Chapter-I

Introduction and Review of Literature
1. Cancer

Cancer is a genetic disorder involving dynamic changes in the genome leading to uncontrolled cell growth, cell division, ability to invade to distant organs through lymph or blood and metastasize. There are more than 100 different types of cancer. Most cancers are named for the organ or type of cell in which they start - for example, cancer that begins in the renal proximal cells is called renal cell carcinoma, cancer that begins in colon is called colon cancer; cancer that begins in basal cells of the skin is called basal cell carcinoma (Tsao et al. 2004).

In essence, human cancer is a genetic disease. The first implications of a genetic basis for cancer are endorsed to David Hansemann and Theodor Boveri. They both observed abnormal numbers of chromosomes arising by multipolar mitoses and suggested that this abnormality is the cause of tumor formation (Hansemann et al. 1890; Boveri et al. 1902).

Cancer is predicted to be progressively an important cause of morbidity and mortality all over the world. If the global cancer rates remain unchanged, the estimated incidence of 12.7 million new cancer cases in 2008 (Ferlay et al. 2010) will rise to 21.4 million by 2030 (IARC 2011).

The origin of the word is credited to the Greek physician Hippocrates who is considered as the “father of Medicine”. Hippocrates used the terms carcinos and carcinoma to describe non-ulcer forming and ulcer forming tumors respectively. The word originated from the Latin for crab, because the swollen veins around a surface tumor appear like the legs of a crab. The exposure of chemical has been associated with the development of cancer as observed by John Hill in 1761, that snuff users developed nasal cancer more frequently than the general population. However, in 1775, Sir Percival Pott observed that occurrence of scrotal cancer in English chimney sweeps is due to the exposure of chemical present in soot. It was later declared as Polycyclic Aromatic Hydrocarbons (PAHs). More importantly, he also proposed a mechanism to reduce the
incidence of these cancers simply by requiring these individuals to bathe on a regular basis (Poirier 2004). Astute clinical observations such as that made by Pott (1775) have been the basis for the discovery of many of the currently known classes of chemical carcinogens in humans (Pott 1775). Examples include the observation by Rehn (Rehn 1895) that workers in the aniline dye industry in Germany frequently developed bladder cancer and more recent observations concerning the induction of angiosarcomas in patients exposed to contrast material used for radiologic imaging studies (Vajdic et al. 1986) and vinyl chloride exposure in the workplace in Louisville, Kentucky (Creech and Johnson 1974).

1.1 History: Induction of neoplasia via chemical is a multistep process involving DNA damage and cell proliferation. Chemical carcinogens impact on different stages of cancer development and function through alteration of cellular and molecular proceedings. There are mainly two types of chemical carcinogens “genotoxic” and “non-genotoxic” to further understand the mechanisms of action of carcinogen (Williams et al. 1983). The epigenetic events are essential for proper development and cellular differentiation within normal tissues. Epigenetics is defined as modifications of DNA or related factors that have information content and are heritable (aside from the DNA sequence itself). Normal epigenetic alterations of DNA include three types of changes, all of which are interrelated: chromatin modifications, DNA methylation, and genomic imprinting (Feinberg et al. 2004).

Genotoxic agents are the chemicals that damage genomic DNA directly, which consequently induce mutation. Genotoxic chemicals are frequently activated within the target cell and induce a dose-dependent increase in neoplasm formation. A second category of carcinogenic chemicals i.e., non-genotoxic agents appear to function through non-DNA reactive or indirect DNA
reactive mechanisms. Although much less is known about the exact mechanism of action of non-genotoxic carcinogens, they modulate cell growth and cell death. The main action of non-genotoxic carcinogens are changes in gene expression and cell growth parameters (Pitot et al. 1981; Kolaja et al. 1996).

1.2 Carcinogenesis—a multi stage process

The induction of neoplasia in rodents by chemical and physical agents involves a multistage process. There are three different stages of carcinogenesis have been defined (Pitot et al. 1981). These include initiation, promotion, and progression.

(A) Initiation: This stage involves a heritable alteration to the genome that facilitates the clonal expansion of the initiated cells in response to a promoting agent. It involves the formation of a mutated, preneoplastic cell that results from the exposure to a particular dose of carcinogen i.e., initiator and may result in mutation, translocation, or amplification in the target cell (Ray and Husain 2002; Hennings et al. 1983). The formation of the preneoplastic, initiated cell is an irreversible, but dose-dependent process. The key event is the covalent binding of the carcinogen to macromolecules, especially to DNA in the cell. Most carcinogens, like aromatic hydrocarbon class are inert. They have to be metabolically active to become reactive with target cell. The reactive metabolites are capable of binding and initiating the carcinogenic response. In this stage, a single somatic cell undergoes non-lethal, but heritable mutation. This initiating mutation may give the initiated cell the growth advantage needed in the second stage of promotion. In contrast to its surrounding cells, the initiated cell can escape the cellular regulatory mechanisms. It is kinetically and pharmacologically compatible with a simple mutational event, possibly involving as few changes as a single base alteration. Initiating agents include chemical, physical or biological agents which are capable of being converted directly or after metabolism to highly reactive metabolite, attacking and binding covalently to positively charged molecules of cellular
components including DNA, RNA, proteins, thiols, and polysaccharides resulting in their structural distortion, mutation, translocation etc. (Cavalieri and Rogan 2002; Pitot and Sirica 1980; Miller 1978). Initiating agents and their metabolites are mutagenic to DNA. Thus, carcinogen administration at particular doses does not induce neoplasia but are capable of initiating cells in experimental models of multistage carcinogenesis (Dragan et al. 1994). (Fig. 1)

(B) Promotion: This stage is an epigenetic event and it involves the selective clonal expansion of the initiated cell through an increase in cell growth either through an increase in cell proliferation and/or a decrease in apoptosis in the target cell population (Schulte-Hermann et al. 1994). The events of this stage are dose dependent and reversible upon removal of the tumor promotion stimulus. The promoter is administered after initiation and cause enhancement of the neoplastic process (Mukhtar et al. 1988). It is the process whereby an initiated tissue or organ develops focal proliferation and acts as precursors for subsequent steps in carcinogenesis (Luebeck and Hazelton 2002). Promotion occupies the greater part of the latent period of carcinogenesis and can be divided into two steps: conversion and propagation (Sun 1990). In the conversion step, the initiated cell is brought into an altered state so that its proliferative advantage over normal cells is expressed in the propagation step, which leads to the formation of a visible tumor (Sun 1990). The biochemical responses to tumor promoters include increased synthesis of DNA, RNA, proteins, phospholipids and prostaglandins, increased phosphorylation of histone, increased activity of protein kinase C, histidine decarboxylase and protease. The essential feature of promotion is to develop mitogenic environment that imposes a differential effect on initiated cells without affecting surrounding cells. Promoters include agents such as various chemicals, drugs, plant products and hormones that do not directly interact with host cellular DNA but somehow influence the expression of genetic information encoded in the cellular DNA (Laird and Jaenisch 1996). (Fig. 1)
Figure 1: Illustration of different stages of Chemical Carcinogenesis
(C) **Progression:** This is the third stage of carcinogenesis that involves cellular and molecular changes which transform the preneoplastic state to the neoplastic state. This stage is irreversible, involves genetic instability, changes in nuclear ploidy, and disruption of chromosome integrity. Increased replicative DNA synthesis and subsequent cell division is important in each of the stages of carcinogenesis (Pitot et al. 1981; Butterworth 1990). Two possible mechanisms have been proposed for the induction of cancer. In one, an increase in DNA synthesis and mitosis by a non-genotoxic carcinogen may induce mutations in dividing cells through misrepair. With continual cell division, mutations will result in an initiated preneoplastic cell that may clonally expand to a neoplasm. In addition, non-genotoxic agents may serve to stimulate the selective clonal growth of already “spontaneously initiated cells” (Ames 1990). For the maintenance of tissue homeostasis, there exists equilibrium between cell proliferation and cell death. The carcinogenesis thus is a result of an imbalance between cell growth and death. (Fig. 1)

2. **Inflammation and cancer**

The role of inflammation in the development of cancer were discovered on the basis of findings that tumors frequently arose at the sites of chronic inflammation and that inflammatory cells were present in tumors (Balkwill and Mantovani 2001). The chronic inflammation and cancer are intricately and inexplicably interlinked to each other and chronic inflammation provides a nidus for the development of cancer. This link between these two are interconnected was not accepted for more than a century, but there has been a current re-emergence in interest in this relation. Several lines of evidence based on a series of findings, from epidemiological studies of patients to molecular studies of genetically modified mice have led to a common acceptance that inflammation and cancer are linked to each other and confirmed by anti-inflammatory therapies that show efficacy in cancer prevention and treatment (Gonda et al. 2009).
2.1 Chronic Inflammation

Chronic inflammation is induced by biological, chemical, and physical factors and is in turn associated with an increased risk of several human cancers (Bartsch and Nair 2006). The fact that constant irritation over long periods of time can result in cancer had already been described in the traditional Ayurvedic medical system, written as far back as 5000 years ago (Garodia et al. 2007). Whether this irritation is the same as what Rudolf Virchow referred to as inflammation in the 19th century is uncertain (Aggarwal and Gehlot 2009). This chronic inflammation is now considered as one of the culprit for diseases such as cancer. For example, inflammatory bowel diseases such as Crohn disease and ulcerative colitis are associated with increased risk of colon cancer (Ekbom et al. 1990; Ekbom et al. 1990a; Gillen et al. 1994), and chronic pancreatitis is related to an increased rate of pancreatic cancer (Ekbom et al. 1993).

Chronic inflammation can be elicited by various stimuli and it enhances the risk of developing cancer. Such stimuli include microbial infections (for example, infection with Helicobacter pylori is associated with gastric cancer and gastric mucosal lymphoma), autoimmune diseases (for example, inflammatory bowel disease viz., crohn’s disease and ulcerative colitis is associated with colon cancer) and inflammatory conditions of unknown origin (for example, prostatitis is associated with prostate cancer). Consequently, treatment with non-steroidal anti-inflammatory agents (NSAIDs) decreases the incidence and the mortality that results from several types of tumour (Koehne and Dubois 2004; Flossmann and Rothwell 2007; Chan et al. 2007).

The hallmarks of chronic inflammation include the presence of inflammatory cells and inflammatory mediators (for example, chemokines, cytokines and prostaglandins) at the site of inflamed tissues, tissue remodelling and angiogenesis. These signs of chronic inflammation are also present in tumours, which supports the firm causal relationship between cancer and inflammation. Among the panoply of inflammatory mediators, TNF-α and NF-kB are the key...
factors involved in cancer-related inflammation (Mantovani et al. 2008). In inflammatory cells as well as in cells at risk of transformation by carcinogens, NF-kB mediates the transactivation of genes encoding inflammatory cytokines (viz., TNF-α), anti-apoptotic factors (viz., BCL-2), cyclooxygenase-2 (COX2), inducible nitric oxide synthase (iNOS) and angiogenic factors (Orlowski et al. 2002, Mantovani et al. 2008).

(A) **Nuclear factor-kappa B (NF-kB)** was discovered by Ranjan Sen and David Baltimore in 1986 as a factor in the nucleus of B cells that binds to the enhancer of the kappa light chain of immunoglobulin (Sen and Baltimore 1986). It is a transcription factor that initiates the expression of a number of the genes that take part in this response. This important factor is itself activated by the degradation of an inhibitory protein, I-kB. NF-kB is maintained in the cytoplasm in its inactive state by association with I-kB. In response to inflammatory signals, I-kB is phosphorylated at two serine residues, creating an E3 binding site. The binding of E3 leads to the ubiquitination and degradation of I-kB and thereby disrupts the inhibitor's association with NF-kB. The liberated transcription factor migrates to the nucleus to stimulate transcription of the target genes. NF-kB ubiquitously expressed in the cytoplasm of all cell types and is present in all cells. In resting state, it is present in the cytoplasm and in response to different stimuli including inflammation, it translocates to the nucleus only when activated, where it regulates the expression of over 200 genes that control the immune system, growth and inflammation (Pahl et al. 1999).

NF-kB consists of a family of Rel-domain-containing proteins; viz., Rel A (also called p65), Rel B, c-Rel, p50 (also called NF-kB1), and p52 (also called NF-kB2). While p100 undergoes phosphorylation-dependent cleavage to form p52 product, p105 is cleaved to form p50. Similarly, a family of anchorin-domain-containing proteins have been identified, that keep NF-kB in its inactive state within the cytoplasm. These include IkBa, IkBb, IkBg, IkBe, bcl-3, p105
and p100. Under resting conditions, NF-kB consists of a heterotrimer of p50, p65 and IkBa in the cytoplasm. The phosphorylation, ubiquitination and degradation of IkBa leads to the release of the p50–p65 heterodimer, which then translocates to the nucleus and binds its specific 10-basepair consensus site GGGPuNNPyPyCC, where Pu is purine, Py is pyrimidine and N is any base. The individual dimers have distinct DNA-binding specificities for a collection of related kB sites (Gilmore 1999; Ghosh 1998).

**Role of NF-kB in cancer:** Numerous pro-inflammatory cytokines and chemokines viz., TNF-α, IL-1, IL-6 and CXCL8 or IL-8 are encoded by target genes of the IKK-β-dependent NF-kB-activation pathway are associated with tumour development and progression in humans and mice (Balkwill and Mantovani 2001). It has been shown that many oncogenes and carcinogens activated NF-kB, whereas agents with known chemopreventive properties can hamper with NF-kB activation (Bharti and Aggarwal 2002). Current studies on mouse give strong and direct genetic evidence that the classical IKK-β-dependent NF-kB -activation pathway to be the molecular link between inflammation and carcinogenesis (Karin et al. 2002) and NF-kB is indeed a essential mediator of tumour promotion (Greten et al. 2004; Pikarsky et al. 2004).

There are two different NF-kB-activation pathways: the classical pathway and the alternative signalling pathway (Bonizzi and Karin 2004) (Fig. 2). Although both pathways can affect tumour development but the accumulating evidences suggest that the canonical pathway mainly associated with the tumor development. This pathway is triggered by bacterial and viral infections, as well as pro-inflammatory cytokines, all of which activate the IKK complex. This complex is composed of two catalytic subunits, IKK-α (also known as IKK1) and IKK-β (also known as IKK2), and a regulatory subunit, IKK-γ (also known as NEMO). The IKK complex phosphorylates NF-kB-bound IκBs, thus targeting them for proteasomal degradation and liberating NF-kB dimers (p65-p50) to enter the nucleus and mediate transcription of target genes.
(Hayden and Ghosh 2004). This reaction mostly depends on the catalytic subunit IKK-β, which carries out IκB phosphorylation (Ghosh and Karin 2002). The alternative NF-kB -activation pathway involves the upstream kinase NF-kB -inducing kinase (NIK) — which activates IKK-α homodimers, independently of either IKK-β or IKK-γ — leading to the phosphorylation and processing of p100, in response to certain members of the TNF family (Bonizzi and Karin 2004; Hayden and Ghosh 2004). The two pathways activate different gene sets and consequently mediate different immune functions (Bonizzi and Karin 2004). The involvement of the classical NF-kB -activation pathway to acute inflammation and cell-survival mechanisms is well accepted, and sustained NF-kB activation in various malignancies has been described (Karin et al. 2002).

![Diagram](source: Karin and Greten 2005)

**Figure 2:** Signalling pathways that lead to the activation of different nuclear factor-xB transcription factors and the biological consequences of these pathways. (Source: Karin and Greten 2005).
(B) Tumor necrosis factor-alpha (TNF-α) is a proinflammatory, pleiotropic cytokine and a powerful activator of immune response. This soluble factor was first isolated from the serum of Bacillus-Calmette-Guerin-infected mice treated with endotoxin, and shown to replicate the ability of endotoxin to induce haemorrhagic tumour necrosis (Carswell et al. 1975). It triggers cell proliferation, cell death, or inflammation through two distinct receptors: TNF receptor (TNFR) I and II (Wajant et al. 2003). TNFRI, which is found on most cells in the body, and TNFRII, which is primarily expressed on haemopoietic cells. TNFRI is activated by soluble ligand, TNFRII mainly by the membrane-integrated form. TNF-α is synthesised as a 26 kDa (233 amino acids) membrane-bound pro-peptide (pro-TNF-α) and is secreted upon cleavage by TNF-α–converting enzyme (TACE). The 26 kDa form is also functional, binding to TNFRII via direct cell-to-cell contact. The major source of TNF-α are activated macrophages and others include fibroblasts, astrocytes, Kupffer cells, smooth muscle cells, keratinocytes and tumour cells. The downstream signalling cascade of these receptors i.e., TNFRI and TNFRII is complex and involving multiple adapter proteins. These proteins are recruited when TNF-α binds to their receptors and further regulate four different signalling pathways: a pro-apoptotic pathway that is induced by binding caspase-8 to FADD; an anti-apoptotic program that is activated by the binding of cellular inhibitor of apoptosis protein-1 (cIAP-1) to TRAF2; AP-1 activation which is mediated through TRAF2 via a JNK-dependent kinase cascade; and NF-kB activation by RIP (Chen et al. 2002) (Fig. 3).

Several mechanisms have been proposed how TNF-α affects tumor progression. Direct cytotoxicity of TNF-α to tumor cells occur but the reverse with TNF-α as an autocrine growth factor has also been observed. Necrosis and regression of tumors are caused via effects on vasculature or via T-cell mediated immunity (Burke and Balkwill 1996). TNF-α also creates
tumor microenvironment fostering tumor development by induction of tumor promoting cytokines, release of MMPs and pro-angiogenic activity (Coussens and Werb 2002).

Figure 3: Illustration of the main TNF receptor signalling pathways (Source: Karin 2006)

(C) Inducible Nitric Oxide Synthase (iNOS) is another important inflammatory mediator linking chronic inflammation and cancer and it is an enzyme that catalyzed the production of NO during arginine metabolism. It was found to be overexpressed in chronic inflammatory diseases and various types of cancer (Kim et al. 2005). During inflammation, induced expression of iNOS in macrophages and epithelial cells leads to production of NO. The expression of iNOS
and the level of NO have been shown to be elevated in various precancerous lesions and carcinomas (Hussain et al. 2004; Hofseth et al. 2003). It has been shown that iNOS can be induced by pro-inflammatory cytokines, such as TNF-α and IL-1 (Hussain et al. 2004), and the transactivated by NF-κB, therefore it may be a downstream mediator of pro-inflammatory cytokines and NF-κB and thus linking inflammation to cancer. Once the inflammation-associated tumors are formed, iNOS expression may be persistently stimulated by cytokines and NF-κB that are prevalent within the tumor inflammatory microenvironment (Li and Verma 2002). It was also suggested that, due to the higher promoter activities of iNOS, excess NO may be produced and cause chronic inflammation, which contributes to the H. pylori induced gastric cancer (Tatemichi et al. 2005). Noticeably, a study using a wide range of in vitro and in vivo models show that iNOS/NO signalling can also induce COX-2, which itself is a promising link between inflammation and cancer (Rao 2004). (Fig. 4)

(D) Cyclooxygenase-2 (COX-2) is an enzymes that plays a key role in the synthesis of lipid inflammatory mediators (prostaglandins and prostacyclines) from arachidonic acid and several studies have indicated that aberrant induction of COX2 and prostaglandins are implicated in the pathogenesis of various type of malignancies. The expression may be induced by a wide range of stimuli, including lipopolysaccharide, proinflammatory cytokines, such as IL-1 and TNF-α, and growth factors, such as epidermal growth factor (Karin et al. 2002; Williams et al. 1999). The products of COX-2 enzyme are prostaglandins, which are key mediators of inflammation (Nathan 2002; Steele et al. 2003). Various nonsteroidal anti-inflammatory drugs affect COX-2 activity by covalent modification or competition for a substrate binding site, and the long-term use of non-steroidal anti-inflammatory drugs was shown by population-based studies to reduce the risk of several cancers (Williams et al. 1999; Buskens et al. 2002; Farrow et al. 1998; Giardiello et al. 1995). COX-2 is also over-expressed in various types of cancer and involved in
cellular proliferation, anti-apoptotic activity, angiogenesis, and an increase of metastasis (Tsujii et al. 1997; Prescott and Fitzpatrick 2000; Eberhart et al. 1994; Ristimaki et al. 1997; Hwang et al. 1998). (Fig. 4)

Figure 4: Schematic representation of the mechanisms for the involvement of inflammation in cancer development.
The functions of COX-2 in linking inflammation to cancer are now becoming the target of intense investigation. A recent study using an oesophageal model of rats indirectly supports that the COX-2 induction might contribute to the progression of cancers from inflammation (Oyama et al. 2004). In this study, specific COX-2 inhibitor celecoxib inhibits the COX-2 pathway and delays the developing progress from esophageal inflammation, metaplasia, to adenocarcinoma. It was reported to be present in 80-90% of colon adenocarcinomas and in 40-75% of premalignant adenomas (Williams et al. 1997; Chapple et al. 2002; Sato et al. 2003; DuBois et al. 1996). Further studies are still warranted to explore the exact role of COX-2 in linking inflammation and cancer and to extend those findings to other types of inflammation associated cancers. Arachidonic acids are the substrate for COX-2 to produce prostaglandins. Interestingly, arachidonic acids can be converted by another enzyme lipoxygenases to leukotrienes, which were suggested to be another missing link between inflammation and cancer (DuBois 2003).

3. Oxidative stress and cancer

Oxidative stress is a condition which takes place due to imbalanced redox status between the production of reactive oxygen species (ROS) and the endogenous antioxidant system able to remove them via scavenging these ROS. ROS are those species that are more reactive than the ground state molecular oxygen. Free radicals are atoms or molecules containing one or more unpaired electron (Moslen and Smith 1992) that are capable of independent existence and are highly reactive. ROS having unpaired electron are known as free radicals and they include superoxide anion (O$_2^-$), hydroxyl (HO$_2$), hydroxyl (OH), and alkoxyl (RO·) radicals. ROS, including superoxide (O$_2^-$), hydroxyl radical (·OH) and H$_2$O$_2$, are constantly generated in aerobic organisms. Usually the endogenous sources of these ROS are oxidative phosphorylation, cytochrome P450 metabolism, peroxisomes and activated inflammatory cell (Klaunig and
Kamendulis 2004; Poli et al. 2004). Enzymatically, ROS can be generated by cytochrome P450, xanthine oxidase, prostaglandin synthase, lipooxygenase and myeloperoxidase (Matsumoto et al. 2003, Takami et al. 2000, von Kruecener et al. 1995) whereas non-enzymatically, it is generated by lipid peroxidation (Tafee and Kensler 1989). Typically, ROS, released by the neutrophils, also acts as a host defending species for destroying exogenous pathogens such as bacteria. ROS can also be generated by ionizing radiation, chemotherapeutic drugs and environmental exposure to chemical oxidants (Klaunig and Kamendulis 2004; Ercal et al. 2001; Kovacic and Osuna 2000).

The human cytochromeP450enzyme system metabolizes a wide array of xenobiotics to pharmacologically inactive metabolites, and occasionally, to toxicologically active metabolites. Impairment of cytochromeP450 activity, which may be either genetic or environmental, may lead to toxicity caused by the parent compound itself (Valko et al. 2007). P450enzymes may also convert the drug to a chemically reactive metabolite, which, if not detoxified, may lead to various forms of hepatic and extrahepatic toxicity, including cellular necrosis, hypersensitivity, teratogenicity, and carcinogenicity, depending on the site of formation and the relative stability of the metabolite, and the cellular macromolecule with which it reacts. Several enzyme systems have been considered in the production of ROS, including NADPH oxidase, xanthine oxidase, (uncoupled) mitochondrial electron transport, and P450 (Finkel and Holbrook 2000; Halliwell and Gutteridge 1990). P450 enzymes are widely studied because of their roles in the metabolism of steroids, fat-soluble vitamins, fatty acids, eicosanoids, drugs, and carcinogens, plus other xenobiotic chemicals (Ortiz de Montellano 2005). Cytochrome P450 is responsible for the bioactivation of DMH which is a procarcinogen and then it leads to the formation of methyl free radicals and the latter will reacts with DNA (Fiala 1977).

Oxidative stress is closely related to all aspects of cancer, from carcinogenesis to the tumor-bearing state, from treatment to prevention. When such oxidative stress exceeds the capacity of
the oxidation-reduction system of the body, gene mutations may result or intracellular signal transduction and transcription factors may be affected directly or via antioxidants, leading to carcinogenesis. The tumor-bearing state is also said to be under oxidative stress associated with active oxygen production by tumor cells and abnormal oxidation-reduction control. The ROS or free radicals are also believed to be involved in all the three stages of carcinogenesis i.e., initiation, promotion and progression (Perchellet and Perchellet 1989).

ROS can randomly react with lipids, proteins and nucleic acids causing oxidative stress and damage in these macromolecules, leading to pathogenesis of chronic diseases, which include cancer (Cooke et al. 2003; Evans et al. 2004). Recently, a lot of evidence indicates that ROS play a central role in the key intracellular signal transduction pathway for a variety of cellular process (Wu 2006).

Endogenous cellular antioxidant defense system against oxidative stress include reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), catalase, glutathione-S-transferase (GST), glucose-6-phosphate dehydrogenase (G6PD) and quinone reductase (QR) (Klaunig and Kamendulis, 2004; Clarkson and Thompson, 2000).

**(i) Reduced Glutathione (GSH):** GSH, a low molecular weight tripeptide, is a cellular antioxidant and the most abundant thiol in mammalian cells. It is present in reduced (GSH) and oxidized (GSSG) forms (Meister and Anderson 1983). The reduced form of glutathione is 10 to 100-fold higher than the oxidized form. An increase in intracellular GSSG can arise from the breakdown of H$_2$O$_2$ by glutathione peroxidase (Cotgreave et al. 1988). Because of the relatively low concentration of GSSG in the cell compared with GSH, a minor elevation in the oxidation of GSH to GSSG can result in a significant elevation in intercellular GSSG levels. Oxidized glutathione can be reduced to GSH by the NADPH-dependent glutathione reductase. GSH is
also used as a cofactor for antioxidant enzymes such as GSH peroxidase, involved in the reduction of peroxides, including membrane lipids peroxides formed upon oxidative insults (Flohe 1978). The GSH/GSSG ratio is normally closely regulated. Disruption of this ratio is involved in several cellular reactions involved in signal transduction and cell cycle regulation under conditions of oxidative stress; the GSH/GSSG ratio tends to decrease either through an increase in the level of GSSG or a decrease in GSH (Schafer and Buettner 2001; Cotgreave and Gerdes 1998; Herlich and Bohner 2000). (Fig. 5)

(ii) Superoxide dismutase (SOD): It catalyzes the reduction of super oxide anion to \( \text{H}_2\text{O}_2 \), which is comparatively less reactive. In skin, it is present in different forms: copper/zinc super oxide dismutase (Cu/Zn-SOD) is found in cytosol while manganese superoxide dismutase (Mn-SOD) is located in the mitochondrial matrix (Vesssey 1993). The Mn-SOD acts as a first line of defense in the detoxification of super oxide anion and is thought to be involved in tumor suppression and cellular differentiation (Church 1993). (Fig. 5)

(iii) Glutathione peroxidase (GPx): This enzyme catalyses the detoxification of \( \text{H}_2\text{O}_2 \) at the expense of the oxidation of GSH to GSSG. It also reduces toxic lipid peroxides to less toxic hydroxy fatty acids utilizing glutathione. There are two forms of GPx one of which is selenium dependent and the other is selenium independent. Se-dependent GPx is highly reactive towards both \( \text{H}_2\text{O}_2 \) and organic hydroperoxides (Flohe 1989). (Fig. 5)

(iv) Catalase: It catalyzes the conversion of hydrogen peroxide \( \text{(H}_2\text{O}_2 \) into water \( \text{(H}_2\text{O} \) and molecular oxygen \( \text{(O}_2 \), thereby reducing the grave damaging effects of \( \text{H}_2\text{O}_2 \) as it can traverse lipid bilayers. \( \text{H}_2\text{O}_2 \) can react with transition metal ions to form extremely reactive hydroxyl and alkoxy radicals which in turn can induce oxidative stress. Catalase is a peroxisomal antioxidant enzyme and is present in all major organs of the body especially liver and erythrocytes (Schobeam and Chance 1976). (Fig. 5)
(v) **Glutathione reductase (GR):** In the cells the ratio of GSH:GSSG is relatively higher in order to cope with oxidative reactions. Reduced glutathione is normally restored by glutathione reductase that is catalyses the reduction of GSSG by utilizing the reducing power of NADPH. It is found in cytosol and mitochondria (Sun 1990). (Fig. 5)

(vi) **Glucose-6-phosphate dehydrogenase (G6PD):** catalyses the formation of 6-phosphogluconolactone from Glucose-6-phosphate using the co-factor NADP that gets reduced to form NADPH (Sun 1990). (Fig. 5)

(vii) **Quinone reductase (QR):** It is also known as DT diaphorase. It catalyses complete two electron reduction of quinones to relatively stable hydroquinones that in turn is estimated or undergoes conjugation to form glucouronate or sulphate (Sies 1988) thus preventing the partial one-electron reduction of quinones to semiquinone free radical intermediates. (Fig. 5)
4. Apoptosis and cancer

Apoptosis is one of the main type of programmed cell death. It was initially described by its morphological characteristics, including cell shrinkage, membrane blebbing, chromatin condensation and nuclear fragmentation (Kerr et al. 1972; Wyllie et al. 1980; Kerr et al. 1994). Now, it has been shown that apoptosis is a gene-directed program which plays important role in developmental biology and tissue homeostasis. There are two different pathways that initiate apoptosis: one is mediated by death receptors on the cell surface sometimes referred to as the ‘extrinsic pathway’; the other is mediated by mitochondria referred to as the ‘intrinsic pathway’.

Figure 6: The molecular mechanisms of apoptosis. Apoptosis pathways can be initiated via different stimuli—that is, at the plasma membrane by death receptor ligation (extrinsic pathway) or at the mitochondria (intrinsic pathway). (Source: Ghavami et al., 2009)
(i) Extrinsic/Death Receptor Pathway: The receptor mediated or extrinsic apoptotic pathway was the first one to be described (Fig. 6). The extrinsic pathway is activated through extracellular ligands by binding to receptors that are located in the cell membrane. Typical death receptors are Fas (fibroblast associated antigen, also called Apo-1 or CD95), tumour necrosis factor receptor-1 (TNFR-1), TRAIL-R1 (TNF-related apoptosis-inducing ligand-R1) or TRAIL-R2 they belong to TNF-R family and contain a cytosolic death domain. Binding of ligands to death receptors causes formation of death inducing signalling complex (DISC) (Kischkel et al. 1995; Ashkenazi et al. 1998) in which the adaptor proteins FADD (MORT1) and/or TRADD bind with their death domain (DD) to a DD in the cytoplasmic region of the receptors, consequently, leads to the recruitment and activation of caspase-8 or -10 (initiator caspases) to the DISC (Boldin et al. 1995; Bodmer et al. 2000). The activated caspase then proteolytically activates downstream effector caspases (also called executioner caspases) i.e., caspase-3 that degrade cellular targets. Activated caspase-8 then directly cleaves pro-caspase-3 or other executioner caspases, eventually leading to the apoptosis. Caspase-8 can also cleave the BH3-only protein Bid. The resulting truncated Bid (tBid) then moves to the mitochondria and induces cytochrome c release, leading to activation of caspase-9 and caspase-3. DISC signalling can be inhibited by expression of c-FLIP, a physiologic dominant negative caspase-8 that leads to the formation of a signalling inactive DISC (Li and Yuan 2008).

(ii) Intrinsic/Mitochondrial pathway: This pathway is activated by several of cellular stresses, including oxidative stress, irradiation, and treatment with chemotherapeutic drugs/cytotoxic drugs (Ghavami et al. 2004; Ghavami et al. 2008; Hashemi et al. 2004). This pathway is mediated by Bax/Bak insertion into mitochondrial membrane, and ensuing release of cytochrome c from the mitochondrial inter-membrane space into the cytosol (Kim 2005). Anti-apoptotic Bcl-2
family members, such as Bcl-2 and Bcl-XL, prevent cytochrome c release, apparently by binding and inhibition of Bax and Bak. BH3-only proteins, such as Bid and Bim, contribute to the pro-apoptotic function of Bax or Bak by inducing homo-oligomerisation of these proteins. Cytochrome c then binds to the Apaf1 and together with (d) ATP causes recruitment of pro-caspase-9 to the complex. The formed multi-protein complex is called apoptosome, which contains several units of Apaf1 and other above molecules (Bratton et al. 2000; Adrain and Martin 2001). Activated caspase-9 in turn activates caspase-3 and initiates the proteolytic cascade (Li et al. 1997). In addition to cytochrome c, mitochondria release a large number of other polypeptides, including apoptosis inducing factor (AIF) (Lorenzo et al. 1999), Endo G, second mitochondrial activator of caspases (Smac/DIABLO) (Du et al. 2000) and HtrA2/Omi (Li et al. 2002) from the intermembrane space. Smac/Diablo and Omi/HtrA2 promote caspase activation through neutralising the inhibitory effects of inhibitor of apoptosis proteins (IAPs) (Van Loo 2002), while AIF and endonuclease G cause DNA damage and condensation (Debatin 2004). (Fig. 6)

Reduced apoptosis is generally associated with tumorigenesis, so genes involved in the negative regulation of this process might have an oncogenic potential (e.g., Bcl-2, Bcl-xL, Survivin), whereas genes involved in the positive regulation of apoptosis might act as tumor suppressor genes (e.g., Bax, Bak). There is now accumulating evidence that some pro-apoptotic genes, in combination with other genes, functions as tumor suppressor. In addition, it appears that in many cases negative regulators of apoptosis might act as oncogenes, but only in cooperation with other proteins apparently, proto-oncogenes. (Fig. 7)

However, the Bcl-2 family includes more than 30 proteins, each of which fulfills either anti-apoptotic or proapoptotic functions, it seems logical that the ratio between these proteins, rather than overexpression of one particular member of this family, might influence tumor formation.
and/or the susceptibility of the tumor cells to undergo apoptosis (Anderson et al. 2005). Accumulating evidence further suggests that downregulation of pro-apoptotic proteins, in combination with the expression of other structural and regulatory proteins, are essential for metastatic progression. It seems that the overall status of the cell death machinery, rather than just the expression levels of the individual proteins, might explain how it affects tumorigenesis (Zhivotovsky and Orrenius 2006). (Fig. 7)
5. Colon cancer

The colon is an important part of the body's digestive system. The colon is a large muscular cylindrical tube (approximately five feet long) that collects and stores waste which then passes into the rectum. The principal functions of the colon are (1) absorption of water and electrolytes from the chyme to form solid feces and (2) storage of fecal matter until it can be expelled.

The colon is a 6-foot long muscular tube that connects the small intestine to the rectum. The large intestine is made up of the cecum, the ascending (right) colon, the transverse (across) colon, the descending (left) colon, and the sigmoid colon, which connects to the rectum. The appendix is a small tube attached to the cecum. The large intestine is a highly specialized organ that is responsible for processing waste so that emptying the bowels is easy and convenient.

Stool, or waste left over from the digestive process, is passed through the colon via peristalsis, first in a liquid state and ultimately in a solid form. As stool passes through the colon, water is removed. Stool is stored in the sigmoid (S-shaped) colon until a "mass movement" empties it into the rectum once or twice a day. It normally takes about 36 hours for stool to get through the colon. The stool itself is mostly food debris and bacteria. These bacteria perform several useful functions, such as synthesizing various vitamins, processing waste products and food particles, and protecting against harmful bacteria. When the descending colon becomes full of stool, or feces, it empties its contents into the rectum to begin the process of elimination. (Fig. 8)

5.1 Colon Carcinogenesis: a multistep process

Colon cancer is one of the most common, best-understood neoplasms from a genetic point of view, yet it remains the leading cause of cancer-related mortality in men and women (O'Brien et al. 2007, Jemal et al. 2009). More than 1 million new cases of colorectal cancer (CRC) are diagnosed worldwide each year (Tenesa et al. 2009). CRC is the 3rd most common malignancy
and 4th most common cause of cancer mortality worldwide (Tenesa 2009). CRC is also the 2nd most common cause of cancer deaths in the United States and other developed countries, despite important advances in detection, surgery and chemotherapy (Jemal et al. 2009; Jemal et al. 2009).

**Figure 8:** Different layers of the normal human colon. (Source: www.penncancer.org)

In terms of epidemiological significance, colonic tumors are limited to hyperplastic polyps, adenomas and adenocarcinomas. Other types of benign and malignant tumors of the colon comprise of less than 5% of the tumor population (Fenoglio-Preiser et al. 1990). Colonic hyperplastic polyps are frequently occurring lesion in the colon is the hyperplastic polyp. If no cellular dysplasia is visible in the microscope, a polyp is scored as a hyperplastic polyp (Rex et al. 1992). It is commonly believed that hyperplastic polyps, especially those located in the rectum and sigmoid colon are benign lesions lacking malignant potential. These polyps consist of a
localized zone of proliferation of intestinal mucosa associated with exaggerated cellular
differentiation and maturation (Ponz de Leon 2002).

Colonic adenomas/adenomatous polyps are well demarcated lumps of epithelial dysplasia that
can be classified into three major histological types: tubular, villous and tubule-villous (Ponz de
Leon 2002). These are benign glandular neoplasms originating from intestinal mucosal
epithelium characterized by incomplete cellular differentiation and by unrestricted cell division.
The adenomas are classified according to the grade of dysplasia as mild, moderate or severe.
Dysplasia predisposes an organ to cancer development. Adenomas are known to be the
precursors of sporadic and hereditary colorectal cancer (Vogelstein et al. 1988; Kinzler et al.
1996). Most CRCs are adenocarcinomas which have developed from adenomas. Only about 5%
of adenomas become malignant (Wilcox et al. 1987). In sporadic cancer the progression from
adenoma to cancer takes approximately 10-15 years (Stryker et al. 1987). Several studies have
shown that removal of adenomas may reduce the incidence of colorectal cancer (Järvinen et al.
2000; Winawer et al. 1993; Thiis-Evensen et al. 1999). Adenocarcinomas of the colon are
malignant tumors arising from the mucosal glandular epithelium. Abundant evidence from
clinical and histopathological studies demonstrates that the majority of colon cancer arises from
pre-existing adenomas over a long period of time. In fact, very often a continuous histological
spectrum with increasing degree of atypia and progressive invasion can be seen in an adenoma
containing a carcinoma (Fenglio and Pascal 1982; Morson 1968). This is often referred to as
adenoma-carcinoma sequence (Morson 1974; Muto et al. 1975). On the other hands, there
appears to be a little evidence to support an evolutionary relationship between hyperplastic and
adenomatous polyps, thus hyperplastic polyps should not be included in the polyp-cancer
sequence (Fenoglio-Preiser et al. 1990; Lane et al. 1971).
Studies on colon cancer support the hypothesis that colon cancer progress through a multi-step process. A small benign adenomatous polyp may arise preferentially from instead of normal mucosa, the hyperproliferative mucosa or abnormal tissue architecture such as aberrant crypt foci (ACF) (Lipkin 1988; Tudek et al. 1989). Subsequent progression of a small adenoma to a large adenoma with increased malignant potential may occur in some cases. Finally, a fraction of larger adenomas may progress to invasive and metastatic cancer (Vogelstein and Kinzler 1993).

These abnormal crypts can be identified by increased size, thicker epithelial lining and increased pericryptal zone after staining the colon with methylene blue (Tudek et al. 1989). But, recent reports has been demonstrated that adenomas arises from another preneoplastic lesions i.e., mucin depleted foci (MDF) which are formed by dysplastic crypts devoid of mucin (Caderni et al. 2003). (Fig. 9)

![Colon Carcinogenesis Diagram](source: Janne et al. 2000)

**Figure 9: Colon Carcinogenesis (Source: Janne et al. 2000).**
5.2 Early Markers of Colon Cancer

**A) Aberrant Crypt Foci (ACF):** ACF were first discovered in the colon of carcinogen treated rodent (Bird et al. 1987) and they have also been observed in patients with sporadic colorectal cancer (CRC) and with familial adenomatous polyposis (FAP) (Pretlow et al. 1991; Roncucci et al. 1991; Nucci et al. 1997). They are identified by their microscopically elevated crypts, thicker colonic epithelial cell lining, increased lumen of the crypt and increased pericryptal zone comparative to normal crypts after staining the colon with methylene blue (Bird et al. 1987). They exhibit preneoplastic features viz., dysplasia (Paulsen et al. 2005; Jen et al. 1994; Siu et al. 1997), hyperproliferation (Bird et al. 1987; Pretlow et al. 1994; Bird 1995), K-ras mutations (Stopera et al. 1992a; Stopera et al. 1992b; Vivona et al. 1993; Pretlow et al. 1993), and overexpression of c-fos (Stopera et al. 1992c), β-catenin (Paulson et al. 2005; Paulson et al. 2001) and cyclin D1 (Paulson et al. 2005). (Fig. 10, 11)

![Figure 10: Morphological appearance of Aberrant Crypt Foci](image)
B) Mucin Depleted Foci (MDF)

MDF were first discovered in the colon of carcinogen treated rats (Caderni et al. 2003) and they have also been observed in patients with FAP and with sporadic CRC (Femia et al. 2008a; Sakai et al. 2011). MDF are characterized by the absence or very limited production of mucins. Mucins are high molecular weight, heavily glycosylated proteins which form a protective layer in the form of gel in intestinal lumen and are synthesized and secreted by the specialized exocrine cells of the colonic crypts i.e., goblet cells (Robbe et al. 2004; Specian and Oliver 1991). MDF are easy to quantify in the entire unsectioned colon and show clear characteristics of dysplasia in histological sections. They also exhibit preneoplastic features viz., dysplasia (Caderni et al. 2003; Femia et al. 2007a; Femia et al. 2008b; Sakai et al. 2011), mutations in β-catenin (Femia et al. 2005) and Apc (Femia et al. 2007a) gene, over-expression of survivin (Femia et al. 2011), COX-2, i-NOS and macrophages (Femia et al. 2009) and reduced expression of MUC2 (a mucin abundantly expressed in the normal colon) and intestinal trefoil factor, a marker of goblet cell lineage (ITF or TFF3), which protects intestinal epithelial cells from various insults and
contributes to mucosa repair (Femia et al. 2008b). While the expression of p21 and p16 (inhibitors of cyclin-dependent kinases) have been found be reduced in ACF as well as MDF (Femia et al. 2007b). On the basis of above evidences, both ACF and MDF are considered to be putative early biomarkers of colon cancer. (Fig. 12)

Figure 12: Topographical appearance of MDF in HID-AB–stained rat and human colon. (Source: Femia et al., 2008; Cadernì et al., 2003).

5.3 Risks Factors for Colon Cancer

Sporadic

Most cases of colorectal cancer are sporadic, and genetic and environmental factors are important. About 20% of all patients with this cancer are estimated to have some component of familial risk without fulfilling the strict criteria for hereditary colorectal cancer (Steward and Kleihues 2003). Family history should therefore always be taken when assessing a patient; the Bethesda guidelines are valuable in this context. However, taking a family history by interview often underestimates family history of colorectal cancer (Lynch and de la Chapelle 2003). Most of the colon cancer cases are sporadic (88–94%) and the causes are older age, male sex, cholecystectomy, ureterocolic anastomosis, hormonal factors: nulliparity, late age at first pregnancy, early menopause. Environmental factors includes diet rich in meat and fat, and poor
in fibre, folate, and calcium, sedentary lifestyle, obesity, diabetes mellitus, smoking, previous irradiation, occupational hazards (eg, asbestos exposure) and high alcohol intake.

Personal history of sporadic tumours includes history of colorectal polyps, history of colorectal cancer, history of small bowel, endometrial, breast, or ovarian cancer (Mitchell et al. 2004).

**Hereditary**

Roughly 5–10% of all colorectal cancers develop in the setting of defined hereditary cancer syndromes. The two main forms are hereditary nonpolyposis colorectal cancer (HNPCC) and familial adenomatous polyposis (FAP) (Lynch and de la Chapelle 2003). Various hamartomatous polyposis syndromes are also associated with an increased risk of such cancer, such as Peutz-Jeghers syndrome, juvenile polyposis syndrome, and Cowden syndrome (Lynch and de la Chapelle 2003; Half and Bresalier 2004).

FAP is an autosomal-dominant disease. In about 80% of affected individuals, a germline mutation can be identified in the adenomatous polyposis coli (APC) gene (Lynch and de la Chapelle 2003; Half and Bresalier 2004). A subset of people with FAP and attenuated FAP has biallelic mutations of the MYH gene (Sieber et al. 2003; Venesio et al. 2004). FAP patients can develop more than 100 colorectal adenomas (50% of patients by age 15 years, 95% by age 35 years); if left untreated, colorectal cancer arises in almost all patients by age 40 years. Extracolonic manifestations, such as periampullary duodenal carcinoma (4–6% of patients) and desmoids (10–20% of patients), are a major cause of mortality and morbidity (Half and Bresalier 2004; Bülow et al. 2004; Parc et al. 2004).

HNPCC is an autosomal-dominant disorder caused by germline mutations of mismatch repair genes. Tumours that arise in the setting of HNPCC typically have a molecular characteristic called microsatellite instability, which helps in making the diagnosis. This instability is defined as frequent mutations in microsatellites, which are short repeated DNA sequences (Grady 2003).
The penetrance of colorectal cancer in HNPCC is 70–85%. Risk is also increased for tumours of the genitourinary system, stomach, biliary system, pancreas, small intestine, and CNS (Lynch and de la Chapelle 2003; Half and Bresalier 2004; Vasen et al. 1999). A genotype-phenotype correlation was suggested for patients with HNPCC (Bandipalliam et al. 2004).

**Colorectal cancer in inflammatory bowel disease**

Colorectal cancer accounts for about a third of deaths related to ulcerative colitis, and risk depends on disease duration (2% of affected people by 10 years, 8% by 20 years, and 18% by 30 years), extent of inflammation, presence of primary sclerosing cholangitis, and backwash ileitis (Itzkowitz and Hapraz 2004; Krok and Lichtenstein 2004). Crohn’s colitis is also associated with increased risk of colorectal cancer; the relative risk is similar to that for ulcerative colitis (Itzkowitz and Hapraz 2004).

5.4 Genetic Model of Colon Carcinogenesis

Specific molecular events underlying the initiation of colorectal tumorigenesis are poorly understood. However, studies from human and animal models suggest that colorectal adenomas and carcinomas arise after somatic mutational events. In fact, even very small benign tumors from either sporadic or familial origin are clonal (Fearon et al. 1987). Similar results were found in early dysplastic lesions and small adenomas in mice treated with AOM (Ponder and Wilkinson 1986). Thus, observations from both human and murine models are consistent with the hypothesis that a somatic mutational event causes one or a small number of cells from within a single intestinal crypt to initiate the neoplastic process by clonal expansion (Nowell 1976). Recently, extensive genetic alterations including both tumor suppressor genes and oncogenes e.g., abnormalities in *APC, MCC, DCC, p53, ras*, have been reported. All inherited mutations identified in the APC gene appear to result in inactivation of the gene, including gross deletions
Levy et al. 1994). Thus, the evidence suggests that germ-line inactivation of the \textit{APC} gene predisposes to colonic neoplasm in human and mice, and argues that the \textit{APC} gene is a tumor suppressor gene. Moreover, studies demonstrated that inactivation of both \textit{APC} alleles is necessary for adenoma development (Ichii et al. 1992; Levy et al. 1994), which is consistent with the recessive nature of a tumor suppressor gene.

The importance of \textit{APC} gene in colorectal cancer research is not limited to FAP or hereditary colorectal cancers. As much as 35-60\% of tumors from patients with no familial predisposition to colorectal cancer display allelic losses involving chromosome 5q21 (Ashton-Rickardt et al. 1989; Solomon et al. 1987; Vogelstein et al. 1988). Chromosome 5q allelic losses are the most frequently detected genetic alteration in small, early adenomas from patients without polyposis (Powell et al. 1992), whereas LOH of 5q can only rarely be noted in adenomas from patients with polyposis (Vogelstein et al. 1988). This suggests that in the majority of colorectal tumors, one or both alleles of the \textit{APC} gene may be inactivated by somatic mutation, and this inactivation may occur at an early stage of tumorigenesis. Considering the existence of small deletion and point mutations that are not detectable by allelic analysis, the actual mutation frequency could be much higher. Moreover, a second gene at chromosome 5q21, the \textit{MCC} (mutated in colorectal cancers) gene, has also been implicated in the development of some sporadic colorectal cancers by somatic mutations that appear to inactivate the gene in about 15\% of cases (Kinzler et al. 1991). Pulse-field gel electrophoresis of constitutional DNA from two FAP patients revealed cytogenetically invisible deletions that exclude the \textit{MCC} from within the
region containing the FAP predisposing mutation (Joslyn et al. 1991). It is speculated that APC protein is a multifunctional microtubule-associated protein and may somehow regulate cellular growth of colonocytes (Bhattacharya and Boman 1995; Munemitsu et al. 1995a; Munemitsu et al. 1995b).

Studies from in vitro and animal models have implicated that ras genes are critical to tumor development in a number of tumor types including colon cancer (Shirasawa et al. 1993). Mutations in ras genes can be identified in about 50% of colon carcinomas and adenomas larger than 1 cm in diameter (Forrester et al. 1987; Shaw et al. 1991; Vogelstein et al. 1988). Adnomas of this size are thought to have increased malignant potential (Muto et al. 1975). Studies of adenomas with various grades of dysplasia have also found that ras mutations are more frequently detected in tumors with increased dysplasia regardless of their polyposis or non-polyposis (Miyaki et al. 1990; Ranaldi et al. 1995). These data favour the hypothesis that K-ras mutations are involved in intermediate to late stage in colon carcinogenesis. However, studies from chemically induced colon cancer models in rats suggested that K-ras mutation appeared early in the neoplastic lesions (Stopera and Bird 1992; Stopera et al. 1992). Mutations in codon 12 of K-ras gene alone were present in 73% of the ACF they obtained from grossly normal colonic mucosa of colon cancer patients (Pretlow et al. 1993). The 12th codon mutations of the K-ras gene account for about 70-80% of total ras mutations detected in colon cancer (Fearon 1993). Currently, there exist two possible hypotheses for the role of observed ras mutations in colon cancer development. Mutations in the ras genes may be an early initiating event in the development of small fraction of adenomas, but these adenomas possess increased potentials to progress to larger adenomas or adenocarcinomas than do those without ras mutations. Alternatively, ras mutations may contribute at a later stage when small adenomas progress to larger ones with elevated malignant potential. Further studies are necessary to understand the
roles of ras oncogene mutations in colorectal tumor development. Allelic losses or LOH in chromosome 17p and 18q are frequently observed in colorectal cancers. Therefore it is reasonable to assume that other tumor suppressor genes in these regions are involved (Vogelstein et al. 1988; Vogelstein et al. 1989). LOH on chromosome 18 centering 18q21 can be detected in more than 70% of colorectal carcinomas, in about 50% of advanced adenomas, but rarely in earlier stage adenomas. A candidate tumor suppressor gene from this region DCC has been identified (Fearon et al. 1990). Perhaps, inactivation of the DCC gene is responsible for some altered growth properties seen in advanced colorectal tumor cells, including invasiveness, altered adhesion and increased metastatic potential. Currently only a limited number of colorectal cancers have been found to harbor DCC gene alterations (Kikuchi-Yanoshita et al. 1992a) and knowledge about DCC protein and its tumor suppressor property is very limited. Allele losses in chromosome 17p were found in more than 75% of colorectal carcinomas, but were rarely detected in adenomas at any stage (Baker et al. 1989; Shaw et al. 1991). In a few cases, allele loss at 17p has been found to be associated with the progression of individual tumors from adenoma to carcinoma (Kikuchi-Yanoshita et al. 1992a; Volgestein et al. 1988). Evidence supports that p53 is one of the tumor suppressor gene at 17p that is associated with the development of colon carcinomas. The p53 gene encodes a 53KD of nuclear phosphoprotein. The wild type protein appears to form a homodimer that binds to specific DNA sequences and acts as a transcription factor (Fields and Jang 1990; Ravcroft et al. 1990), and seems to function by reacting to DNA damage, causing cell cycle arrest and leading to apoptosis (Canman et al. 1994; Guillouf et al. 1995; Lane et al. 1994; Lowe et al. 1993). Dimerization of the p53 gene product may in part explain the dominant negative effect seen in some cases when wild type and mutant p53 co-exist (Eliyahu et al. 1988). However, in almost 90% of colon carcinomas with LOH on 17p, a single missense point mutation could be detected in the remaining p53 allele (Baker et al. 1990). Thus,
point mutation in one p53 allele, coupled with the loss of the remaining wild type allele, both occur frequently in the later stages of the progression to colon carcinomas. Finally, p53 mutations are not rate limiting for the development of early stage colon adenomas (Malkin et al. 1990). Also, p53 deficient mice did not spontaneously develop tumors in the intestine. Mutations in the other genes may be necessary before mutated p53 alleles can exert a phenotypic in colonic epithelial cell (Donehower et al. 1992).

5.5 Colon specific carcinogens in rodents: DMH and its metabolites

The synthetic compounds 1,2-dimethylhydrazine, chemically related to the naturally occurring carcinogen cycasin (Laqueur 1965; Laqueur 1975), have proved to be of great value in their reliable and specific ability to produce colon tumors in several rodent species (Druckrey et al. 1967; Thurnherr et al. 1973; Ward et al. 1974). With animal models such as these, it becomes possible to study modifying influences on the initiation and development of colon cancer under strictly controlled laboratory conditions, and thus, by drawing appropriate analogies, to approach the understanding of the etiology, also to attain the goals of prevention, diagnosis and better management of colon cancer in man (Weisburger 1973). The potency and organo-specificity of pro-carcinogens, such as 1,2-dimethylhydrazine and azoxymethane, are to a great extent determined by the chemical reactivity and the availability of sufficient amounts of their ultimate carcinogenic forms in the cells of the target tissues. The amount of the ultimate carcinogen, in turn, is a function of the activities of the metabolic pathways leading to its formation, the activities of detoxication pathways and also of the biological half-lives of all of the metabolic species involved. Thus, for example, the procarcinogen 1,2-dimethylhydrazine has been postulated by Druckrey (Druckrey et al. 1967; Druckrey 1970; Preussmann et al. 1969) to undergo a series of chemical transformations in vivo, which include as intermediates the
Compounds azomethane, azoxymethane, the proximate carcinogen methylazoxymethanol, and the ultimate carcinogen methyldiazonium ion. The latter, a highly reactive species, forms methyl carbonium ions, which, presumably, are responsible for the methylations of macromolecules and other compounds both in vivo (Hawks and Magee 1974; Miller 1964; Thurnherr et al. 1973) and in vitro (Matsumoto and Higa 1966; Thurnherr et al. 1973). (Fig. 13)

**Mechanism of action of DMH in Rodent Model**

1,2-dimethylhydrazine

↓

Azomethane

Cyt P450

N-oxidation

Azoxymethane

Cyt P450

Methylazoxymethanol

Methyldiazonium ion + Formaldehyde

Methyl Carbonium ion + N2

forms adduct with DNA

Colon Cancer

*Figure 13: depicts metabolism and mechanism of action of DMH.*
The histopathology of DMH induced colon adenocarcinomas is similar to those seen in colorectal cancer patients (Pozharisski et al. 1979). There is evidence that induced colon tumors follow an adenoma to carcinoma sequence, resembling that of human colorectal tumors (Madara et al. 1983). The early histopathologic changes after DMH administration include the following:
1) Aberrations of nuclei of epithelial cells at the bases and the Proliferative compartments of the crypts, which peak at 24 hours after DMH administration
2) DNA synthesis depression, down to 20% of its normal value only 30 minutes after the administration of the drug
3) Elevated cell proliferation, characterized by elongated heights of the mucosal crypts and expansion of the proliferative compartment of the colonic crypts after repeated weekly injection
4) The development of aberrant crypt foci (ACF) after repeated weekly injections (Blakey et al. 1985; Wargovich et al. 1983). ACF are commonly believed to be the precursor lesions of colonic neoplasm (Mclellan and Bird 1988) and are frequently used as a quantitative biomarker for colonic neoplasia (Mclellan and Bird 1988).

However counter-arguments also exist. The distribution of ACF does not seem to match that of arising colon tumors; instead, it seems to match well with the distribution of gut associated lymphoid tissue (GALT) (Carter et al. 1994; Hardman and Cameron 1994). About 80% ACF collected from mice or humans were shown to harbor K-ras mutations (Pretlow et al. 1993; Singh et al. 1994) yet the occurrence of K-ras mutation was rare in dysplastic crypts (Jen and Powell 1994) and early stage adenomas (Vogelstein et al. 1988). This did not seem too consistent with the hypothesis that ACF were tumor precursors. In several experiments ACF were found to correlate poorly with colonic tumor occurrence (Hardman et al. 1991; Trorup et al. 1994). Very recently, the susceptibility genes for ACF and adenomas might be two non related parallel events. The following three possibilities could explain these inconsistencies:
1) Only a small fraction of ACF represents true premalignant lesions progressing to colon cancer
2) Factors associated with the GALT promote carcinogenesis and thereby the few ACF in the region subsequently progress to tumors at much higher rate than elsewhere in the colon
3) ACF and premalignant lesions of adenomas represent two parallel and distinct events that are initiated by colon carcinogens, and ACF seldom if ever progress to adenomas. Nevertheless, there seems to exist little doubt that ACF are indicators of colon carcinogen exposure.

6. CHEMOPREVENTION

Cancer chemoprevention is defined as the use of natural or synthetic agents to prevent, inhibit or reverse the carcinogenesis process before malignancy. Dr. Michael Sporn is widely credited with launching the modern era of cancer chemoprevention research (Sporn et al. 1976). In 1976, he first pointed out that the target of clinical efforts should be the process of carcinogenesis rather than the state of cancer. He advocated the treatment of precancerous conditions and coined the term “chemoprevention”. Chemoprevention may be conducted at variety of time points in this process to reduce occurrence of in situ or invasive cancers (primary intervention at earlier stages in the process) or cancer morbidity and/or mortality (secondary intervention at later stages in the process).

The development of cancer occurs over years and involves multiple genetic and phenotypic alterations that lead to invasive cancer. Chemoprevention is based on the fact that intervention is possible during the development and progression of pre-cancerous cells through use of non-cytotoxic nutrients and/or pharmacological agents during the time period between tumor initiation and malignancy (Pezzuto 1997).

There are three sequential levels of disease prevention depending on whether the interventionist addressed to healthy individuals and there is prevention of occurrence of the disease (primary
prevention) or patients in preclinical or premalignant stage (secondary prevention). At later stage, attempts can be made to prevent local recurrences as well as invasion and metastasis of malignant cells (tertiary prevention). In particular, primary prevention means preventing the occurrence of diseases. Secondary prevention means early detection and intervention, preferably before the condition is clinically apparent and has the aim of reversing, halting or at least retarding the progress of a condition. Tertiary prevention means minimizing the effect of diseases by preventing complications and premature deteriorations. Chemoprevention of cancer is a young discipline that is progressively emerging from its pioneer stage. It is reassuring that many drugs and food constitutes, some of which are widely consumed by the population, potentially possess cancer preventive mechanisms and are effective in preclinical models. Chemoprevention aims to directly modulate specific steps in the carcinogenic process, block mutagenic carcinogens, prevent DNA damage by free radicals, suppress epithelial cell hyperproliferatin, and/or modulate epithelial cell differentiation and apoptosis. An increasing number of nutrient and noncurrent compounds present in fruits, vegetables and cereal grains have been found to interfere with the process of cancer development in laboratory research (Sultana et al. 2003; Block 1992; Bresnick et al. 1990). Epidemiologists have found that population that consume large quantities of plant-derived foods have lower incidence rates of various types of cancer.

6.1 Classes of Chemopreventive agents

Cancer is a multistep process that occurs over an extended time frame, therefore, there are number of possible stage at which the process could be halted, slowed downer even reversed. Wattenberg (1985) developed a scheme to classify the chemopreventive agents. According to it, there are three major types of chemo preventive agents.
(A) Metabolic Inhibitors

This includes a group of compounds that prevent the formation of carcinogens from precursors. The best-studied inhibitors in this category include ascorbic acid, caffeic acid, ferulic acid, gallic acid, N-acetylcysteine, proline, and thioproline (Stoner et al. 1997). Although these compounds were suggested to “act predominantly to prevent the formation of nitrosamines from secondary amines and nitrite in an acidic environment”, their ability to prevent the formation of heterocyclic amines is now being recognized. Thus, antioxidants (catechins, flavonoids, caffeic acid) and organosulphur compounds (diallyl sulphide and dipropyl disulphide), have been reported to limit heterocyclic amine formation under various conditions (Kucuk 2002; Mehta et al. 2010).

(B) Blocking agents

These are typically those compounds that can inhibit initiation either by inhibiting the formation of carcinogens from precursor molecules or reactive metabolites from the parent carcinogens, or by preventing the ultimate electrophilic and carcinogenic species from interacting with critical cellular target molecules, such as DNA, RNA, and proteins (Wattenberg 1985). Initiation of carcinogenesis, involving damage to the DNA can be prevented or reduced by blocking agents that are particularly effective if administered before the carcinogen. Blocking mechanisms include alterations to the profile of both phase I and II drug metabolizing enzymes altered rates of DNA repair and scavenging of reactive oxygen and other free radical species (Boone et al. 1990). Even if DNA has been damaged, they can still be effective at limiting further adduct formation. They prevent carcinogens from modifying DNA and causing mutations. This is usually achieved by increasing the expression of detoxification and antioxidant enzymes in target tissues, though alterations in the pharmacokinetics of xenobiotics may also serve to protect against tumorigenesis (Wattenberg 1983). Such responses are thought to represent a form of
cellular adaptation to chemical and oxidative stress. They prevent cancer-producing compounds from reaching or reacting with critical target sites in the tissues. They prevent carcinogens activation, enhance detoxification of carcinogenic agents, or trap cancer-producing compounds before they reach or react with target sites in tissues (Greenwald 2002; Wattenberg 1983).

Example of blocking agents includes: Ellagic acid, caffeic acid, ferulic acid, p-hydroxyl Cinnamic acid, indole-3-acetonitrile, indole-3-carbinol, benzyl isothiocyanates, phenyl isothiocyanates, coumarin, quercetin and B-carotene. (Fig. 14)

(C) Suppressing Agents

These agents act in either promotion or progression stage of the carcinogenic process to inhibit malignant expression of initiated cells. Because carcinogenesis is a multistep process that often progresses slowly in the early stages and, hence, there is great potential for suppressing its development. Suppressing agents can be classified as compounds that inhibit polyamine metabolism; induce terminal cell differentiation; modulate signal transduction; modulate hormonal/growth factor activity; inhibit oncogene activity; promote intercellular communication; restore immune response; induce apoptosis; correct DNA methylation imbalances; inhibit basement membrane degradation; and inhibit arachidonic acid metabolism (Hail et al. 2008; Stoner et al. 1997). They reduce the consequences of altered gene expression by reducing proliferation of initiated cells or restoring apoptosis to normal levels, thus preventing the accumulation of damaged cells (Surh 2003; Manson et al. 2000; Wattenberg 1985).

Suppressing agents prevent the evolution of the neoplastic process in cells already altered by carcinogenic stimuli (Wattenberg 1996) Some act by producing differentiation; others specifically counteract the consequences of genotoxic events, in particular, oncogene activation, and still other specifically counteract the consequences of genotoxic events, in particular, oncogene activation, and still others selectively inhibit the proliferation of neoplastic cells (Moon et al.
1993; Reddy et al. 1993). Post-initiation events generally are less well understood than those that occur during the initiation phase, and for this reason the classification of suppressing agents is more difficult. Suppressing agents alter signaling pathways that control apoptosis or cell proliferation. They also protect during the initiation phase of heterocyclic amine-induced carcinogenesis. These include various polyphenols, retinoids, carotenoids, and vitamins (Wattenberg 1993).

Example of Suppressing agents includes: Soya bean protease inhibitor, benzyl-isothiocyante, B-sitosterol, caffeine, fumaric acid and selenium.

Indole-3-carbinol, a breakdown product of glucobrassicin vegetables, and curcumin, a major component of the spice turmeric, exhibit both blocking and suppressing mechanisms of action (Chinni et al. 2001; Hudson et al. 2003). (Fig. 14)

6.2 Chemoprevention by plants and natural compounds

Plants have been used for medicinal purposes for centuries. Herbal medicine is based on the fact that plants contain natural substances that can promote health and alleviate illness (Adlercreutz 1998). Human beings rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts or their active components (Winston 1999). Self-prescribed herbal preparations are widely used today for a host of common ailments and conditions, such as anxiety, arthritis, colds, coughs, constipation, fever, headaches, infections, insomnia, intestinal disorders, stress, ulcers and weakness (Halsted 1999; Mc Nutt 1995).

Research interest has focused on various plants that possess hypolipidemic, antiplatelet, antitumor or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cancer (Craig 1999). In different plants, wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins,
plant sterols, curcuminoids, and phthalides have been identified (Ren et al. 2003; Park and Pezzuto 2002).

Figure 14: depicts the targets of different types of chemopreventive agents (Source: Surh 2003)