Cancer is formed as uncontrolled multiplication of cell and the invasion of these cells to other tissues. Cancer is a genetic disease that results from multiple genetic defects due to dietary factors, infectious agents, environmental factors and various other causes which affect lifestyle. Carcinogenesis is a multistage process and during the process of malignant transformation, cells gradually evolve from the benign to the malignant phenotype (Smith et al., 2006).

Cancer is not just one disease but many diseases. There are more than hundred different types of cancer. Most cancers are named after the organ or type of cell from where they originate and can be broadly grouped into the following different types.

- **Carcinomas**, the most common types of cancers, which arise from the cells that cover external and internal body surfaces. Lung, breast, and colon are the most frequent cancers of this type.
- **Sarcomas** are cancers arising from cells found in the supporting tissues of the body such as bone, cartilage, fat, connective tissue and muscle.
- **Lymphomas** are cancers that arise in the lymph nodes and tissues of the body's immune system.
- **Leukaemias** are cancers of the immature blood cells that grow in the bone marrow and tend to accumulate in large numbers in the bloodstream.
- **Adenomas** are tumors that come from glandular tissue like the thyroid, the pituitary gland the adrenal gland.
Origins of Cancer

To understand cancer, it is helpful to know what happens when normal cells become cancer cells.

The body is made up of many types of cells. These cells grow and divide in a controlled way to produce more cells as they are needed to keep the body healthy. When cells become old or damaged, they die and are replaced with new cells.

However, sometimes this orderly process goes wrong. The genetic material (DNA) of a cell can become damaged or changed, producing mutations that affect normal cell growth and division. When this happens, cells do not die when they should and new cells may be formed when the body does not need them. The extra cells may form a mass of a tissue called a tumor (National cancer institute, 2008).

![Loss of Normal Growth Control](image)

Not all tumors are cancerous; tumors can be benign or malignant.
- **Benign tumors** are not cancerous. They can often be removed and in most cases they are not reformed. Cells in benign tumors do not spread to other parts of the body.

- **Malignant tumors** are cancerous. Cells in these tumors can invade to nearby tissues and spread to other parts of the body. The spread of cancer from one part of the body to another is called metastasis.

Cancer is an important problem and draws attention of health organizations worldwide. The most recent estimates of the global cancer burden suggest that there are 10.9 million new cases, 6.7 million deaths, and 24.6 million persons alive with cancer (Max Parkin *et al.*, 2005).

Colon cancer is one of the leading causes of cancer-related deaths worldwide. It ranks second in incidence to lung cancer in men and breast cancer in women. There is no difference in incidence between men and women, and this kind of cancer is prevalent in individuals aged over fifty. (Max Parkin *et al.*, 2005).

Studies based on cancer registries in India (Mumbai, Bangalore, Chennai, Delhi, and Bhopal) have shown increasing trends for colon and rectum, throughout the period of observation in most of the registries. In order to have better understanding of the etiology of this cancer, more epidemiological studies are needed in the near future on a priority basis (Yeole, 2008).

**ETIOLOGY OF COLON CANCER**

Colon cancer often results from a combination of genetic predisposition and environmental factors. Hereditary risk contributes to approximately 20% of cases (Narayan and Roy, 2003). The main inherited
predisposition syndromes are familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancer (HNPCC), and other types of tumors with a familial history (Ramsoekh et al., 2007). Environmental and dietary factors account for 80% of cases. Such factors include a diet with low fiber, vegetables, and folate and high in fat, red meat, heavy alcohol consumption, a sedentary occupation, and cigarette smoking (Campos et al., 2005). In this process, a number of genetic changes are observed, including the inactivation of the tumor suppressor genes and the activation of specific oncogenes. Identification of persons at risk can be achieved by a combination of a detailed family history, testing with molecular and mutational analysis. The etiology of colon cancer involves the interaction of molecular changes and environmental factors, with a great emphasis on diet components. There is a rapid increase in colon cancer incidence in several populations previously considered to be at low risk for this disease (Willett, 1989) and with a 20-fold difference in incidence between high-incidence and low-incidence regions (Tomatis, 1990), and the changes in incidence patterns observed in migrant studies (Haenszel and Kurihara, 1968; Whittemore et al., 1985) suggest that environmental factors, including those related to diet (Doll and Peto, 1981; Nelson, 1996), contribute to the etiology of colon cancer. Given the roles of the colon and rectum as conduits for ingested food and the many potentially anticarcinogenic substances contained in fruit (Steinmetz and Potter, 1991), vegetables (Steinmetz and Potter, 1996), and cereals (Slavin et al., 1999), these food groups are among the most widely studied in relation to colorectal cancer risk.

Several lifestyle factors are likely to have a major impact on colorectal cancer development although the precise mechanisms have yet to be yet identified. Physical inactivity and to a lesser extent, excess body
weight, are consistent risk factors for colon cancer. Exposure to tobacco products early in life is associated with a higher risk of developing colorectal neoplasia. Diet and nutritional factors are also clearly important. Diets high in red and processed meat increase the risk of colon cancer. Excess alcohol consumption, probably in combination with a diet low in some micronutrients such as folate and methionine, appear to increase risk. A number of micronutrients acting as antioxidants have been shown to be protective against colon cancer e.g. selenium, vitamin C, and vitamin E (Kune and Watson, 2006). There is also recent evidence supporting a protective effect of calcium and vitamin D in the etiology of colorectal neoplasia (Oh et al., 2007). Although the majority of more than 20 case–control studies [reviewed in (Steinmetz and Potter, 1996)] have shown an inverse association between fruit and vegetable consumption and colorectal cancer risk yet seven prospective cohort studies (Michels et al., 2000 and Shibata et al., 1992) have obtained inconsistent results.

However, some micronutrients or phytochemicals in fiber-rich foods may be important; folic acid is one such micronutrient that has been shown to protect against the development of colorectal neoplasia and is currently being studied in intervention trials of adenoma recurrence. The overwhelming evidence indicates that primary prevention of colon cancer is feasible. Continued focus on primary prevention of colorectal cancer, in combination with efforts aimed at screening and surveillance, will be vital in attaining the greatest possible progress against this complex, yet highly preventable disease (Martínez, 2005).

There are also specific host factors leading to a significant increase in the risk of colorectal cancer which include familial polyposis syndromes,
familial non-polyposis syndromes, benign colonic pathologies, and family history of colon cancer.

MOLECULAR GENETICS AND PATHOGENESIS OF COLON CANCER

Colon cancer, a classical model for the genetic basis of cancer, is now providing researchers with the opportunity to view epigenetic events in the context of human neoplasia. Epigenetic is the study of modifications in gene expression that do not involve changes in DNA nucleotide sequences (Verma and Srivastava, 2002). Knowledge of genetic and epigenetic changes of tumorgenesis help us to use approaches such as therapeutics and dietary interventions to prevent cancer development.

Molecular genetics of colon cancer is an area of intensive research not only for understanding about colorectal cancer but also an insight into the process of cancer development in general. From the analysis of the molecular genesis of colon cancer, three key themes concerning the molecular pathogenesis of cancer have been established. Firstly, cancer emerges via a multistep progression at both the molecular and the morphologic levels (Fearon and Vogelstein, 1990). Secondly, loss of genomic stability is a key molecular and pathophysiological step in cancer formation (Lengauer et al., 1998). Thirdly, hereditary cancer syndromes frequently correspond to germ-line forms of key genetic defects whose somatic occurrences drive the emergence of sporadic colon cancers (Kinzler and Vogelstein, 1996).

Molecular genetics of colon cancer has provided a strong support and evidence for the multistep process of carcinogenesis. Genetic alterations in the multistep colon carcinogenesis are believed to start from partial deletion
of long arm of chromosome 5 (Fearon and Vogelstein, 1990). Such deletions are associated with hyperplasia of the colonic epithelium. The earliest step in the neoplastic pathway is a shift of proliferation from the normally restricted zone at the base of the colonic crypt and the retention of cells capable of proliferation to the top of the colonic crypt. This appears to be mediated by a mutation in the \textit{APC} gene. The gene for familial adenomatous polyposis \textit{FAP} or \textit{APC} has been localized in 5q21-q22 segment (Nishisho et al., 1991). Its gene product is believed to be a negative regulator of colonic epithelial proliferation. Another genetic locus at the 5q arm has been found to be mutated in a number of colon cancers and is called MCC (mutated colon cancer) (Kinzler et al., 1991). Its action is believed to be similar to the FAP gene, which has been mapped to a site closed to the FAP gene. DNA hypomethylation has been discovered in small adenomas and may represent the next step in the sequence (Feinberg et al., 1988). Further alterations are seen in the \textit{ras} oncogene (commonly \textit{K-ras}, located on chromosome 12p), chromosome 17 (possibly \textit{p53}) and chromosome 18 (Vogelstein et al., 1988). Allelic deletions of long arm of chromosome 18 have been observed with high frequency in colon cancers as compared to polyps. The gene locus is named DCC (deleted in colon cancer) and the gene product of DCC locus has a homology to neural cell adhesion molecule, therefore may have a role in cell-cell and cell-matrix interactions and transmembrane signaling (Fearon et al., 1990). DCC deletion is believed to be a late event in the adenoma-carcinoma sequence. Critical lesions in the \textit{p53} gene appear to be responsible for malignant transformation and the appearance of genetic instability of neoplastic cell, which increases the likelihood that additional genetic events will occur that contribute to a progressively more aggressive neoplastic phenotype. The \textit{p53} gene is located on the short arm of
chromosome 17 and its product is a nuclear phosphoprotein. The wild type protein binds to specific DNA sequences and functions in control of cell cycle progression. Deletion and mutations of \textit{p53} are commonly seen in carcinomas but not adenomas, suggesting it to be another late event in the sequence (Baker \textit{et al.}, 1990).

**GENOMIC INSTABILITY**

Genomic instability plays an essential role in the development and progression of colon cancer. Based on different forms of genomic instability, colon cancer can be broadly be divided into two groups. In the first group, tumors are characterized by the presence of defective DNA mismatch repair (MMR). These tumors show the presence of high-level microsatellite instability (MSI-H) and the absence of protein expression for one of the several proteins involved in the MMR pathway (Baudhuin \textit{et al.}, 2005). The most commonly affected gene in sporadic colon cancer with defective MMR is \textit{hMLH1}, with the primary mechanism of gene inactivation being hypermethylation of the promoter (Cunningham \textit{et al.}, 1998). These tumors account for ~15% of sporadic colon cancers. The majority of sporadic colon cancers (85%), however, are proficient in DNA MMR but show another form of genomic instability at the gross chromosomal level, which has been called chromosomal instability (CIN). Such CIN represents the end result of a number of processes, including mutations in mitotic checkpoint genes, microtubule spindle defects, and telomere dysfunction (Grady, 2004). Tumors with CIN are most often aneuploid, have an abnormal karyotype, and are microsatellite-stable (MSS), whereas the majority of MSI-H tumors are believed to be near-diploid and with few, if any, karyotypic abnormalities. Recent studies, however, suggest that some MSI-H tumors
may also show evidence of CIN, although the extent and nature of this 
overlap remains uncertain (Goel, 2003 and Camps et al., 2006).

MSI and CIN have been considered to be mutually exclusive forms of 
genomic instability. The distinction seems to be of clinical importance 
because recent data strongly suggests that biological behavior, including 
response to therapy, differs between MSI-H and MSS tumors (Ribic et al., 
2003). However, the genomic classification of colon cancers based on MSI 
and CIN status may be overly simplistic, and tumors may have evidence of 
both types of instability or neither. For example, recent data indicate that the 
CpG island methylator phenotype can be found in up to 25% of sporadic 
colon tumors and that those tumors may not progress independently through 
either the MSI or the CIN pathways (Goel, 2003; Toyota et al., 1999 and 
Samowitz et al., 2005). An even more precise genomic and epigenetic 
classification of tumors, beyond the simple MSI versus CIN dichotomy, may 
prove to further delineate important subgroups of colon cancers with respect 
to clinical behavior.

The type and degree of genomic instability in colon cancers correlates 
with their clinical and phenotypic characteristics. Supporting this idea are the 
ample data that MSI-H and MSS colon tumors differ in their pathologic 
features, their prognosis, and response to therapy (Ribic et al., 2003 and Raut 
et al., 2004). A more refined classification of colon cancer that recognizes 
the possible overlap between CIN and MSI-H tumors may prove to be of 
clinical relevance.

ABERRANT CRYPT FOCI (ACF)

Since the first report of ACF in a rodent model by Bird in 1987, there 
has been increasing interest in their biology. ACFs are pre-polyp
abnormalities identified in single crypts by high magnification chromoendoscopy using either methylene blue or indigo carmine. Colonic ACF may represent early lesions capable of progression to colon cancer and/or be predictive markers of future risk. Kinzler and Vogelstein (1996) place ACF as the earliest identifiable morphological change on the pathway to colon cancer. However, it has been shown that ACFs are a heterogeneous group of lesions and some may be important in colon cancer development. Elucidation of the distribution of ACF in the general population, and the association of ACF with personal characteristics will advance the understanding of their biological meaning.

The mechanism by which ACF increase in size seems to be a process of crypt division, which begins at the base of the crypt and proceeds upwards until two crypts are generated. Thus, the number of crypts per ACF, also termed “crypt multiplicity”, would be an important parameter for evaluating ACF progression. Bouzourene et al., (1999) demonstrated the crypt multiplicity to be significantly lower from proximal toward distal colon, which was opposite to that of ACF density, and was significantly larger when it was associated with carcinoma or adenoma than with nonneoplastic diseases. Also, no gradient in ACF density and crypt multiplicity was observed according to the distance from the tumor (Pretlow et al., 1991).

Increased mitotic activity, which has been proposed as a biomarker of colon cancer at early stages, was observed in a majority of ACFs. Most of the crypts showed a mitotic pattern similar to that of normal adjacent crypts (Roncucci et al., 1993). In some of the dysplastic foci, mitotic activity was seen to distribute throughout the crypts, as reported in adenomas. The above
findings may be consistent with the assumption that ACF are the preneoplastic lesions (Murray et al., 1999).

The potential benefit of understanding ACF is significant. If the number of ACFs is a good indicator of future colon cancer risk, then it can be used clinically for the assessment of risk, screening guidelines, and intervention strategies. Also, if ACFs, or an identifiable subset of these lesions, are strongly associated with progression to colon cancer, then their removal may reduce future risk. ACFs are beginning to be used as markers in intervention trials for risk-reduction of colon cancer. A better understanding of the relation of ACF to risk factors for colon cancer will provide more guidance for design of future intervention trials.

COLON CARCINOGENESIS: staging, and treatment

Staging: Chemically induced neoplasia appears to be a complex phenomenon, which proceeds through multiple stages, including initiation, promotion, and progression (Maskens, 1983). Early detection of colorectal cancer provides the greatest odds of beating the disease. Prognosis is highly dependent on the stage of the colorectal cancer. Basically, stage I (Duke's) indicates that the cancer has penetrated only the most superficial layer of the bowel wall (the mucosa). Stage II means, that the cancer has penetrated into the muscular layer of the bowel wall. Stage III indicates that the cancer has spread to nearby lymph nodes, and stage D indicates spread to other sites (metastases) commonly the liver and the lung.

These stages, along with the functional and genetic changes reported to occur in the early stages of progression to sporadic colon cancer are outlined in Figure 1.
Fig 1. Staging of colon carcinoma according to Duke's system; stage 0 net accumulation of epithelial cells, stage I shows that cancer cells are limited on superficial layer, stage II indicates it has penetrated into the muscular layer, stage III indicates that the cancer has spread to nearby lymph nodes and stage IV shows metastases.

Initiation involves the formation of a mutated, preneoplastic cell from a genotoxic event. Formation of the preneoplastic initiated cell is an irreversible, but dose-dependent process. Promotion involves the selective clonal expansion of the initiated cell through an increase in cell number, 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 may be reversible upon removal of the tumor promotion stimulus. Progression, the third stage, involves cellular and molecular changes that occur from the preneoplastic to the neoplastic state. This stage
is irreversible, involves genetic instability, changes in nuclear ploidy, and disruption of chromosome integrity.

Increasing evidence suggests that this multistage process may result from the accumulation of genetic alterations in certain proto oncogenes or other genes, thereby leading to progressive disordering of the mechanisms that normally regulate cellular growth and differentiation (Weinberg, 1989).

Most of the chemical carcinogens do not have the potency to cause cancer in their usual condition. The non-cancer causing form of the chemical is called a procarcinogen. Procacinogens are frequently complex organic compounds that the human body attempts to dispose of when ingested. Hepatic enzymes chemically change the procarcinogen in several steps to yield a chemical that is more easily excreted. These chemical changes result in modification of the procarcinogen (with no cancer forming ability) to the ultimate carcinogen (with cancer causing competence). Ultimate carcinogens have been shown to have a great affinity for DNA, RNA, and cellular proteins, and it is the interaction of the ultimate carcinogen with the cell macromolecules that causes cancer (Boffa et al., 1982).

There are a few tests and procedures that are employed to determine the stage of the cancer. Treatment and prognosis depend upon stage of the cancer. Colon cancer staging is based upon how far the cancer has penetrated into the lining of the colon, whether the cancer involves lymph nodes adjacent to the colon, or the cancer has spread to other organs.

Initial staging usually involves a colonoscopy (a test in which a flexible tube with a camera is guided through the colon), abdominal and pelvic Computerized Tomography (CT) scan (specialized x-rays), and a chest x-ray. Once a cancer is diagnosed, the extent of the disease (stage) needs to be determined to form the basis for therapeutic decisions. These
include chest x-rays to find if the cancer has spread to the lungs; Computerized Axial Tomography (CAT) scans help to determine the degree of metastases, particularly with regard to the liver; and the histopathology which indicates the depth of penetration into the lining of the colon, and whether there is lymph node involvement or not.

Surgery has the most feasible treatment option in colon cancer due to the following reasons:

- The cancerous part of the colon and the associated lymph nodes can be removed, providing a cure for those with early stage disease.
- It provides the opportunity to examine the abdominal area for signs of cancer spread.
- It can prevent complications from the tumor such as obstruction (blockage) of the colon and bleeding.

Adjuvant chemotherapy is usually recommended for patients in whom it is suspected that residual cancer remains in the body after the primary tumor has been removed. Even if the tumor has been removed completely tiny cancer cells may remain in the body and grow, causing relapse after surgery. This is most likely in patients who have positive lymph nodes (stage III disease). In such patients, chemotherapy can prevent the relapse and prolong survival. Some of the most important drugs which are used for colon cancer therapy are fluoropyrimidines, irinotecan and oxaliplatin (Meyerhardt et al., 2005). Like chemotherapy, radiation therapy may be helpful for patients who are at high risk of recurrence, such as those in whom cancer has perforated the wall of the colon or spread to adjoining organs. It may also be
used in treating advanced stages of the disease and in treating some metastases, particularly if they are painful (Braendengen et al., 2008).

However, 40% of patients who undergo systemic chemotherapy for advanced cancers still do not achieve shrinkage of their tumors. Therefore, new strategies are warranted in order to improve these results. Much of the basic cancer research during the era of molecular biology has focused on the dissection of molecular pathways resulting in tumor growth and progression. The rationale for this approach is that it allows the identification of pathways that might be disrupted with new biological targeted therapies. Among all the biological agents that are being evaluated in cancer treatment, three different strategies are in advanced development in the treatment of colon cancer and have already shown unequivocal evidence of efficacy. Large phase II and III clinical trials are evaluating the role of epidermal growth factor receptor (EGFR) pathway inhibitors, vascular endothelial growth factor receptor (VEGFR) pathway inhibitors and cyclooxygenase-2 (COX-2) inhibitors, both in advanced disease and in the adjuvant setting.

EGF was identified in 1962, and was purified and characterized by Stanley Cohen in 1980, work for which he later received the Nobel Prize in Physiology and Medicine. EGFR is a tyrosine kinase receptor that belongs to the ErbB family and is abnormally expressed and activated in cancer cells in many tumor types including colon cancer. Following stimulation by its natural ligands, EGFR initiates signal transduction cascades that promote cell division, migration and angiogenesis, and inhibit apoptosis (Arteaga, 2003). Therefore, there is a clear rationale for the development of agents capable of blocking the activity of EGFR.

One novel approach in the treatment of solid tumors involves therapeutic agents that inhibit the neovascularisation process of growing
tumors. There is strong evidence that links tumor growth and metastasis with the angiogenesis process in most human tumors, including colon cancer (Tomisaki et al., 1996 and Tanigawa et al., 1997). Moreover, a clear correlation between the microvessel density in the pathology specimens and progression-free and overall survival in patients with colon cancer has been demonstrated. The formation of a vascular network in the tumor growth involves different complex pathways (Carmeliet, 2003) including angiogenic factors, membrane receptors and signal transduction cascades that lead to the vessel formation and stabilization processes. VEGF is the most potent and specific angiogenic factor, and its expression in colon cancer has been demonstrated to correlate with recurrence and prognosis (Takahashi et al., 1995).

COX-2 isoform has been reported to play an important role in the pathogenesis of colon cancer. COX-2 is virtually undetectable in normal colon, but is upregulated in 40% of adenomas and 85% of colon cancer (Eberhart et al., 1994). Moreover, strong COX-2 expression is a marker for poor survival in colon cancer (Sheng et al., 1997). COX-2 inhibitors decrease the number and size of polyps and may prevent the progression from adenomatous polyp to invasive carcinoma. This effect has been clinically studied in patients with familiar adenomatous polyposis (FAP).

Several options are currently available for the treatment of patients with metastatic colorectal cancer (mCRC), including different regimens of chemotherapeutic compounds (fluoropyrimidines, irinotecan and oxaliplatin) and targeted therapies such as bevacizumab and cetuximab. Interestingly, most recent trials that attempt to expose patients to all five drug classes (fluoropyrimidines, irinotecan, oxaliplatin, bevacizumab and cetuximab) target an overall survival (OS) well over 2 years (Ponz-Sarvisé et al.,
When administered alone new targeted therapies have demonstrated activity in different *in vitro* and *in vivo* studies. However, the clinical use in patients when administered as a single agent is not so brilliant (Au *et al.*, 2007). On the other hand, the combination of these drugs with classical chemotherapies has shown better clinical profiles (Lenz *et al.*, 2007; Ogino *et al.*, 2005).

There is also the challenging possibility of combining different targeted therapies in order to overpass tumor resistance. Combining targeted therapies against different pathways is also a possibility. The cross-talk at a molecular level of the different networks implicated in cell biology is almost unknown. However there are more data that implicate different molecular networks when studying resistance to targeted therapies against one pathway.

**Metabolic activation**

![Flowchart](image)

*Fig1: Pro-carcinogens are converted to reactive electrophiles or detoxified to soluble stable products and excreted*
DMH AND COLON CARCINOGENESIS

In 1962, Laqueur, discovered that rats fed cycasin, a plant product, developed intestinal cancer. The active substance was identified and soon a similar compound, methylazoxymethanol acetate (MAMA) was synthesized that was more effective than the natural product (Nigro and Bull, 1985). In 1970 Druckrey found that two chemicals structurally related to MAMA, dimethylhydrazine (DMH) and azoxymethane (AOM) were even more potent intestinal carcinogens (Nigro and Bull, 1985). This provided a momentum to further research on the role of chemical carcinogens and use of these carcinogens to induce cancer like conditions in animal models.

DMH has been extensively studied (Freeman et al., 1978 and Srihari et al., 2008). Evidence from animal studies has shown that experimental colonic tumors induced by procarcinogen 1, 2-dimethylhydrazine (DMH) are of epithelial origin with a similar histology, morphology and anatomy to human colonic neoplasms (Femia and Caderni, 2008). DMH and its metabolite AOM are the agents widely used in experimental models of colorectal carcinogenesis in rodents. They are highly specific indirect colorectal carcinogens that induce the initiation and promotion steps of colorectal carcinogenesis yielding colorectal tumor lesions in a dose-dependent manner in rats, mice and hamsters (Shirai et al., 1983; Shinchi and Isamu, 1985).

DMH is metabolically activated in liver by series of reactions through intermediates azomethane, AOM and methylazoxymethanol (MAM) to the ultimate carcinogenic metabolite, highly reactive methyldiazonium ion (Fiala et al., 1987). MAM is excreted into the bile and transported to the colon or enter directly into epithelial cells of the colon from the blood circulation (Shinchi and Isamu, 1985; Fiala et al., 1987 and Rubio et al., 1980). Some studies have demonstrated that rat colon epithelial cells are capable of metabolising DMH into carcinogenic metabolite without previous
metabolism by other tissues or colon bacteria (Glauert and Bennink, 1983; Oravec et al., 1986). Although intestinal flora (Onoue et al., 1997; Goldin and Gorbach, 1981) and bile acids (Narahara et al., 2000) have influence on the incidence of tumors, the latter were induced also in germ-free rats (Onoue et al., 1997) and isolated segments of rat colon (Rubio et al., 1980).

The ultimate carcinogenic metabolite of DMH is responsible for methylation of DNA of epithelial cells in the proliferative compartment of the intestinal crypts (Swenberg et al., 1979). Metabolically activated DMH modifies not only nucleic acids but also histones and other DNA-binding proteins in the target cells (Boffa et al., 1982).

Colon specific susceptibility for DMH is a result of delayed or incomplete repair of damaged DNA in the colon compared to other organs (Swenberg et al., 1979), leading to accumulation of mutations, and in a small proportion of cells giving rise to colon cancer. DMH alkylates DNA and the promutagenic lesions O₆-methylguanine (O₆-MeG) has been detected in DNA from various rat and mouse tissues following exposure to DMH (Herron and Shank 1982; James and Autrup 1983). Knowledge of the genetic hallmarks of tumorigenesis provides an opportunity to use approaches such as dietary intervention to prevent cancer development.

Higher susceptibility to colon versus small intestine has been shown in experiments where segments of colon that were transposed to the middle part of small intestine developed tumors but segments of small intestine that were transposed to the colon did not develop tumors (Gennaro et al., 1973). Tumors are distributed in all parts of the colon, but in a majority of cases are observed in the distal part of colon (Shirai et al., 1983; Veceric and Cerar, 2004; McGarrity et al., 1988 and Park et al., 1997). Gross tumors are initially detected in the distal colon at 16 weeks but in proximal colon after 22 weeks (McGarrity et al., 1988). The tumor incidence can be modulated by the amount of carcinogen administered and the number of applications.
With increasing doses of the carcinogen, the latency period decreases and the tumor incidence increases (Shinchi and Isamu, 1985; Maskens and Dujardin-Loits, 1981). Colonic tumor induced by procarcinogen DMH are of epithelial origin.

Injections of DMH could result in increased colonic crypt cellularity, colonic crypt cell proliferation and colonic crypt proliferative prior to the development of colon cancer (Richards, 1977 and Heitman et al., 1983). Arutiunian et al (1997) reported a tendency of free-radical processes during DMH-induced carcinogenesis in rats. This procarcinogen thus provides an adequate model for kinetic and therapeutic studies of the colon cancer.

Precursor compounds (DMH)
(procarcinogen)

\[ \text{Carcinogen (Azoxy methane, Methyldiazenonium) } \]

Reaction with (colonocytes) cellular targets

\[ \text{Carcinogen-DNA adduct } \]

Neoplastic manifestation

\[ \text{Cancer (Adenocarcinoma)} \]

**Fig2: mechanism of action of colon carcinogen DMH**
OXIDATIVE STRESS AND COLON CARCINOMA

The gastrointestinal tract is particularly susceptible to oxidative stress attack, which lead to carcinogenesis. Oxygen radicals were found to enhance carcinogenesis at all stages in colon carcinogenesis: initiation, promotion, and progression. The reduction of oxygen to reactive oxygen species is believed to be a mechanism by which ionizing radiation, certain chemicals and carcinogens can induce adverse biological effects (Cerutti, 1985). During reduction of oxygen to water in the normal biological system, the electrons are transferred from the electron transfer chain. The monovalent reduction of dioxygen involves the sequential addition of four single electrons, which at each intermediate stage result in the production of potentially damaging molecular species:

\[
\begin{align*}
O_2 + e^- & \rightarrow O_2^- & \text{(Superoxide anion radical)} \\
O_2^- - e^- & \rightarrow O_2^{2-} & \text{(Peroxy anion)} \\
O_2^{2-} + 2H^+ & \rightarrow H_2O_2 & \text{(Hydrogen peroxide)}
\end{align*}
\]

By the interactions of divalent cations, such as iron and possibly copper the hydroxyl radical (OH) may be formed:

\[
\begin{align*}
O_2 + Fe^{3+} & \rightarrow O_2 + Fe^{2+} \\
H_2O_2 + Fe^{2+} & \rightarrow OH+Fe^{3+}
\end{align*}
\]

The hydroxyl radical (OH) is highly reactive and capable of causing severe damage by extracting electrons.

The possible detrimental effects of these events are kept in check by an efficient and complex multifactorial protective mechanism (Diplock,
Cellular antioxidant enzymes and the free radical scavengers normally protect a cell from toxic effects of the ROS. However, when generation of the ROS overtakes the antioxidant defense of the cells, oxidative damage of the cellular macromolecules (lipid, proteins and nucleic acids) occurs, leading finally to various pathological conditions.

Antioxidant status is the balance between pro-oxidants and the antioxidant system. This balance is dynamic and, in the human body, is probably tipped slightly in favor of oxidation, which is essential for the production of energy. A serious imbalance oxidation is defined as oxidative stress; it may result from excessive production of reactive oxygen species (ROS) and free radicals and/or weakening of the antioxidant system due to lower intake or endogenous production of antioxidants or from increased utilization.

Three classes of antioxidants have been identified: primary antioxidants (e.g. Superoxide dismutase, Glutathione peroxidase, Caeruloplasmin, Transferrin and Ferritin) prevent the formation of new free radical species; Secondary antioxidants (e.g. Vitamin E, Vitamin C, β-carotene, Uric acid, Bilirubin and Albumin) remove newly formed free radicals before they can initiate chain reactions. These chain reactions can lead to cell damage and further free radical formation. Tertiary antioxidants (e.g. DNA repair enzymes and Methionine sulphoxide reductase) repair cell structures damaged by free radical attack.

Oxidative stress is a term used to describe any challenge in which pro-oxidants predominate over antioxidants, it may be due to either increased production of ROS or deceased level of antioxidants (enzymatic or nonenzymatic) or both (Venkatesh et al., 2001). Oxidative stress is related to
a variety of diseases like Cancer, Cataract, Diabetes mellitus, neurodegeneration, heart ailments etc (Irashad and Choudhari, 2002).

Free radicals are highly reactive compounds that are created in the body during normal metabolic functions or introduced from the environment. Free radicals are inherently unstable, since they contain extra energy. To reduce their energy load, free radicals react with certain chemical in the body, and in the process, interfere with the ability of cells to function normally. Hydroxyl radical is the most reactive oxygen radical which is produced from water when exposed to X-rays or gamma-rays, or from hydrogen peroxide present in our body. Superoxide radical is produced from oxygen when an electron is attached. Nitric oxide is produced in our body and nitrogen dioxide is found in polluted air and smoke. The vascular endothelial cells which from the lining of our blood vessels, the phagocytes which are part of our immune system, and some brain cells also produce nitric oxide.

The electron transport system of aerobic respiration, peroxisomal hydrogen peroxide-generating enzymes, cytochrome P-450, oxidizable elements like Fe$^{+++}$ and Cu$^{++}$ and redox cycling by certain xenobiotics are also important endogenous processes that serve as sources of ROS (Diplock, 1987). Oxidative stress is defined as an imbalance between generation of ROS and decreased antioxidant defense systems (Oberley, 2002). Oxidative stress develops particularly in inflammatory reactions because the inflammatory cells, neutrophils, and macrophages produce large amounts of ROS. It has been known for a long time that oxidative stress in inflamed tissue can lead to the development of malignant tumors.
GLUTATHIONE REDOX CYCLE

One of the mechanisms dealing with toxic effects of oxygen is the glutathione redox cycle (Fahey and Sundquist, 1991). Glutathione, a widely distributed tripeptide (L-glutamyl-L-cysteinyl-glycine), is synthesized in virtually all the animals cells by the sequential action of the two enzymes, -glutamylcysteine synthetase and glutathione synthetase. It is present in high concentrations in the mammalian cells as reduced glutathione (GSH), with minor amounts of oxidized glutathione (GSSG), mixed disulfides of GSH, and other cellular thiols and thioesters (Kosower and Kosower, 1978).

Glutathione is involved in the regulation of several cell functions including amino acid transport (Vina and Vina, 1983), enzyme activities, thiol-disulfide balance (Ziegler, 1985), synthesis of DNA precursors (Holmgren, 1979), and also the cell proliferation (Kavanagh et al., 1990).

GSH-related enzyme viz: GSH-Px, phospholipids hydroperoxide GSH-Px and GSH-S-transferase play major roles in scavenging the free radical peroxide reduction and detoxification of xenobiotics through the formation of GSH-S-conjugates (Sies and Ketterer, 1988; Dolphin et al., 1988).

The ability of GSH-Px to reduce hydrogen peroxide depends on the availability of glutathione. The level of glutathione (GSH) is maintained by the reduction of GSSG by NADPH (nicotinamide adenine dinucleotide phosphate) via glutathione reductase and by de novo synthesis from glutamic acid, cysteine and glycine. It has been suggested that GSH-Px may be inactivated during oxidative stress (Condell and Tappel, 1983) which makes the cells prone to oxidative damage. The function of GSH-Px and thereby the net antioxidant capacity of the glutathione redox cycle depends on various factors such as, selenium status of the body for de novo synthesis of
GSH-Px for its action as a cofactor, level of intracellular glutathione, a sufficient glutathione reductase activity and sufficient level of NADPH.

Because of the constant exposure to free radicals, both plants and animals have developed numerous antioxidant compounds and systems to protect themselves.

Antioxidant compounds can be divided in three forms: Enzymes (superoxide dismutase (SOD), catalase, and glutathione peroxidase), Antioxidant Phytochemicals (plant foods) and Vitamins, Minerals (vitamin C and vitamin E as well as minerals like selenium).

CELLULAR ANTIOXIDANT DEFENSE SYSTEM AND SELENIUM

The level of dietary intake of all the antioxidant micronutrients directly affects the circulating level of these nutrients and the activities of the antioxidant metalloenzymes. Thus, low intakes of one or more of antioxidant nutrients could reduce the body’s defenses against free radical damage and increase susceptibility to health problems associated with free radical damage (Halliwell, 2001). The selenoenzyme, glutathione peroxidase that requires selenium essentially in the active site of the enzyme, catalyses the reduction of hydrogen peroxide. This enzyme is localized both in mitochondrial and cytosolic compartments. It is also capable of catalyzing the reduction of a wide range of lipid hydroperoxides to the corresponding hydroxyl acids (Gebicki et al., 2002).

The hydroxyl radical generation occurs primarily into the regions of high oxygen reduction in cell, i.e. mitochondria and smooth endoplasmic reticulum. Hydroxyl radical attacks the unsaturated fatty acids of the intracellular membrane phospholipids. This finally leads to a change in the
three dimensional conformation of the phospholipids molecules so that the structural integrity of the membrane is lost.

Dietary intake of selenium induces the synthesis of selenium-containing antioxidant enzyme viz: cytosolic glutathione peroxidase (GSH-Px) (Rotruck et al., 1973; Flohe et al., 1973) and phospholipids-GSH-Px (Ursini et al., 1985). Selenium status of the animals is therefore very important in equipping the body against the lipid peroxidation damage as the excess of selenium is also toxic to the body.

**SELENIUM AN ESSENTIAL TRACE ELEMENT**

Selenium is an essential trace nutrient for humans, animals and bacteria. It is, a group VIA element, mainly found in metal sulfide deposits in the copper mines. In the earth’s crust, selenium occurs as selenides of silver, copper, lead, mercury and other metals mixed with the mineral sulfides. In the elemental form, it is biologically inactive and is available to the biosphere only after various natural processes convert it into selenides, selenites, selenates and organic selenium. It is taken up by plants from the soil present in the form of selenate, selenite or organic selenium thus enters the food chain.

Initially, selenium was viewed as a potential toxic element. As early as 1934, Franke reported that subacute administration of selenium to the experimental animals resulted in the marked decrease in food intake leading to a decreased growth rate. Klaus Schwartz and colleagues at the National Institutes of Health discovered selenium’s nutritional essentiality in rats in 1957, and similar findings were reported in chicks that same year (Patterson et al., 1957). Selenium’s role as a component of glutathione peroxidase was discovered in 1973, and the form of selenium at the active site was found to
be selenocysteine in 1978. Protein-bound selenocysteine is the predominant form of selenium in rats fed sodium selenite (Hawkes et al., 1985), and many selenocysteine-containing proteins have been reported, including several novel genes identified also seem to code for selenocysteine proteins (Behne and Kyriakopoulos, 2001). Further research revealed that not only the organic (selenomethionine, selenocysteine, amino acid chelates, yeast), but also the inorganic selenium compounds viz. selenite, selenate and selenocyanide were effective as antioxidants. Presently, selenium is considered to be an essential trace element having an important role in antioxidant mechanism. Different functions of selenium are also thought to be mediated by selenium binding proteins.

**DISTRIBUTION OF SELENIUM IN MAMMALIAN BODY**

Immediately after the administration, selenium was taken up by the erythrocytes. Jenkins and Hidiroglou (1972) observed that selenite was metabolized in the erythrocytes by a glutathione dependent system to form hydrogen selenide or similarly reduced selenide which was then readily shifted to the plasma proteins. Studies also showed that lipid fractions of the serum lipoproteins were also associated with the $^{75}$Se binding. $^{75}$Se-selenite in human plasma enters all the tissues including the bones, the hair, and the blood cells (Buescher et al., 1960). It was noticed that local administration of selenite and intravenous injections led to the same metabolic pathways (Behne et al., 1991).

Once in the systemic circulations, selenium is taken up by different organs, tissues, cells and cell organelles depending upon the requirement and binding affinities. Selenium has been reported to bind number of proteins including glutathione peroxidase and type-I iodothyronine-5'
monodeiodinase (Shamberger, 1983; Behne et al. (1988). Behne et al. (1988) demonstrated thirteen selenium containing protein subunits with molecular weights ranging from 12 to 75 kDa in the tissue homogenates. Various reporters have suggested that selenium binding proteins are associated with chemopreventive effects of selenium (Morrison et al., 1989; Gasparian et al., 2002) and some important biological functions in specific target tissues (Behne et al., 1988).

CHEMOPREVENTION OF CANCER AND ROLE OF SELENIUM

Cancer chemoprevention refers to the use of pharmacological agents to inhibit, delay or reverse the multi-step process of carcinogenesis. The last two decades in particular have witnessed explosive growth in this emerging field of cancer chemoprevention. Extensive efforts to evaluate possible application of various chemopreventive agents, in individuals at high risk of neoplastic development have been carried out. Epidemiological studies suggest a protective role of several agents in reducing the risk of cancer (Naithani et al., 2008). The protective action of all these agents is explained as a combination of various proposed mechanisms involving anti-oxidant, anti-inflammatory, immunomodulatory action, apoptosis induction, molecular association with carcinogen, cell cycle arrest, cell differentiation induction, antimicrobial effect, and anti-angiogenesis etc. Large numbers of candidate substances such as phytochemicals and their synthetic derivatives have been identified by a combination of in vitro and in vivo studies in a wide range of biological assays.

Chemoperevention has obvious common elements with chemotherapy, but also distinct differences. Chemoprevention focuses on reduction of incidence and is related to classical epidemiology, whereas
chemotherapy focuses on prognosis and is related to clinical epidemiology. Chemotherapy can be either systemic or, in certain cases, localized, whereas chemoprevention is almost always systemic. In chemotherapy the outcome is generally a high frequency event (like death or metastasis), whereas in chemoprevention it is usually of low frequency (incidence cancer cases); this is reflected in the required sample size in the corresponding studies. Lastly, chemotherapy is applied to seriously ill patients, for whom side-effects, even serious ones, may be acceptable, whereas chemoprevention is generally administered in cases where serious side-effects are unacceptable.

Many classes of agents have shown promise as chemopreventive agents. These include antioxidants and other diet derived agents. Various dietary antioxidants have shown considerable promise as effective agents for cancer prevention by reducing oxidative stress which has been implicated in the development of many diseases, including cancer (Naghma et al., 2007). Therefore, for reducing the incidence of cancer, modifications in dietary habits, especially by increasing consumption of fruits and vegetables rich in antioxidants are increasingly advocated. Accumulating research evidence suggests that many dietary factors may be used alone or in combination with traditional chemotherapeutic agents to prevent the occurrence of cancer, their metastatic spread, or even to treat cancer. The reduced cancer risk and lack of toxicity associated with high intake of fruits and vegetables suggest that specific concentrations of antioxidant agents from these dietary sources may produce cancer chemopreventive effects without causing significant levels of toxicity. Moreover, the study of the antioxidant system and oxidative stress is crucial in understanding the chemoprevention of colon cancer.
Ganther (1999) reported the metabolism of selenium compounds is a prerequisite for cancer prevention. After intestinal absorption, dietary selenite is reduced by thiols (e.g., glutathione) and NADPH-dependent reductase, through selenodiglutathione to highly toxic H$_2$Se. In turn, H$_2$Se, is converted to selenophosphate, and then incorporated as selenocysteine into numerous selenoproteins, such as glutathione peroxidase. Selenium compounds exert their biological effects either directly or indirectly by being incorporated into enzymes and other bio-active proteins. The main inorganic dietary form of selenium is sodium selenite (Na$_2$SeO$_3$). In the organic forms selenomethionine and selenocysteine, a selenium atom is present in the position occupied by a sulfur atom in the amino acids methionine and cysteine. Se-methylselenocysteine is selenocysteine modified by the replacement of the hydrogen atom with a methyl group on the selenium atom. Selenium-containing enzymes do not appear to be as important as selenium metabolites in cancer chemoprevention (Medina et al., 2001 and Ganther, 1999). Extensive studies have concluded that selenium compounds directly converted to mono-methylated forms, (methylselenol, CH$_3$SeH) or related intermediates (e.g., aromatic selenol) are powerful chemopreventive agents.
In the 1960s, the code for the incorporation of amino acids into proteins by tRNA assigned 20 amino acids to 61 of the 64 possible triplet codons (e.g., cysteine as UGU or UGC). In the 1990s it was discovered that UGA not only acts as a stop codon, but as a codon for the incorporation of selenocysteine into amino acids. This discovery made UGA the second known dual-function codon, along with AUG (which acts as an initiator as well as a codon for methionine incorporation). The discovery also made selenocysteine the "21st amino acid".

Non-specific incorporation of selenium into protein can be toxic (Hatfield, 2002). Se-methylselenocysteine, however, cannot be specifically or non-specifically incorporated into protein, and is rapidly metabolized to
Exogenous selenium from both organic and inorganic sources must be converted into hydrogen selenide (H$_2$Se) before it is incorporated into selenoproteins (as selenocysteine).

There are nearly 30 known selenoproteins containing selenocysteine. Selenocysteine is the active site of the antioxidant enzymes glutathione peroxidase and thioredoxin reductase, for example. Thioredoxin reductase not only maintains cell proteins in a reduced state, it is essential for providing the deoxyribonucleases required for DNA synthesis (Holmgren, 1989). Deiodinases (D1, D2 and D3) required for activation and deactivation of T4 thyroid hormone to T3 forms are selenium-containing enzymes (Bianco, 2002). Thiol (–SH) compounds can be antioxidant reducing agents (by hydrogen atom donation), but selenium (which sits below sulfur in the same column of the periodic table) is more nucleophilic (electron-rich) and therefore more able to release the hydrogen. Selenium has a 15% longer bond-length than sulfur, which facilitates the formation of selenium-sulfur bonds at the catalytic sites (Gromer, 2003). The presence of selenocysteine rather than cysteine in thioredoxin reductase not only increases catalytic activity 100-fold, but optimizes the activity at physiological pH (Zhong, 2000).

However, though in literature, selenium has been shown to have anticancer action but there is a paucity of information with regard to the action of selenium in delaying the chain of molecular events leading to the development of malignant tumors and in particular to colon tumors. Selenium affects oxidative stress, DNA methylation, DNA repair, inflammation, apoptosis, cell proliferation, carcinogen metabolism, hormone production, angiogenesis and immune function (Taylor, 2004). Selenium is
enzymatically methylated to monomethylated, dimethylated and trimethylated metabolites that use s-adenosylmethionine as the methyl donor. It affects DNA methylation and its intake correlates with various type of cancer.

Cancer begins with DNA mutation, aberrant DNA methylation or defective cell-cycle control. DNA is normally protected from cancer-causing substances by methyl groups, but selenium deficiency (like folic acid deficiency) can result in decreased DNA methylation and therefore increased DNA damage and mutation (Davis, 2000). When DNA is damaged, p53 either stimulates DNA repair or causes cells to self-destruct (apoptosis) if the DNA damage is irreparable. Among the most striking genetic changes are those in the gene for p53 protein, located on chromosome 17p, initially identified because of the high frequency of allele loss in this region of chromosome 17 (Nanda et al., 1990). Nigro et al. (1989) showed that p53-encoding gene mutations in colorectal and other cancers occurred in specific conserved regions of the gene and might be present in well over 50% of colorectal cancers. Selenium also promotes the activity of p53 protein, which is often called "the guardian of the genome". The thioredoxin reductase system promotes p53 induction of DNA repair enzymes (Seo, 2002). Cells exposed to selenomethionine have shown a 3-fold increase in p53 activity (Longtin, 2003).

Healthy wound-healing involves a well-coordinated immune/inflammatory response. Neutrophils and macrophages (immune system cells) enter the wound and fight bacteria by creating free radicals like hydrogen peroxide, peroxinitrite and the hydroxyl radical. Antioxidant enzymes like selenium-containing glutathione peroxidase and thioredoxin reductase protect neutrophils, macrophages and other tissues form the attack
by free radicals which were released to destroy the pathogens. Macrophages release growth factors to promote tissue re-growth. With chronic inflammation, however, these natural mechanisms run amuck into cycles of tissue regeneration and destruction, creating an environment conducive to cancer-development (Coussens, 2002 and Karin, 2005). Continuous exposure to the free radical peroxynitrite leads to DNA mutation. Growth factors from macrophages promote proliferation of new cancer cells. An estimated 15% of cancers are attributed to inflammation associated with chronic infections, such as hepatitis, papillomavirus, and the gastric bacterium *Helicobacter pylori* (Kuper, 2000).

Selenium compounds have been shown to block DNA transcription factors that would otherwise worsen the inflammatory response (Jozsef, 2003 and Yoon, 2001). And selenium compounds are very effective at protecting cells (particularly endothelial cells) from peroxynitrite-induced DNA damage (less than 5% of which is due to hydroxyl radical production) (Roussyn, 1996). Glutathione peroxidase and possibly other selenoproteins directly reduce peroxynitrite to nitrite (Sies, 1997). Because thioredoxins increase transcription, inhibit apoptosis and stimulate cell proliferation & angiogenesis while providing antioxidant defense, but are overexpressed in some cancer cells to promote their growth and increase resistance to therapy.

**SELENIUM AND APOPTOSIS**

Selenium is an essential dietary component for animals including humans, and there is an increasing evidence for the efficacy of certain forms of selenium as cancer-chemopreventive compounds. In addition, selenium appears to have a protective effect at various stages of carcinogenesis
including both the early and later stages of cancer progression. Mechanisms for selenium-anticancer action are not fully understood; however, several theories have been proposed which include antioxidant protection, enhanced carcinogen detoxification, enhanced immune surveillance, and modulation of cell proliferation (cell cycle and apoptosis), inhibition of tumor cell invasion and inhibition of angiogenesis.

Epidemiological studies, preclinical investigations and clinical intervention trials support the role of selenium compounds as potent cancer chemopreventive agents. Induction of apoptosis and inhibition of cell proliferation are considered important cellular events that can account for the cancer preventive effects of selenium. Toxicity should always be considered a determining factor in the selection of potential chemopreventive agents.

Apoptosis, or programmed cell death is a highly regulated process with distinct morphologic and biochemical features (Hengartner, 2000; Que and Gores, 1997). In nontechnical terms, the cell uses a genetically controlled program to cause its own death in little more than a few hours (Que and Gores, 1997). It has been suggested that the incidence of certain diseases, such as cancer, is increased by an inhibition of normal apoptotic process (Sjostrom and Bergh, 2001; Wong et al., 1999). Martin et al., (2002) reported that a low rate of apoptosis was strongly associated with a higher prevalence of colorectal adenomas.

The possibility that selenium may increase anticancer apoptotic activity has been suggested by several carcinogenesis studies (Lanfear, 1994; Zhong, 2001; Chun et al., 2006). It is unclear how selenium might induce apoptosis; however, several selenium metabolites, such as selenocystine, hydrogen selenide, methylselenol, and selenodiglutathione,
are being investigated for their anti-apoptotic activity (Fleming et al., 2001; Ip et al., 2002; Chen and Wong, 2008).

Selenide and methyselenol are metabolized from organic sources of selenium (naturally obtained through diet), such as selenomethionine, selenocysteine, and methylselenocysteine. Selenodiglutathione is metabolized from inorganic sources of selenium, such as selenate and selenite (Lu, 2001).

It has been suggested that methyselenol may directly activate caspases, which are thought to be downstream executors of the apoptosis (Ghose et al., 2001). It has been observed that selenodiglutathione is associated with an increased expression of Fas ligand, a well-known mediator of apoptosis (Fleming et al., 2001; Ghose et al., 2001). Fas ligand from tumor cells signals the immune system to induce apoptosis. Chen and Wong (2008) proposed selenocysteine induced caspase-independent apoptosis in MCF-7 breast carcinoma cells, which was accompanied by poly(ADP-ribose) polymerase (PARP) cleavage, caspase activation, DNA fragmentation, phosphatidylinerine exposure and nuclear condensation. Moreover, SeC induced the loss of mitochondrial membrane potential (ΔΨm) by regulating the expression and phosphorylation of Bcl-2 family members. Loss of ΔΨm led to the mitochondrial release of cytochrome c and apoptosis-inducing factor (AIF) which subsequently translocated into the nucleus and induced chromatin condensation and DNA fragmentation. MCF-7 cells exposed to selenocysteine (SeC) shown increase in total p53 and phosphorylated p53 on serine residues of Ser15, Ser20, and Ser392 prior to mitochondrial dysfunction. Silencing and attenuating of p53 activation with RNA interference and pifithrin-alpha treatment, respectively, partially suppressed SeC-induced cell apoptosis.
Pathways to apoptosis for methylselenocysteine, a lower homologue of selenomethionine, have also been suggested (Ip et al., 2002; Ghose et al., 2001). Ip et al. (2002) propose that methylselenocysteine increases the expression of cyclin D1 and cdk5, two proteins associated with increased apoptosis. In addition to these promoting factors, selenium down-regulates AKT2, which transmits survival signals. The combination of these signals increases the probability of cell death through apoptosis. Because most colorectal cancers arise from benign adenomas (Day and Morson, 1978), an opportunity for early detection and intervention exists if modifiable risk factors for the adenoma can be identified. Prior to induction of apoptosis, selenium compounds may alter the expression and/or activities of a number of cell cycle regulatory proteins, signaling molecules, proteases, mitochondrial associated factors, transcriptional factors, tumor suppressor genes, polyamine and glutathione levels. Depending on the form, selenium compounds can target separate pathways but more efforts are needed to learn about disrupting different pathways converging to apoptosis. Numerous selenium compounds are known to inhibit carcinogenesis in several animal models but not all of these have been examined for their efficacy to induce apoptosis or vice versa in the corresponding target organ. Thus, in view of the lack of information with regard to the precise role selenium in chemoprevention of cancer, the present study has been designed to investigate the potential of selenium in delaying the events leading to colon carcinogenesis.