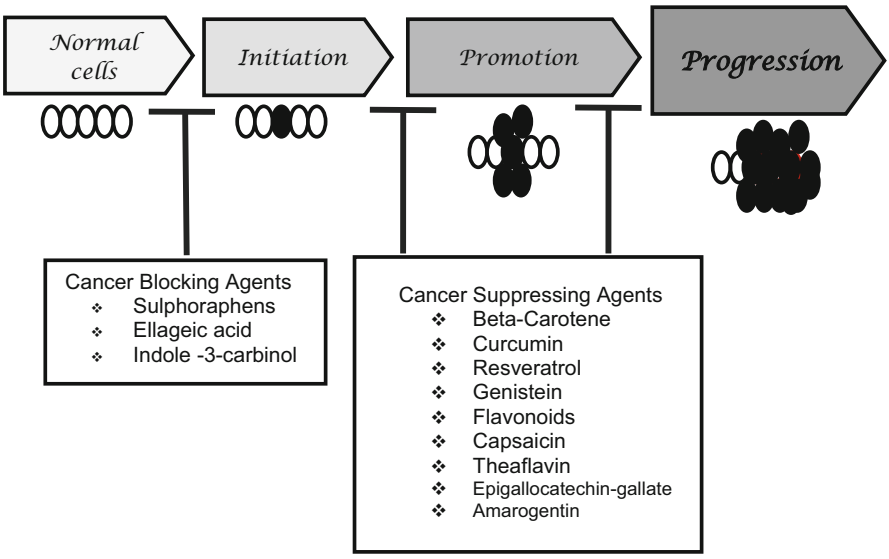

Fig. 9.5 Dietary phytochemicals that block or suppress different stages of carcinogenesis. Blocking agents block metabolic activation of pro-carcinogens and restrict the step of initiation. Cancer-suppressing agents can suppress either promotion or progression step (edited and modified from Ref. (Kotecha et al. 2016))

potential cancer chemopreventive compounds belong to different structural and functional chemical classes.

Chemopreventive agents can be classified according to their mechanism of action and must have the following properties (Fig. 9.5):

- Prevent absorption or metabolism of carcinogens (blocks *initiation*).
- Prevent carcinogens to react with specific cellular targets (blocks *initiation*).
- Suppress the expression of neoplasia in cells exposed to carcinogens (blocks *promotion*).
- Delay or prevent the conversion of initiated cells to preneoplastic cells and ultimately to neoplastic cells (blocks *promotion/progression*).
- Inhibit tumor progression by inhibiting cell proliferation and blocking metastasis (blocks *progression*). List of few such important chemopreventive compounds found in several daily used fruits, vegetables, and spices is listed in Table 9.4.

9.8 Conclusion

There are numerous causes of cancer. Altering molecular pathways like cell fate, cell survival, and genome maintenance are important for the development of cancer. Their key regulatory genes have been identified as the molecular targets to diagnose or understand the treatment procedure for cancer. Different natural compounds could

prevent the process of carcinogenesis. Healthy lifestyle and food habit may help keep this dreadful disease away.

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Appendix

Angiogenesis: In this physiological process, new blood vessels are formed from preexisting blood vessel. This process is guided mainly by VEGF (vascular endothelial growth factor)-mediated pathway. Other factors are also involved like VEGFR, FGF, PDGF, and PDGFR. This process is mainly found in case of invasive tumor.

Hypoxia and normoxia: Normoxia is the condition in which normal oxygen level in a cell remains between 10 and 21%, whereas in hypoxic condition it is reduced to less than 5%. Hypoxia is quite evident in the core area of a tumor due to lack of vascularization (McKeown 2014).

MicroRNA: This is popularly known as miRNA. This is one type of noncoding RNA, i.e., small noncoding RNA containing ~ 22 nucleotide length, which helps in gene silencing and alteration of gene expression.

Microsatellite DNA markers: It is a stretch of DNA motif (containing 1–6 nucleotides or more) repeated almost 5–50 times in a genome of an organism. It has higher mutation rate than other areas of the genome. For example, ATATATATAT is a dinucleotide microsatellite; GCTGCTGCTGCTGCT is a trinucleotide microsatellite. These microsatellites are located throughout the human genome at an average of approximately 30-Kb interval.

Noncoding RNA: These RNAs are not transcribed into proteins, but these have important role in gene transcription regulation. There are several types of noncoding RNAs such as transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), as well as small RNAs such as microRNAs, siRNAs, snRNAs, and the long noncoding RNAs.

Polycomb ring finger oncogene: This is BMI1 protein. It has one ring finger domain. It plays important role in self-renewal pathway, chromatin remodeling, and DNA repair pathway. Its overexpression is reported in several cancers, e.g., hematological malignancies, breast, ovarian, bladder, prostate, colorectal, and skin.

Stem cells: These are cells with self-renewal property and are capable to differentiate into other cell types. For example, hematopoietic stem cells are present mainly in bone marrow and can differentiate into different types of blood cells like red blood cells (RBC), white blood cells (WBC), and platelets.

Ubiquitination: In eukaryotes, a small (8.6 kDa) regulatory protein ubiquitin is found that helps in the process of ubiquitination. In this process, ubiquitin protein

binds to the target protein and that protein is degraded via proteasome-mediated pathway or changes its cellular location or prevents interaction with other proteins.

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