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Cancer encompasses a number of disorders whose hallmark is the uncontrolled proliferation of cells that invade and damage surrounding tissues and organs. The genesis of cancer lies in alterations in oncogenes, tumor suppressor genes, and/or DNA repair genes. Oncogenes encode proteins that promote the loss of growth control and the conversion of the cell to a malignant state. Therefore, oncogenes can be considered as accelerators of cell proliferation and tumorigenesis. Tumor suppressor genes encode proteins that restrain cell growth and prevent cells from becoming malignant. Thus, tumor suppressor genes exercise negative regulation over cell growth and promote cell death. Owing to its morbidity this disease has enormous impact on human health and therefore has been the focus of research for a very long time.

Skin Cancer

Skin cancer or skin neoplasms are abnormal skin growths which are classified into three major types: Squamous Cell Carcinoma (SCC), Basal Cell Carcinoma (BCC) and Melanoma. SCC and BCC form the ‘Nonmelanoma’ category of skin cancer (NMSC). Since skin cancers develop in the epidermal layer, therefore such tumors are clearly visible. This makes most of the skin cancers detectable at early stages and have good prognosis. Both the forms of NMSCs i.e. SCC and BCC have a good prognosis and the survival rates are over 95%. SCC is a malignant tumor of the epidermal keratinocytes whereas BCC is a malignant tumor of the basal cells of the epidermis. The occurrence of SCC is less common than BCC but metastasize more frequently than BCC (Preston and Stern, 1992). BCC makes up about three-quarters of NMSCs and SCC make up most of the rest.

Most SCCs are readily treated and produce few sequelae. However, a subset of high risk lesions is responsible for the observed morbidity and mortality
associated with SCC. The overall risk for nodal or distant metastasis for SCC is in the range of 2-6%, however, rates as high as 47% have also been reported for cases with extensive perineural invasion. Lymph node metastasis is associated with significant morbidity and once lung metastases occur the disease adopts an aggressive and incurable form (Veness et al., 2005). However, if left untreated these tumors can become destructive and invade the nearby tissues. A subset of SCC is categorized as high risk because it has been associated with higher rates of recurrence, metastasis and even death in certain cases. The risk analysis of SCC is based upon certain tumor related factors (intrinsic factors), patient related factors (extrinsic factors) or a combination of both. For example, the SCCs of lips and ears have a higher rate of recurrence and metastasis than the SCCs at other sites. Organ transplant recipients are at a greater risk for developing SCCs which have a higher metastatic tendency (Euvrard et al., 2006). Melanoma is a cancer of melanocytes, the pigment cells of the epidermis. Melanoma is the least common but the most morbid type of skin cancer. They frequently metastasize and are fatal once they spread (Jerant et al., 2000).

Epidemiology of Skin Cancer

Among the cutaneous malignancies, NMSCs is the most frequent malignancy worldwide (American Cancer Society, 2010). The highest incidence of NMSCs is seen in white population. Epidemiological studies suggest that an inverse relation exists between the degree of skin pigmentation and incidence of NMSCs. This may be due to the protective effect of melanin on UV light induced damage (Yamaguchi et al., 2008). Globally, the highest incidence of skin cancer has been reported in Australia, which is nearly four times that prevalent in US, Canada and UK. Skin cancers account for 80% of the newly reported cancers in Australia (Australian Institute of Health and Welfare, 2008). According to the American Cancer Society, more than 2 million
NMSCs are diagnosed annually (American Cancer Society, 2010). Studies suggest that one in every five Americans will develop skin cancer in their lifetime (Robinson, 2005). The studies also reveal that one American dies of melanoma almost every hour (World Health Organisation, 2010). Of the 8000 skin cancer deaths that occur annually in America, almost 3000 are attributable to aggressive or high-risk SCC (Brantsch et al., 2008). Cancer registries indicate that approximately 75 percent of skin cancer deaths occur in patients with melanoma (American Cancer Society, 2010).

In people of African and Asian descent the incidence of SCC is rare, but it is the most common form of skin cancer in these people. This is accompanied by a higher mortality rate, perhaps due to delayed diagnosis because tumors are more likely to occur in sun protected areas, including the scalp and sites of previous injury and scarring (McCall and Chen, 2002). In dark skinned people, incidence of SCC is higher than that of BCC. SCC frequently occurs at sites that have not been exposed to sun and is often aggressive and BCC occurs at sites frequently exposed to the sun (Fleming et al., 1975). Studies over the years have revealed that the incidence of both melanoma and NMSC is lower among Indians due to the protective effects of melanin. However, there are indications that the occurrence of NMSCs is on a rise in India as well. Among the NMSCs, SCC is more predominant in the Indian population than BCC. In India, development of SCC has been associated with certain dermatological conditions such as discoid lupus erythematosus [DLE] (Ghosh et al., 1997), lupus vulgaris [LV] (Betti et al., 2002), lichen planus (Singh et al., 1998), necrobiosis lipoidica diabcticorum (Pavitharan, 1998), lichen simplex chronicus (Masood and Manzoor, 2000) and psoriasis (Kumar et al., 2004). Despite the easy availability of protective sunscreens, topical steroids and growing awareness among patients, still there are several Indian reports regarding the development of SCC from chronic DLE lesions. There are
numerous reports from various parts of India regarding malignant transformation of LV into SCC. Long standing cases of LV undergo malignant changes to form SCC, which is termed as ‘lupus carcinoma’ (Leleva et al., 1981; Salodkar et al., 1992; Das et al., 2007). A large number of cases have been reported from India regarding the malignant progression of actinic keratoses to SCC in albinos (Sivalingam et al., 2009). ‘Kangri’ cancer that occurs following the use or exposure of kangri, an indigenous fire pot tucked in between the thighs to generate warmth during the winter months is typically seen to occur on the lower extremities and the abdominal wall, also exhibits SCC pattern. Typically, this type of cancer is observed exclusively among the impoverished people of Kashmir using Kangri. These tumors are aggressive with a substantial risk for local to regional metastasis in 20-50% cases (Teli et al., 2009).

**Risk Factors for Development of Skin Cancer**

Exposure to UV radiation is one of the leading causes of all types of skin cancers. The incidence of both melanoma and NMSC is on a rise owing to the depletion in ozone levels (World Health Organization, 2010). It was suggested that there could be an increase of 2-4% in incidence of skin cancer for every 1% reduction in ozone layer with the increase being larger for SCC than for BCC (Fears and Scotto, 1983). BCC and SCC often carry a UV-signature mutation (thymidine dimers) indicating that these cancers are caused by UV-B radiation via the direct DNA damage. However the malignant melanoma is predominantly caused by UV-A radiation via the indirect DNA damage due to the generation of reactive oxygen species (ROS). Natural (sun) & artificial UV exposure (tanning salons) have both been associated with the development of skin cancer (Australian Institute of Health and Welfare, 2008). The World Health Organization estimated that worldwide about 65000 people die from sun related diseases, mostly from malignant skin cancer (World Health
Another significant risk factor for the development of skin cancer is the use of tobacco and tobacco related products which are rich sources of potent carcinogens like polycyclic aromatic hydrocarbons (PAHs). The risk of skin cancer doubles with smoking (Morita, 2008). Chronic non-healing wounds, especially burns have the likelihood of developing into SCC.

Individuals with genetic predisposition for certain dermatological conditions like ‘Congenital Melanocytic Nevi Syndrome (CMNS)’ can develop skin cancer. CMNS is characterized by the presence of nevi or moles of varying size that either appears at or within 6 months of birth. Nevi larger than 20 mm in size are at higher risk for becoming cancerous. Patients with dermatological conditions like DLE (Ghosh et al., 1997), LV (Betti et al., 2002), lichen planus (Singh et al., 1998), necrobiosis lipoidica diabetorum (Pavitharan, 1998), lichen simplex chronicus (Masood and Manzoor, 2000), psoriasis (Kumar et al., 2004), actinic keratoses (Sivalingam et al., 2009) are at risk for developing skin cancer. Viral infections particularly infection of the human papilloma virus is often associated with SCC of the genitals, anus, mouth, pharynx, and fingers (Karagas et al., 2010).

Animal Models of Carcinogenesis

The induction of cancer is a multistage process and its stages have been defined experimentally as initiation, promotion and progression [Figure: 1] (Slaga et al., 1980; Yuspa and Shields, 1997). Several models exist to study carcinogenesis and its chemoprevention. One of the better understood and characterised models is the mouse skin tumor promotion model. The tumor induction protocol involves the application of a sub-threshold dose of a carcinogen such as 7,12-dimethylben(z)anthracene (DMBA) followed by the repetitive application of a tumor promoter such as 12-tetradecanoylphorbol-13-acetate (TPA). Within 10 weeks, benign papillomas begin to appear and a small percentage of these papillomas eventually progress to malignant SCCs. The
papillomas are benign neoplastic lesions comprising of hyperplastic keratinocytes and supporting stromal cells. Virtually all of the papillomas contain Ha-ras mutation (Quintanilla et al., 1986). The appearance of papillomas/tumors on the skin surface enables the easy monitoring of the number, size and growth of these tumors throughout the study design. The papillomas which progress to SCC are easily visualized and quantified. A small fraction of these can metastasize to distant sites. Carcinomas invade through the basement membrane and are characterised by loss of differentiation, increased mitotic activity and nuclear atypia.

Initiation is an irreversible genetic event and usually consists of a single mutation, caused by the exposure to endogenous and exogenous carcinogens. If the resulting DNA damage is left unrepaird and allowed to replicate, it can lead to mutations which may give the mutated cell a growth advantage relative to normal cells. The initiation stage is characterised by the generation of a cell or cell population which is altered genetically by the covalent binding of the ultimate carcinogen to DNA. However, during this stage the epidermis exhibits a normal appearance without the presence of papillomas (Kemp, 2005). The promotion stage follows the initiation stage and involves the selective clonal expansion of the initiated cells, a result of the altered expression of genes whose products are associated with hyperproliferation, tissue remodeling, differentiation and inflammation (Slaga et al., 1980). Promotion occurs over a long period of time and it is reversible in its early stages. Benign papillomas begin to erupt on the skin surface during the promotion stage. The progression stage involves the conversion of benign tumors into malignant neoplasms capable of invading adjacent tissues and metastasizing to distant sites (Slaga, 1984). Progression stage is characterised by dysplasia, and ultimately by invasion and metastasis, due to additional genetic alterations (such as loss of tumor suppressor function) and progressive genomic instability (Hursting et al., 1999).
Stages of carcinogenesis (mouse skin model)

Initiation
- Covalent binding of carcinogen to DNA, cell replication and fixation of mutation
- Mutation induction in critical target genes, e.g., Ha-ras
- Phenotypically normal epidermis

Promotion
- Expansion of initiated cells through epigenetic mechanisms
- Altered gene expression, enzyme activities
- Development of clonal outgrowths, benign papillomas

Progression
- Clonal expansion of pre-neoplastic cells
- Mutations in oncogenes and tumor suppressor genes
- Invasion and metastasis
- Gene amplification e.g. mutated Ha-ras gene
- Alteration in differentiation

Figure 1: Multistage carcinogenesis in mouse skin

Landmarks were achieved in cancer research using mouse models. Research over the past several decades has revealed that there is a high degree of genetic and biological similarity between the process of neoplastic development in mice and humans. To a very large extent, the tumors in mice develop in the same tissues and with same histopathological alterations as observed in humans. The multistage nature of carcinogenesis has been demonstrated in both rodents (Balmain et al., 1992; Dragon and Pitot, 1992; Yuspa, 1994) and human cancers (Fearon and Vogelstein, 1990). However, the mouse skin tumor model has certain limitations. It is well known that cancer in mice and humans has different causes and is affected by differences in tissue organization, telomere length, ability of cells to senescence and immortalize (Balmain and Harris, 2000). Even though the multistage nature of carcinogenesis has been proven in both animal and human models, the temporal nature of initiation, promotion and progression can not be ascertained. It is known that multiple
mutational events are required in the formation of tumor. Epidemiological studies have indicated that four to six genetic hits are necessary for tumor development (Peto et al., 1975). Throughout the life span, humans get exposed to mixture of agents that may act simultaneously at different stages of carcinogenesis. Also, it is known that promotional events which increase cell proliferation and decrease apoptosis can influence subsequent initiation events. Also, an individual’s genetic background very strongly influences his or her susceptibility to a carcinogen (Gonzalez, 1995).

Rather, than occurring in three discrete and predictable stages, human carcinogenesis should be considered as an interplay between the alterations in genes regulating cell proliferation, cell survival, apoptosis, differentiation and DNA repair etc (Stanley, 1995). Since, both environmental and genetic factors influence carcinogenesis, one can control these variables using isogenic mice and providing them identical exposure (in an experimental animal model).

Skin Tumor Induction Using DMBA as a Carcinogen and TPA as a Promoter

DMBA belongs to the class of PAHs and is a well known pro-carcinogen, pro-mutagen (Miller, 1978) and teratogen (Currie et al., 1970). It exhibits a high degree of cytotoxicity in vivo, particularly in the adrenal gland (Huggins et al., 1961, Huggins and Mori, 1961; Wheatley et al., 1966) and fetal brain (Kellen et al., 1976) as well as in vitro to cells in culture (Diamond et al., 1968; Diamond, 1971). Amongst all the PAHs tested, DMBA is the most potent mouse skin carcinogen in the conventional mouse skin initiation promotion regimes (DiGiovanni and Juchau, 1980). It is an indirect acting carcinogen and undergoes metabolic activation by cytochrome P450 (CYP) monooxygenase system to exert its mutagenic and carcinogenic activity. In the two stage carcinogenesis model, active metabolites of DMBA bind to bases in DNA forming carcinogen-DNA adducts. TPA treatment that follows DMBA application causes the fixation of mutation in critical genes associated with cell
proliferation, apoptosis, differentiation etc. In the complete carcinogenesis protocol, DMBA acts both as the initiator and the promoter.

Figure 2: Chemical Structure of DMBA and TPA

Figure 3: Metabolism of DMBA by CYP 1A1, CYP 1B1 and mEH (Miyata et al., 1999)
Cytochrome P450 1B1 (CYP1B1) oxidizes DMBA to 3, 4-epoxide. This is followed by the hydrolysis of the epoxide by microsomal epoxide hydrolase (mEH) to the proximate carcinogenic metabolite, DMBA-3, 4-diol. This metabolite is then further oxidized by either CYP1B1 or cytochrome P450 1A1 (CYP1A1) to the principal ultimate carcinogenic metabolite, DMBA-3,4-diol-1,2-epoxide, which is capable of producing DNA adducts (Dipple and Nebzydoski, 1978; Tierney et al., 1978; Wislocki et al., 1980). Other ring hydroxylations and methyl hydroxylations of DMBA result in inactive metabolites that do not bind DNA (Figure: 3).

DNA adducts are the covalent addition products resulting from the binding of reactive chemical species to DNA bases. When the chemical species is a carcinogen/carcinogen metabolite, adduct so formed is known as carcinogen-DNA adduct. The formation of covalent DNA adducts represents a key early event in the cascade of molecular alterations that leads to the transformation of a normal cell into a cancer cell. Carcinogen-DNA adduct formation may be taken as an index for carcinogenicity, since it is the first step towards cancer (Gangar et al., 2006b). The covalent modification of DNA bases can affect the biological processing of DNA specifically repair, replication and transcription. Upon replication of un-repaired DNA, the modifications may eventually lead to mutations and ultimately cancer, especially if the adduct is located in a proto-oncogene or a tumor suppressor gene (Dipple, 1991). The high cancer incidence observed in people with deficiencies in any variety of DNA repair enzymes potentiates the already established role of DNA adducts in cancer initiation (Benhamou and Sarasin, 2000). DNA adducts are not DNA mutations but are direct inducers of mutations.

The initiated cell with an irreversible alteration in the genotype may remain dormant-latent for a long period of time and sometimes not lead to the formation of a tumor if not treated with a tumor promoter. Tumor promotion is considered to be an interplay of several processes that include stimulation of proliferation, altered gene expression (Pitot, 1989), generation of ROS (Ito and
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Hirsoe, 1987) and inhibition of intercellular communication (Loch-Caruso and Trosko, 1985). Tumor promoters block apoptosis in a variety of cell types like fibroblasts, myeloid and monocytic leukemias and carcinomas derived from prostate, breast and liver (Wright et al., 1994). The capacity to induce a state of resistance to apoptosis may be a common feature of tumor promoters. Treatment of tumor promoters affects the expression/function of key molecules involved in the apoptotic cascade. The application of TPA to mouse skin causes biochemical alterations and changes in cellular functions. Induction of epidermal ornithine decarboxylase (ODC) is an important biochemical alteration elicited by TPA (Verma et al., 1980). The ornithine decarboxylation reaction catalyzed by ODC is the rate limiting step in polyamine biosynthesis (Kern et al., 1999). Polyamines (putrescine, spermidine and spermine) are critical for cell survival and normal skin homeostasis. The majority of polyamine molecules are cationic and are bound to polyanionic macromolecules such as DNA, RNA, and phospholipids (Igarashi et al., 1982), resulting in far-reaching effects upon cellular processes including DNA replication, DNA repair, transcription, translation etc. ODC is a transcriptional target of the oncogene Myc (Bello-Fernandez et al., 1993) and is up regulated in a wide variety of cancers. The polyamines are associated with increased cell growth and reduced apoptosis (Gerner et al., 2004). Protein kinase C (PKC) is the receptor for phorbol ester tumor promoters and is activated in response to TPA (Blumberg et al., 1987). It is suggested that PKC is involved in TPA induced ODC. Tumor promoters modulate the activity of PKC which in turn leads to the induction of cell proliferation associated genes such as c-myc, c-fos and c-jun. c-fos and c-myc is induced in culture cells (Greenburg and Ziff, 1984) and in mouse skin (Rose-John, 1988) in response to phorbol esters.

There are reports indicating that topical application of TPA stimulates DNA synthesis in epidermis and that indomethacin inhibited TPA induced DNA synthesis in mouse epidermis (Furstenberg and Marks, 1978; Furstenberger et al., 1984). TPA application causes inflammatory reactions in skin (Blumberg
et al., 1983). Painting of dorsal skin of CD-1 mice exhibited a dose related increase in vascular permeability (Nakadate et al., 1985). Expression of transcription factors like NF-kappa B and AP-1 which have implications in carcinogenesis also increases following TPA treatment (Dong et al., 1997)

**Xenobiotic Metabolising Enzymes-Critical Players in Initiation of Carcinogenesis**

A xenobiotic is a compound that is foreign to the body, such as drugs, chemical carcinogens and various other compounds that have found their way into our body by one route or another. Majority of these compounds are acted upon by the metabolic enzymes, with liver being the main organ involved. Occasionally, a xenobiotic may be excreted unchanged. Over 30 different enzymes are known to be involved in this process. The xenobiotic metabolism can be considered to take place in phases, each of the phase with its distinct functions and its special enzymes involved (Figure: 4). The two phases are: Phase I and Phase II. The overall purpose of the two phases of xenobiotic metabolism is to increase the water solubility and facilitate the excretion of the xenobiotic. Hydrophobic xenobiotics, such as PAHs are extremely lipophilic and would persist in adipose tissue almost indefinitely, if remained unchanged or converted to more polar forms. Detoxification, is sometimes synonymous with some of the changes involved in the metabolism of xenobiotics, however it is not always correct to strictly adhere to it because in many cases the reactions to which the xenobiotics are subjected actually increase their biological activity and toxicity.

In Phase I, metabolic reactions convert xenobiotics from inactive to biologically active compounds. Therefore, the original xenobiotic is referred to as the pro-drug or pro-carcinogen. The carcinogen (pro-carcinogen) undergo metabolic alteration (activation or de-activation) by a mixed function oxidase enzyme system (MFO) to form a series of metabolites through epoxidation, hydroxylation, glucuronidation, sulfation, epoxide hydration and glutathionation (Vainio et al., 1991). The MFO system commonly called as the
Cytochrome P450 system is present in the smooth endoplasmic reticulum and in the nuclear envelope of the cell. This phase of xenobiotic metabolism is generally considered as the activation phase. However, there are instances where the phase I reactions convert the active compounds to less active or inactive compounds, prior to conjugation. Phase-I enzymes (cytochrome P450, cytochrome b5 etc) primarily oxidize, reduce or hydroxylate the xenobiotic to a more polar form suitable for excretion. In this process, sometimes the phase-I metabolic reactions convert pro-drugs or pro-carcinogens to the active form capable of reacting with cellular macromolecules.

Phase II reactions (catalysed by Glutathione-S-transferase (GST) Uridine diphosphosphate glucuronosyl-transferases (UDP-GT)) comprise of conjugation reactions that convert the active products of phase I reactions to less active or inactive species, which are rendered suitable for excretion in bile or urine. Rarely conjugation may actually enhance the biological activity of a compound. If not detoxified, the active metabolite is then available for interaction with cellular macromolecules. The ultimate carcinogen formed is a strong electrophilic agent that can undergo non-critical binding, spontaneous decomposition or critical binding with nucleophilic centers in cellular material, such as the nucleic acid bases and the amino acid residues of proteins. Binding to DNA may be eliminated by the DNA repair system if it occurs prior to DNA replication and the cell then retains its normal properties. On the other hand, the DNA binding may lead to changes in the genome responsible for transformation and thereby an initiated cell will give rise to latent tumor cells eventually leading to tumor formation. Thus, modulation of carcinogen biotransformation enzymes is a reliable marker in the evaluation of chemopreventive potential of a drug and several agents have been screened for their anticancer activity based on this concept (Dasgupta et al., 2004; Gangar and Koul, 2007).
Cytochrome P450 (CYP)

In phase I, the major reaction involved is hydroxylation catalyzed by members of a class of enzymes referred to as monooxygenases or Cytochrome P450 (CYP). Hydroxylation may terminate the action of a drug, though this is not always the case. Other reactions catalyzed by this family of enzymes include deamination, de-halogenation, de-sulfuration, epoxidation, peroxygenation and reduction (Guengrich, 2001).

\[
RH + O_2 + NADPH + H^+ \rightarrow R-OH + H_2O + NADP
\]

Reduced Cytochrome P450 $\rightarrow$ Oxidised Cytochrome P450

The effect of this reaction is rendering the generally lipophilic substrates more hydrophilic by hydroxylation. In addition to xenobiotics, endogenous
compounds such as certain steroids, fatty acids, eicosanoids and retinoids are also the substrates that are subjected to metabolism by these monooxygenases. Some of the CYP that are of importance in carcinogen activation and detoxification are: CYP1A1, CYP1A2, CYP1B1, CYP2C9 and CYP3A4. NADPH is used to yield reduced CYP and the enzyme that catalyses this reaction is called NADPH-CYP reductase. Here, electrons are transferred from NADPH to NADPH-CYP reductase and then to CYP. This leads to reductive activation of molecular oxygen and one atom of oxygen is subsequently inserted into the substrate. Cytochrome b5 is another hemoprotein like the CYP that is found in the endoplasmic reticulum. Cytochrome b5 can also act as an electron donor and replenish the reduced CYP store (Porter, 2002).

**Aryl Hydrocarbon Hydroxylase (AHH)**

AHH is an isoform of CYP that catalyses the oxidative biotransformation of PAHs to reactive metabolites such as phenols, dihydrodiols, quinones, and epoxides (Sims, 1967; Jerina et al., 1970; Selkirk et al., 1971) that can bind to cellular macromolecules. AHH has been found in several human tissues including liver (Kuntzman et al., 1966; Kapitulnik et al., 1977), lung (Prough et al., 1977), placenta (Juchau et al., 1973), pulmonary alveolar macrophages (McLemore et al., 1977), lymphocytes (Gurtoo et al., 1975), and skin (Levi et al., 1972; Alvares et al., 1973). Inducibility of AHH by environmental carcinogens may correlate with susceptibility to tumorigenesis in experimental animals and in human populations. AHH may be a critical determinant of cutaneous carcinogenic responses to PAH such as B(a)P by transforming the parent compound into proximately reactive metabolites skin *in vivo* (Gelboin et al., 1970).

**Glutathione-S-Transferase (GST)**

This enzyme catalyses the conjugation reaction of the activated metabolite with glutathione (GSH). GSTs play a protective role by covalent binding and removing electrophiles.
A number of toxic electrophilic metabolites are conjugated to GSH and rendered inactive. If the toxic metabolites are not conjugated to GSH they become free to attack the cellular macromolecules such as DNA, RNA and protein. GSH conjugates are subject to further metabolism before excretion. The glutamyl and glycyl groups of GSH are removed and an acetyl group (from acetyl CoA) is added to the amino group of the remaining cysteinyl moiety. The resulting compound is a mercapturic acid, a conjugate of L-acetylcysteine which is then excreted in the urine. GSTs play an important role in metabolism and detoxification of carcinogens such as PAH oxides and diol epoxides (Hayes et al., 2005). Most of the GSH conjugates are less toxic and readily excreted from the body. However GSTs convert dichloromethane and short-chain alkyl halides to unstable and mutagenic GSH conjugates (Strange et al., 2001).

**Uridinediphosphate glucuronosyltransferase (UDP-GT)**

Glucuronidation is a major pathway for detoxification of numerous carcinogens including PAHs, aryl and heterocyclic amines (Hecht, 2002). Uridinediphosphate glucuronic acid is the glucuronyl donor and glucuronosyl transferases present in both the cytoplasm and endoplasmic reticulum are the catalysts. Glucuronic acid is conjugated to large number of potentially reactive compounds including phenols, dihydrodiols, quinones and quinols (Mulder et al., 1990). Conjugates can include ether O-glucuronides, ester O-glucuronides, N-glucuronides, S-glucuronides and C-glucuronides (Turesky et al., 1991). Like sulfation and GSH conjugation, glucuronidation produces polar conjugates that are readily excreted. Glucuronidation is probably the most frequent conjugation reaction. Glucuronidation catalyzed by glucuronyl transferases is a major pathway of metabolism of estrogens (Zhu and Conney, 1998) and androgens (Belanger et al., 1998) in the breast.
DT-Diaphorase (DTD) or NAD(P)H Quinone Oxidoreductase 1 (NQO1)

DT-diaphorase (DTD) or NAD(P)H quinone oxidoreductase 1 (NQO1) catalyses two-electron reduction of a wide variety of substrates including PAH o-quinone to inactive products such as PAH-hydroquinone (Ross and Siegel, 2004). It also provides protection against benzene induced hematotoxicity by converting benzene derived quinones to hydroquinones (Palackal et al., 2005). Skin tumors caused by B(a)P and DMBA are increased in NQO1 knock-out mice, indicating that it is somehow involved in detoxification of PAHs (Long et al., 2001). NQO1 activates some of the anti-tumor agents, such as mitomycin C and streptonigrin, to reactive metabolites that are toxic in tumor cells as well as normal cells (Sharp et al., 2000).

Inter-individual differences exist in levels of expression and catalytic activity of enzymes that activate and/or detoxify PAHs and other carcinogens, thus establishing the reasons for variation in response to PAH exposure. Factors affecting such variations include induction and inhibition of these enzymes by chemicals and also by genetic polymorphisms of enzymes. Some individuals have defective enzymes that activate PAHs but they have normal enzymes that detoxify them. On the other hand, some individuals have normal activating enzymes but defective detoxifying enzymes. Regulation of hepatic and extra-hepatic metabolizing enzymes will be useful in inhibiting the initial steps that lead to the formation of a latent cell, which over a period of time expresses itself as a transformed cell. Although hepatic enzymes are always likely to play a major role in the metabolism of carcinogens, enzymes expressed locally in the target tissue would also have an important influence in modulating the levels of DNA reactive species. Agents that can block carcinogen activation or enhance detoxification can act as possible chemo preventive agents against cancer, since they would ultimately inhibit the events downstream from carcinogen activation and DNA adduct formation, which would automatically reduce the frequency of mutations and undo the loss of control of cellular proliferation that is controlled by tumor suppressor genes and oncogenes.
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Involvement of Transcription Factors in Carcinogenesis

Transcription factors are regulatory proteins that recognize specific DNA sequences, bind them and recruit the correct RNA polymerase to carry out RNA synthesis, consequently regulating the expression of genes. The involvement of transcription factors cannot be denied in the process of tumorigenesis because they control the expression of genes involved in cell survival, cell proliferation, cell adhesion, differentiation, cell growth, inflammation, invasion and angiogenesis.

Nuclear Factor- kappa B (NF-κB)

Nuclear Factor- kappa B (NF-κB) is a nuclear transcription factor bound to an enhancer element of the immunoglobulin kappa light chain gene in B cells (Sen and Baltimore, 1986). It contains a DNA binding domain/dimerization domain –Rel homology domain and a nuclear localization domain. NF-κB consists of subunits that form two classes of proteins-class I and class II which in turn form dimers with each other. Class I proteins include p50, p52, p100 and p105 and Class II proteins include c-Rel, Rel B and RelA/p65 (Aggarwal et al., 2006). Except RelB which only forms homodimers, all other proteins can form both homo and heterodimers. RelA-p50 is the most common heterodimer formed. NF-κB exists in the cytoplasm in an inactive form, bound to an inhibitory protein (IκBa, IκBβ) belonging to the IκB family. The IκB proteins mask a nuclear localization signal on the NF-κB proteins thereby preventing the translocation of NF-κB to the nucleus. The inhibitory complex with the IκB proteins can be degraded in response to certain stimuli. Activation results in phosphorylation of IκB by IκB kinases, subsequently leading to rapid ubiquitination and degradation of IκB in the proteasome. Degradation of the inhibitory protein unmasks the nuclear signals on the p65/p50 complex resulting in rapid translocation to the nucleus where it binds to specific kB recognition elements in the promoter region of target genes (Baeuerle and Baltimore, 1989).
NF-κB genes are members of a proto-oncogene family and many functions of their encoded proteins have implications in cancer and its therapy. NF-κB has been implicated in carcinogenesis because it controls the expression of genes involved in cell survival, proliferation, adhesion, differentiation, growth, transformation, inflammation, invasion and angiogenesis (Mitisiades et al., 2002). NF-κB is activated in response to various stimuli such as cytokines, growth factors, hormones, carcinogens, tumor promoters, radiation, oxidative and chemical stress etc. Carcinogens like DMBA, B(a)P diol epoxide, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone have been shown to activate NF-κB (Shishodia et al., 2003) and tumor promoters like phorbol ester and okadaic acid have also been shown to activate NF-κB (Dong et al., 1997). NF-κB is also activated by hypoxia (Koong et al., 1994) and acidic pH (Schutze et al., 1992), which are the characteristics of tumor microenvironment. Constitutively expressed NF-κB is a hallmark of cell lines derived from skin, colon, ovary, breast, lung, prostate tumors (Rayet and Gelin, 1999; Collins et al., 2000). Activated NF-κB is also noted in tumors obtained from patients with cervical, breast, colon and renal cancer (Zhong et al., 1998; Petro et al., 2000; Nair et al., 2003). Constitutively expressed NF-κB has also been postulated in Hodgkin’s lymphoma, acute myelogenous lymphoma, T-cell lymphoma, Burkitt’s lymphoma (Garg and Aggarwal, 2002).

**Activator Protein 1 (AP-1)**

Activator Protein (AP-1) is a redox sensitive nuclear transcription factor that comprises of members of jun, fos, ATF (activating transcription factor) and MAF (musculoaponeurotic transcription factor) protein families. Likely as NF-κB, AP-1 is important in traversing tumor promotion and progression because of its ability to alter gene expression in response to carcinogens and tumor promoters including TPA, UV radiation and ROS (Dong et al., 1997). These proteins are capable of forming many heterodimers and homodimers, which then determine the genes that are regulated by AP-1 (Chinenov and Kerppola, 2001; Vogt, 2002). AP-1 proteins are basic leucine zipper (bZIP) proteins.
because they dimerize through a leucine zipper and contain a basic domain for interaction with the DNA backbone.

AP-1 proteins are primarily considered to be oncogenic, but are also known to exhibit anti-oncogenic effects. AP-1 proteins like c-fos, c-jun and fos B have been shown to transform cells in culture (Jochum et al., 2001). c-fos when overexpressed in mice caused osteosarcoma formation by the transformation of chondroblasts and osteoblasts (Wang et al., 1991; Grigoriadis et al., 1993). c-jun is essential in the development of skin and liver tumors, as inhibiting c-jun activity in basal keratinocytes of skin or in hepatic cells, interferes with the development of chemically induced papillomas and liver tumors (Young et al, 1999; Eferl et al., 2003). Some AP-1 proteins that possess weak or no transforming activity include Fra1-2 (Berger et al., 1995) and jun D (Vandel et al., 1995) respectively. Some of the jun and fos proteins not only lack transforming activity but some proteins can also suppress tumor formation (Deng and Karin, 1993). jun B and jun D have anti-oncogenic effects. In the multistage development of tumors, regulation of cell proliferation by AP-1 is of considerable importance (Park et al., 1999; Liu et al., 2002) c-jun is a positive regulator of cell proliferation (Schreiber et al., 1999; Wisdom et al., 1999) and AP-1 complexes comprising of c-jun induces the transcription of positive regulators of cell cycle progression like cyclin D1 or repress negative regulators such as p53 and p16 (Schreiber et al., 1999; Bakiri et al., 2002). AP-1 does not always promote cell proliferation but also has anti-proliferative activities (Shaullin and Karin, 2002). jun B and jun D are considered as negative regulators of cell proliferation (Passegue and Wagner, 2000; Weitzmann et al., 2000).

In vitro studies have indicated that increased AP-1 expression can cause apoptosis in specific cell types, including human tumor cells (Shaullin and Karin, 2002). Oncogenic AP-1 proteins can behave as negative regulators of apoptosis in liver tumors (Eferl et al., 2003). The apoptosis regulating activity of AP-1 is probably due to the differential regulation of pro-apoptotic and anti-
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apoptotic genes. The decision as to whether AP-1 is oncogenic or anti-
oncogenic depends upon the antagonistic activities performed by the different
AP-1 proteins and is also influenced by tumor type, tumor stage and the genetic
background of tumors (van Dam and Castellazi, 2001; Bakiri et al., 2002). AP-
1 activity can be regulated by dimer composition, transcription, post-
translational modification and interactions with other proteins (Eferl and
Wagner, 2003).

Signal Transducers and Activators of Transcription (STAT)

Signal transducers and activators of transcription (STAT) are a family of
transcription factors which are localized in the cytoplasm in an inactive form
and get translocated to the nucleus on activation. STAT get activated in
response to a wide spectrum of growth factors, cytokines and hormones to
modulate genes involved in cell proliferation, apoptosis, differentiation,
survival and other biological functions (Levy and Darnell, 2002). STAT
activation is dependent upon tyrosine phosphorylation which induces
dimerization between two STAT molecules. Activated dimer STATs
translocate to the nucleus and bind to consensus promoter sequences of target
genes thereby regulating their transcription (Bromberg, 2001). Tyrosine kinases
such as janus kinases, receptor tyrosine kinases (RTKs) and non-RTKs can
activate STAT proteins by tyrosine phosphorylation (Darnell, 1997).

So far, STAT family comprises of seven members, which are encoded by seven
distinct genes: STAT 1 (α and β splice isoforms); STAT 2; STAT 3 (α and β
splice isoforms); STAT 4; STAT 5α; STAT 5β and STAT 6 (Akira, 1999).
Constitutive activation of STATs has been described in a large number of
human cancer lines and primary tumors including blood, head, neck, breast,
lung, skin etc (Bromberg, 2002). STAT 1 functions as a negative regulator of
neoplastic transformation whereas STAT 3 and STAT5 are implicated in the
oncogenic transformation of cells. STAT 1 and STAT 3 have antagonistic
effects on cell proliferation and apoptosis (Stephanou and Latchman, 2005).
The differential functions of STAT proteins is due to their preferential
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activation by certain ligands, their binding to and activation of specific genes and/or due to distinct functional domains.

STAT 1 has been recognized to be a principal target of both type I and type II interferon (IFN) (Stark et al., 1998). Majority of the normal functions of STAT 1 are closely associated to the biological effects of IFNs since its utilization is specific to IFN ligands in vivo. Inadequate function of STAT 1 can result in dysregulated cell growth and disturbed immune functions which are important to the process of tumor formation (Bromberg et al., 1996). Loss of STAT 1 expression or its inappropriate activation has been observed in malignant cells obtained from tumors of different histological type such as that of breast cancer, head and neck cancer, melanoma, leukemia and lymphoma (Bromberg and Darnell, 2000; Battle and Frank, 2002; Stephanou and Latchman, 2005).

The anti-oncogenic function of STAT 1 was convincingly demonstrated using STAT 1 knock out mice. STAT 1 deficient mice did not exhibit increased tendency for spontaneous tumor formation, but manifested increased susceptibility to both chemically induced and transplanted tumors compared to wild type animals (Kaplan et al., 1998; Lee et al., 2000a; Lee et al., 2000b). This data suggested that STAT 1 might function as a tumor suppressor. Evasion of apoptosis is one of the mechanisms by which inactivated STAT1 may contribute to cancer development in few cell types (Sahni et al., 2001). STAT 1 deficient mice exhibited increased susceptibility to chemically induced carcinogenesis, partly because of the suppression of pro-apoptotic genes whose expression is partly dependent on IFN-γ and transcriptionally active STAT 1(Kaplan et al., 1998). Studies have revealed that activated STAT 1 (phosphorylated at serine 727) can positively influence apoptosis in cells (Janjua et al., 2002). Anticancer drugs like doxorubicin and cisplatin which induce apoptosis in malignant cells also potentiate STAT 1 phosphorylation (Thomas et al., 2004).
Activated STAT proteins (STAT 3) are persistently tyrosine phosphorylated because of deregulated positive STAT effectors such as tyrosine kinases or negative regulators of phosphorylation such as phosphatases (Bromberg, 2002). Oncogenic transformation by activated STAT 3 is due to upregulation of genes known to be involved in apoptosis and proliferation, including Bcl-xl, c-Myc, cyclin D1, survivin (Niu et al., 2002; Kanda et al., 2004; Schlette et al., 2004). Phosphorylated STAT 3 also contributes to the invasiveness of ovarian cancer cells because it is one of the components of focal adhesions which are the sites of cell contact with the extracellular matrix (Silver et al., 2004). The involvement of STAT 3 in initiation of skin carcinogenesis has been tested both in vitro using DMBA treated primary keratinocytes and in vivo by topical treatment of this mutagen. It was observed that STAT 3 mutant keratinocytes underwent enhanced apoptosis following DMBA treatment, compared to their control counterparts (Chan et al., 2004). In Ha-ras initiated keratinocytes, it was observed that the introduction of STAT 3 decoy molecules, leads to enhanced apoptosis with a concomitant decrease in Bcl-xl levels. Inhibition of STAT 3 in cancer derived cell lines containing ample phosphorylated STAT 3 leads to apoptosis.

STAT 3 is required for keratinocyte proliferation during the promotion stage of two-stage skin carcinogenesis. Application of TPA alone to mouse skin causes epidermal hyperproliferation and enhanced BrdU uptake in wild animals, however, less proliferation was observed in mice lacking STAT 3 (K5Cre. Stat 3\(^{\text{fl/fl}}\)) (Chan et al., 2004). Alongside, an increase in the expression of STAT 3 targets like cyclin D1, c-Myc was observed in wild animals while a delay in increase in these proteins was observed in K5Cre. Stat 3\(^{\text{fl/fl}}\) mice.

p53

p53 is a transcription factor which is considered as one of the important tumor suppressor genes. The activity of p53 gives rise to a variety of cellular outcomes, most notably cell cycle arrest and apoptosis. In a healthy cell, the level of p53 is very low. When a cell sustains genetic damage as occurs if the
cell is subjected to chemical carcinogens, UV light, genotoxic agents etc (Han et al., 2008) the level of p53 rises rapidly. The increase in p53 protein is due to an increase in the level of translation of preexisting p53 mRNAs and decrease in the protein’s degradation through alteration in protein-protein interaction. In unstressed cells, p53 levels are kept low through a continuous degradation of p53. A protein called Mdm2, binds to p53 and prevents its activity and transports it from the nucleus to cytosol. The Mdm2 oncoprotein binds to the N-terminus of p53 and represses its transcriptional activity. Also Mdm2 acts as ubiquitin ligase and covalently attaches ubiquitin to p53 and thus marks p53 for degradation by the proteasome (Haupt et al., 1997). The activation of p53 is marked by two events; increase in the half-life of p53 leading to a quick accumulation of p53 in stressed cells and second, a conformational change enables p53 to be activated as a transcriptional regulator in these cells. The critical event leading to the activation of p53 is the phosphorylation of its N-terminal domain.

It has been observed that approximately fifty percent of all human cancers contain cells with mutations or deletions in both alleles of the p53 gene. These tumors bearing mutated p53 tend to be more invasive with a high metastatic risk and are correlated with a poor prognosis. Following treatment with ionizing radiation, p53 gets phosphorylated on multiple residues. Mdm2 also undergoes rapid phosphorylation by the ATM kinase, as a result the phosphorylated Mdm2 is unable to promote p53 degradation (Maya et al., 2001). p53 encodes a protein called p21 that inhibits the cyclin dependent kinases (CDKs) that normally drives the cell through the G1 checkpoint. As the level of p53 rises in response to genetic damage, the level of p21 also rises and the progression of the cell through the cell cycle is arrested. This halt in the cell cycle allows the cell to repair the DNA damage, before it can proceed for DNA replication in the S phase. When both copies of p53 gene are mutated, the p53 protein formed is no longer functional and the cell progresses through the cell cycle while sustaining the DNA damage. Failure to repair DNA damage and
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allowing the cells to replicate can result in mutations which can have implications in carcinogenesis. Alternatively, p53 can direct a genetically damaged cell to undergo apoptosis. It does so by activating the expression of bax, a protein actively involved in carrying out apoptosis. p53 has been described as "the guardian of the genome because of its role in maintaining genome stability (Read, 1999).

Dual Role of Reactive Oxygen Species in Cancer

Oxidative stress results from the metabolic reactions that use oxygen, and it has been defined as the disturbance in the equilibrium status of pro-oxidant/antioxidant systems in intact cells leading to the enhanced formation of ROS. $\cdot O_2^-$, $H_2O_2$, $OH$ are the commonly produced ROS formed by the univalent reduction of $O_2$ (Harman, 1993) Hydroxyl (OH) and superoxide ($O_2^-$) radicals are chemical species with one or more orbital electrons with unpaired spin states and are called ‘free radicals’ and hydrogen peroxide ($H_2O_2$) and singlet oxygen ($O_2$) are ‘non-radicals’. Several important cellular processes like signal transduction and phagocytic activity of phagocytes are facilitated by ROS. An important cellular site for ROS production is the electron transport chain which is present in the inner membrane of mitochondria. The drug metabolising enzymes which includes the CYP monooxygenase system located in the endoplasmic reticulum is also another important site for ROS production. Other less active sites of ROS production include plasma membrane, nuclear membrane, and xanthine-xanthine oxidase system (Conklin, 2004).

The production of ROS is kept under control by the antioxidant defense system of the cell which includes the primary enzymatic defense and the secondary non-enzymatic defense. Antioxidant defense system is a protective and programmed response to prevent cellular damage from oxidative stress. Superoxide dismutase (SOD) (Fridovich, 1983; Fridovich, 1986), catalase (Chance et al., 1979) and peroxidases (Meister and Anderson, 1983) comprise the primary defense and smaller molecules like GSH, alpha-tocopherol, ascorbate constitute the secondary defense (Blake et al., 1987). The iron-
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containing SOD (Fe-SOD) and the manganese-containing (Mn-SOD) enzymes are characteristic of prokaryotes and copper-containing enzyme (Cu-SOD) and zinc-containing enzyme (Zn-SOD) are characteristics of eukaryotes. SOD catalyses the dismutation of superoxide into oxygen and \( \text{H}_2\text{O}_2 \). Catalase is a hemoprotein which catalyses the decomposition of \( \text{H}_2\text{O}_2 \) to water and oxygen, thereby protecting the cell from oxidative damage (Deisseroth and Dounce, 1970). Glutathione peroxidase (GPx) catalyses the reaction of hydroperoxides with reduced glutathione (GSH) to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide (Chance et al., 1979). It is a selenoenzyme; two-third of which (in liver) is present in the cytosol and one-third in the mitochondria. Glutathione Reductase (GR) catalyses the reduction of the oxidized substrate (GSSG) into reduced form (GSH). It is essential for glutathione redox cycle because it maintains adequate levels of GSH. Reduced glutathione (GSH) is a ubiquitous tri-peptide endowed with the functions of detoxifying electrophiles, maintaining the essential thiol status of proteins by preventing oxidation of –SH groups or by reducing disulfide bonds induced by oxidative stress; scavenging free radicals and several other functions (Schirmer et al., 1989). GSH is an important constituent of intracellular protective mechanisms against a number of noxious stimuli and is known to be a major low molecular weight scavenger of free radicals in cytoplasm. This implies that cells have intact pro-oxidant/anti-oxidant systems that continuously generate and detoxify oxidants during normal metabolism. When excess of oxidative events occur for example during administration of certain drugs or exposure to carcinogens, mutagens etc, the pro-oxidant systems outbalance the anti-oxidant systems causing damage to lipids, proteins, carbohydrates and nucleic acids consequently interfering with vital cellular functions. Mild chronic oxidative stress may alter the antioxidant systems by inducing or repressing proteins that participate in these defenses and by depleting cellular stores of anti-oxidants such as GSH and vitamins such as vitamin E, vitamin C etc.
Oxidant carcinogens interact with multiple cellular targets including membranes, proteins and nucleic acids. Excessive ROS production leads to the oxidative modification of cellular macromolecules (lipids, proteins, nucleic acids) with potentially deleterious effects. They cause structural damage to DNA and have the potential to mutate cancer-related genes resulting in the malignant transformation of cells and development of cancer. At the same time, oxidants activate signal transduction pathways and alter the expression of growth and differentiation related genes. There are studies that have established that DNA damage by ROS is responsible for mutagenesis, oncogenesis and aging. ROS induced lesions in DNA include base modifications, strand breaks and abasic sites (Ahmad et al., 2005). The carcinogenic effects of ROS have been primarily attributed to the genotoxic effects of ROS, but ROS are also known to play a significant role in the promotion stage of carcinogenesis. It is known that several oxidants and free radical generators are tumor promoters. Tumor promoters like TPA generate ROS and the involvement of H$_2$O$_2$ in tumor promotion has been extensively proved. In mouse skin tumorigenesis, oxidative stress induced by ROS has been linked to tumor promotion (Cerruti, 1985; Nishigori et al., 2004). Topical application of tumor promoters to mouse skin enhances the production of H$_2$O$_2$ in the epidermis which is responsible for their tumor promoting activity (Cerruti, 1985; Perchellet and Perchellet, 1989,). O$_2^-$ radicals also formed in keratinocytes of mouse skin in response to application of TPA (Pence and Reiners, 1987).

However, now the dual role of ROS in carcinogenesis has been recognized in the living systems (Valko et al., 2004). Interestingly, reports on cancer chemopreventive agents suggest that they enhance peroxidative damage in the tumorous tissue thereby exercising their anti-oncogenic action (Balasenthil et al., 1999; Subapriya and Nagini, 2003). The detrimental/beneficial effects of ROS depend upon the state/type of tissue and the concentration of the anticancer agent being used. During their course of action, anti-neoplastic agents produce numerous electrophilic moieties creating a pro-oxidant milieu.
in the cell leading to oxidative stress-induced lipid peroxidation which can then attack key targets in the cell. This oxidative damage can slow cell cycle progression of cancer cells and cause cell cycle checkpoint arrest (Conklin, 2004).

**Oxidative Stress and Cell Cycle**

The cell cycle consists of four phases: G1, S, G2 and M. During the G1 phase, the cell prepares for DNA synthesis (Figure 5). The S phase is the synthesis phase during which DNA synthesis occurs. During the G2 phase, the cell prepares for mitosis. During the mitotic phase ‘M’, the cell divides into two daughter cells.

![Cell Cycle Diagram](image)

**Cell Cycle**

G1: cell prepares for DNA synthesis; S: DNA synthesis; G2: cell prepares for mitosis; M: cell undergoes mitosis. The cell cycle safeguards include the restriction points (R) and checkpoints (C). Restriction point is passed only when all the requirements for DNA synthesis are met.

Checkpoint arrest occurs in response to DNA damage and leads either to repair of the damaged DNA or apoptosis if the damage is too severe.

**Figure 5: Cell Cycle (Conklin, 2000)**

The cell cycle has restriction and checkpoints which function as safeguards in the cell cycle. G1 and G2 checkpoints ensure that the integrity of the genome is maintained and in case of any damage, cause cell cycle arrest. The S checkpoint causes a halt in the cell cycle, if a problem is encountered with DNA replication and the M checkpoint arrests the cell cycle if a problem with the mitotic spindle assembly occurs. In case of a cell cycle arrest, the cell can meet either of the two fates; it can correct the error or undergo apoptosis.
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Cyclin dependent kinases (CDKs) are enzymes that along with cyclins enable ordered progression through the cell cycle. The CDKs are the catalytic subunits which get activated when they combine with the regulatory subunits called cyclins. Once activated, the CDKs phosphorylate their protein substrates which allow progression through the cell cycle.

Figure 6: Cyclin dependent kinases and cyclins in cell cycle (Conklin, 2004)

One of the major effects of oxidative stress is its ability to reduce the rate of cell proliferation by (1) inhibiting the transition of cells from G0 phase to G1 phase, (2) prolonging G1 phase, (3) delaying progression through S phase by inhibiting DNA synthesis, (4) inhibiting cell cycle progression through restriction point and (5) causing cell cycle arrest at checkpoints (Gonzalez, 1992; Kurata, 2000; Schackelford et al., 2000). Cell proliferation in normal and cancer cells growing in culture is inhibited in the presence of pro-oxidant milieu. It has also been reported that oxidative stress slows tumor growth in laboratory animals (Bartoli and Galeotti, 1979; Masotti et al., 1988). High growth rate in normal liver tissue (Wolfson et al., 1956; Cheeseman et al., 1986) and tumors (Bartolli and Galeotti, 1979; Dianzani, 1993) is associated with low levels of lipid peroxidation (LPO). Oxidative stress mediates this cell cycle arrest by targeting key enzymes and proteins which are essential in cell cycle progression. These include cyclin dependent kinases (CDKs).
(Schackelford et al., 2000), DNA polymerases (Hauptlorenz et al., 1985) and cyclins etc (Figure: 6)

**Oxidative Stress and Apoptosis**

ROS can induce apoptosis by causing damage to cellular components like DNA and some studies suggest that ROS are downstream mediators of apoptosis (Johnson et al., 1996). The two major pathways of oxidative stress induced apoptosis are the mitochondrial mediated apoptosis initiated by cytochrome c and the CD95 death receptor pathway initiated by ligation of the death receptor by its ligand CD95 (Debatin, 2000; Kaufmann and Earnshaw, 2000; Solary et al., 2000). Following the initiating event (cytochrome c release or CD95 ligation), apoptotic process occurs by the action of caspases which are cysteine dependent aspartate directed proteases (Player et al., 1979). The initiating signals of CD95 ligation or cytochrome c release activate initiator caspases (caspase 8 and caspase 9 respectively) which subsequently activate the effector caspases (caspases 3, 6 and 7). Excessive ROS generation can lead to opening of the mitochondrial permeability transition pore with the consequent release of cytochrome c from the intermembrane space into the cytosol culminating in the activation of the caspase cascade and hence the apoptotic mode of cell death (Jeong et al., 2008).

Several chemopreventive compounds like N-(4-hydroxyphenyl) retinamide (Hail and Lotan, 2001), celecoxib (Chan et al., 1998), indomethacin (Kusuhara et al., 1999), epigallocatechin gallate (Yang et al., 2002), curcumin (Morin et al., 2001), tamoxifen and capsaicin (Hail and Lotan, 2002) promote the generation of ROS leading to oxidative stress which appears to be associated with apoptosis induction in various cell types (Isenberg et al., 2000; Macho et al., 2000; Armstrong et al., 2001). Electron transport chain in the inner mitochondrial membrane is the major site for ROS generation in a cell (Fleury et al., 2002) and, under certain conditions, elevated mitochondrial ROS generation can serve as an apoptotic signal (Skulachev, 1996). Through direct i.e. inhibition of mitochondrial respiration (Hail and Lotan, 2001; Hail and
Lotan, 2002) and/or indirect i.e. promotion of the dissipation of mitochondrial inner transmembrane potential (Hail and Lotan, 2001; Hail and Lotan, 2004). Chemopreventive agents disrupt the mitochondrial function to trigger the intrinsic pathway of cell death. Enhanced ceramide production promoted by N-(4-hydroxyphenyl)- retinamide (Maurer et al., 1999) and celecoxib (Chan et al., 1998) lead to excessive generation of ROS, impaired mitochondrial function, and triggering of intrinsic pathway of apoptosis.

ROS can have several divergent effects depending on the extent of their production and mechanisms available to scavenge them in a given cell type. ROS can serve as mitogenic stimuli, senescence promoters, or cell death mediators (Fleury et al., 2002). However, reports are also available which point out that apoptosis does not necessarily require ROS and that their generation is a late event after cells have already been committed to the apoptotic cascade (Jacobson, 1996; Clutton, 1997). There is evidence pointing out that excessive oxidative stress inhibits caspase activity which is responsible for the diminished response of anticancer drugs to kill tumor cells (Lee and Shachter, 2000; Shacter et al., 2000). Electrophilic aldehydes are generated in the cells in response to antineoplastic agents. These aldehydes bind to the active site of caspases and inhibit their activity, which is plausible for the reduced efficacy of antineoplastic agents during oxidative stress (Lee and Shachter, 2000; Shacter et al., 2000).

**Prevention and Treatment of Cancer: Mechanism Based Strategies**

**Strategies Targeting Initiation**

There are several important means of intervention of carcinogenesis at the initiation stage. Direct acting carcinogens do not require metabolic activation to form reactive electrophilic moieties capable of attacking and damaging DNA. Formation of electrophilic DNA reactive species is the common denominator of all carcinogen activating systems (Macleod and Slaga, 1995). Indirect acting carcinogens exert DNA damage through electrophilic intermediates (Miller and
Miller, 1981). Activation leads to the formation of proximate or ultimate carcinogen and deactivation leads to its detoxification and consequent loss from the body. The detoxification step (Phase II) follows the activation step (Phase I) and serves to protect the cell by conjugating the pro-carcinogens or their reactive metabolites and facilitating their excretion from the body. Epoxide hydrolase transforms the epoxides into dihydrodiols. GST conjugate the dihydrodiols to GSH. (Hayakawa et al., 1974; Keen et al., 1976). Therefore, anti-initiating agents could either be suppressors of Phase I enzymes, inducers of Phase II enzymes or both i.e. dual agents. ROS are sometimes involved in the activation of carcinogens and are also generated during the metabolic activation of carcinogens which may attack DNA with deleterious consequences (Perchellet et al., 1995). Thus scavenging ROS could also constitute an effective strategy for modulating carcinogenesis during the early stages.

Medicinal plants, herbs, vegetables and fruits contain chemical constituents that modulate the metabolic activation of chemical carcinogens. Cruciferous vegetables such as cauliflower, broccoli and cabbage are rich in isothiocyanates which are known to interfere with the metabolism of nitrosamines (Morse et al., 1989; Hecht, 1995). Diallyl sulfide, a constituent of garlic has been shown to be a potent inhibitor of CYP2E1, which is involved in the metabolism of several chemical carcinogens like nitrosamines (Brady et al., 1991), dimethylhydrazine etc. (Chee et al., 1994). Other plant compounds including, flavonoids, isoflavonoids and coumarins have the potential to modulate carcinogen activating enzymes such as CYP (Bickers et al., 1982). Investigations conducted by Cai et al., (1997) have revealed that several naturally occurring coumarins can block skin tumor initiation in response to PAHs such as B(a)P and DMBA, by inhibiting their metabolic activation mediated by the CYP enzymes. While employing CYP inhibitors as chemopreventive agents, it is considered that activation of carcinogen occurs predominantly in the target tissue. Therefore, selectively blocking CYP i.e. in
the target tissue would be better and effective in decreasing the frequency of initiating events in the target tissue without substantially altering CYP activity elsewhere.

Conjugation of reactive electrophilic moieties to endogenous cellular nucleophiles can facilitate their excretion and minimize the risk posed by carcinogen. Enhancing the detoxification of chemical carcinogens by enzymes such as GST and UDP-GT is enhanced by several constituents of garlic, onions, cruciferous vegetables, spices etc. Diallyl sulfide and s-allyl-cysteine are present in garlic, and enhance GST levels in colon and liver (Sparnins et al., 1988). Thus these compounds have tumor inhibiting activity due to the dual effect of decreased carcinogen activation and enhanced carcinogen detoxification. Isothiocynates also have a dual role of suppressing carcinogen activation and enhancing carcinogen detoxification (Hecht, 1995).

Resveratol, an important constituent of grapes and berries, has demonstrated anti-tumor activity against two-stage mouse skin carcinogenesis, by enhancing carcinogen detoxification mediated by phase II enzymes such as quinone reductase (Jang et al., 1997). Plants with medicinal utility like Ashwagandha have the potential to inhibit skin and forestomach tumorigenesis by suppressing the carcinogen activation mediated by CYP and cytochrome b5 and enhancing carcinogen detoxification mediated by GST (Padmavathi et al., 2005). Studies carried out by Bhuvaneswari et al., (2005), suggested that modulation of xenobiotic-metabolizing enzymes by tomato and garlic combination plays a key role in mitigating the mutagenic and carcinogenic effects of DMBA.

There are reports available in literature regarding the scavenging of ultimate electrophiles by several compounds present in the cell itself or compounds available via exogenous sources. Sulphydryl groups, like cysteine are effective scavengers of the metabolite of B(a)P, Benzo(a)pyrene diol epoxide (BPDE) (Kootstra et al., 1980). Riboflavin also promotes detoxification of BPDE by enhancing its hydrolysis (Wood et al., 1982). Ellagic acid, belongs to the class of polyphenols and blocks the mutagenicity of BPDE by reacting with it (Wood
et al., 1982). A major polyphenolic antioxidant in green tea, epigallocatechin-3-gallate (EGCG) has demonstrated anticarcinogenic action in several models including mouse, skin, forestomach, liver breast, colon etc (Katiyer and Mukhtar, 1996). It has been reported that EGCG can trap the active metabolites of several pro-carcinogens (Stoner and Mukhtar, 1995) and also enhances the repair of damaged DNA (Weisburger, 1995).

ROS can act as both initiators and promoters of tumorigenesis by damaging critical cellular macromolecules such as DNA, proteins and lipids and by acting as cell signaling molecules (e.g. Nitric oxide) (Trush and Kensler, 1991; Perchellet et al., 1995). Therefore antioxidants could serve as effective agents for combating cancer. Polyphenolic compounds found in green tea, spices, fruits and vegetables have been shown to effectively inhibit TPA promotion in mouse skin (Perchellet et al., 1995). Intracellular antioxidant defenses like GSH, SOD, catalase, GPx are induced in response to several anti-tumor agents. Ascorbic acid, alpha tocopherol, and selenium exert anti-tumor effects by decreasing ROS production and enhancing antioxidant defenses (Perchellet et al., 1995).

Normal mammalian cells have the ability to repair DNA using different strategies like base excision repair, nucleotide excision repair, mismatch repair etc, depending upon the kind of damage and its location in the genome (Mitchell et al., 1995). Small lesions such as alkylated bases can be repaired by base excision repair (Friedburg et al., 1995), UV photodimers can be repaired by nucleotide excision repair (Sancar, 1993) and errors resulting due mis-replication during normal replication can be corrected using mis-match repair mechanism (Modrich et al., 1994). Enhancement in the rate and fidelity of DNA repair should result in a decrease in initiating events. Therefore, inducing DNA repair could be effective in combating tumorigenesis at the early stages.
Strategies Targeting Promotion and Progression

Tumor promoters are not inducers of mutations but alter the expression of genes associated with proliferation, apoptosis, tissue remodeling, inflammation, angiogenesis, differentiation (Kelloff, 2000). The identification of mechanisms by which tumor promoters alter gene expression has helped to determine critical targets that could be used to develop new prevention strategies. In addition to cell proliferation, apoptosis has emerged as an equally important target for cancer chemoprevention (Thompson et al., 1992). Changes in gene expression occur in response to tumor promoters, which is an effect of the modulation in signal transduction pathway. For example, TPA enhances the activity of PKC, which is a significant event in carcinogenesis (Blumberg, 1988). By activating PKC, tumor promoters enable the cell to bypass the normal cellular mechanisms for regulating cell proliferation. It is also known that several oncogenes (like ras), hormones, growth factors and cytokines also activate the PKC pathway and lead to the evasion of cell proliferation control mechanisms (Fischer and DiGiovanni, 1995). Tumor promoters can elicit the production of proinflammatory cytokines such as tumor necrosis factor and several interleukins and other inflammatory molecules such as eicosanoids, nitric oxide which are involved in inflammation and carcinogenesis (Fischer and DiGiovanni, 1995). Therefore, antipromotion/antiprogession agents would belong to one or more of the following categories: (1) inducers of apoptosis, (2) inducers of differentiation, (3) inhibitors of angiogenesis, (4) inhibitors of ROS, (5) inducers of differentiation, (6) modulators of signal transduction and (7) inhibitors of arachidonic acid metabolism. It has been identified using two stage carcinogenesis model in mouse skin that several dietary components act through diverse mechanisms to alter tumor promotion and progression. Retinoids particularly, all-trans retinoic acid inhibits tumorigenesis in mouse skin by inhibiting TPA induced promotion (Weeks et al., 1979; Gensler et al., 1986). Data indicates that retinoids reduce elevated polyamine levels by inhibiting the induction of epidermal ODC influencing cell differentiation and
growth (Verma et al., 1979 Pegg, 1988). Synthetic retinoid, fenretinide, exerts antitumor effects by inducing apoptosis in transformed cells (Lotan, 1996). Apart from exercising control at the initiation stage, EGCG appears to induce apoptosis by reducing the expression of apoptotic inhibitors such as bcl-2 and by increasing the expression of pro-apoptotic proteins such as bax and caspase-9 (Masuda et al., 2001). At high doses, phenylisothiocyanate can induce apoptosis in vivo and in vitro (Kong et al., 2000; Bonnesen et al., 2001; Yang et al., 2002).

The induction of terminal differentiation in proliferating cells results in the production of cells that are no longer capable of proliferation but are more susceptible to apoptosis. Therefore, inducers of differentiation such as retinoids, carotenoids have the potential of arresting the growth of a neoplasm at the promotional stage (Leszczyniecka et al., 2001). Experimental evidence has suggested that NF-kB is involved in cancer initiation, promotion and progression. NF-kB is a transcription factor that regulates the expression of genes which contribute to tumorigenesis, such as inflammatory, anti-apoptotic and positive regulators of cell proliferation and angiogenesis (Karin et al., 2002). Curcumin blocks NF-kB signaling, thereby suppressing cell survival and cell proliferation genes like bcl-2, cyclin D1, IL-6, cyclooxygenase-2, matrix metalloproteinase-9 (Aggarwal et al., 2004). Curcumin also enhances apoptosis via activation of caspase 3 and 9 and decreasing bcl-2 expression (Notarbartolo et al., 2005).

Likely as other cells of the body, tumor cells also require access to adequate levels of nutrients and removal of cellular waste material in order to continue surviving and support their growth and proliferation. When solid tumors reach a size of 2-3mm approximately, their further growth gets restricted unless the tumor is able to develop its own blood supply. Tumors also require access to blood vessels (and lymphatic vessels) in order to metastasize (Hasina and Lingen, 2001). Therefore, inhibition of angiogenesis appears to be another important chemopreventive mechanism. Several chemopreventive agents that
exert inhibitory control over carcinogenesis via several other mechanisms also do so by acting as anti-angiogenesis agents. Such agents include certain NSAIDs like aspirin, sulindac, all-trans-retinoic acid, 9-cis-retinoic acid, tamoxifen etc (Sharma et al., 2001). EGCG inhibits endothelial cell proliferation as well as tumor vascularization (Jung and Ellis, 2001).

**Natural Products as Anticancer Agents**

Natural products constitute a rich source of agents that have immense medicinal value. More than half of the currently available drugs are natural compounds or are related to them. Traditional medicines which are based largely on products derived from plants and animals are the primary source of health care in the rural areas of developing countries (Chan, 2003; Kong et al., 2003). In rural areas of India, 75% of the population is dependent on herbal medicines for healthcare. In a study conducted by World Health Organisation (2008), it was concluded that at least 65-80% of the world population relies on traditional forms of medicine (like herbal) for its primary health care needs. There is an ever increasing interest in the pharmacological evaluation of various plant products for chemoprevention and therapy of cancer (Da Rocha et al., 2001; Mann, 2002; Gupta et al., 2004) Over 60% of the anticancer drugs available today (etoposide, teniposide, vinblastine, vincristine etc) are natural in origin (Newmann and Cragg, 2007).

Chemically synthesized and highly purified medicines exhibit pronounced effects because of their enhanced potency. However, when used for a longer duration, they may exert undesirable side effects that may sometimes be lethal. Cancer therapy is most often accompanied by life threatening side effects rendering the treatment process highly disadvantageous to the patient. The current treatment options for cancer using cytotoxic drugs suffer from the disadvantage of being highly toxic to a wide spectrum of tissues such as heart, lungs, bone-marrow, kidney, brain etc (Sporn and Suh, 2000). Adriamycin, a commonly used drug in chemotherapy poses threat to the overall functioning of the body because its use increases the risk of heart failure, liver damage and
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suppresses the hematopoetic functions of bone marrow. Adriamycin (as well as other chemotherapy medications) can increase the risk of developing leukemia later in life (Kristi and Schenstadt, 2007). Thus, arises the need for using agents that act selectively on the tumor cells without causing any detrimental effects to the overall health of the patient. Since, majority of the ill effects surface upon prolonged use of the anticancer agent, therefore development of new dose scheduling regimens that allow their beneficial use over extended time periods could prove very useful.

It must be understood that the processes employed in the manufacture and/or synthesis of pure principal substances sometimes destroy or reduce the original physiological properties of the medicinal agent vis-a-vis when the same are used in their crude or natural state. When used in its entirety, the therapeutic action of the herb or part of the herb is quite different from the medicinal substance or substances isolated thereof. For example, iodine is released relatively slowly from dried seaweed and therefore is less likely to cause rash as sometimes occurs in cases where patients exhibit high sensitivity to iodine when released rapidly when used in the purified state. Extracts have the benefit of being less toxic and being more or equally powerful in combating the disease owing to the presence of myriad number of compounds which when present together act in synergism and enhance the medicinal properties. The therapeutic activity of a medicinal plant does not lie exclusively in one single component or a few components; rather one substance is so dependent on the presence of another substance that the plant or part of the plant when used in its entirety often yields better results than any single component if used in isolation. It is also known that the so called physiologically inert material that constitutes the bulk of the plant material is a lot of times an indispensable factor that contribute significantly to the medicinal value of the plant (Steinmetz, 1961). Tumor cells use multiple cell survival pathways to prevail and thus agents that can suppress multiple pathways have great potential for treatment of cancer.
A research group at Linus Pauling Institute, in 2000, conducted a study on the anticancer potential of ginseng crude extracts and purified ginsenosides (isolated from American ginseng) on a breast cancer cell line. When the cancer cells were exposed to the ginsenosides in a six-day assay, none of the ginsenosides enhanced the growth of the cells, but the crude fraction not only inhibited proliferation of the cancer cells at all doses but actually caused significant tumor cell death when administered at high doses. In a ten-day assay, one ginsenoside enhanced the proliferation of the cancer cells, but all other ginsenosides were either neutral or inhibitory. The crude fraction had the greatest inhibitory effect such that the after 10 days, the number of cancer cells had been reduced by 98 percent compared to the control cell cultures (Sharon and Williams, 2000). When used in its entirety, the plant or part of the plant has the ability to target multiple targets simultaneously, thereby minimizing the likelihood of resistance to treatment.

It is a common epidemiological observation that populations consuming large amount of vegetables, fruits and other plant products in their diet, have a lower risk of cancer. This is believed to be so, because different vitamins and other micronutrients in vegetables, fruits and other natural plant products prevent carcinogenesis by interfering with the actions of mutagens, carcinogens and tumor promoters. These natural inhibitors of carcinogenesis do not cause any harm and are very safe (Koul et al., 2005). Crude herbal preparations may not work like magic bullets but definitely exert beneficial effects with little or no side effects. They also tend to be more effective for long standing health problems that do not respond well to synthetic medicines. Therefore there is a need to reassess the therapeutic potential of herbal preparations supported by scientific methodology, which can lead to their rational use. The identification of such therapeutic targets involving the use of extracts would bolster the ongoing efforts directed in exploiting the beneficial effects of extracts in treatment of diseases without any fear of undesirable side effects.
Treatment of Cancer

Surgery, chemotherapy, radiation therapy, immunotherapy and hormone therapy are the treatment options available for cancer. Cancer treatment is usually done by adopting one or more (combination therapy) of the above mentioned treatment modalities. Depending upon the type of cancer, location, how far it has spread, and other factors like age and health of the patient, the oncologist decides the treatment plan. Often, the treatment of cancer is accompanied by undesirable side effects causing discomfort to the patient. At times, the side effects are extremely harmful and debilitating such that the side effects prove to be more threatening than the disease itself. The current treatment options for cancer using cytotoxic suffer from the disadvantage of being highly toxic to a wide spectrum of tissues such as heart, lungs, bone-marrow, kidney, brain etc (Sporn and Suh, 2000). Nausea, vomiting, diarrhea, fatigue, skin and hair changes, cardiomyopathy, pulmonary toxicity, neuropathy, bleeding, low blood counts etc are some of the many adverse effects which occur following cancer treatment.

Surgery can be very successful in treating some kinds of cancer, but it cannot be used in all cases. For cancers that are localized (e.g.skin cancer) or other cancers that have not metastasized, surgical removal of the tumor may be a good option. However, for aggressive cancers those are not localized and have spread to distant sites in the body, or in cases where tumor cannot be removed without damaging vital organs, such as the liver or brain, surgery may not prove to be beneficial. In cases where cancer takes an aggressive form and the prognosis is not good, palliative surgery may be an option to relieve pain that may be caused by the cancer. One of the major side effects of surgical based therapy is the spread of the disease that follows the removal of the primary tumor (Bertagnolli, 2005). A primary tumor sends chemicals to signal new blood vessels to grow into it, but at the same time also sends a chemical signal that prevents other tumors from growing in other parts of the body. Once the primary tumor is removed, there is nothing to stop other tumors from growing.
elsewhere. That is why some cancer patients often get worse after undergoing tumor removal.

Radiotherapy involves the use of high energy waves (X rays, gamma rays) or particles (α and β particles) emitted from radioactive elements to destroy cancer cells. Radiotherapy is used as an adjuvant therapy because it is used in combination with other treatment modalities. It is used as a curative (where the therapy has survival benefit) and palliative treatment (where cure is not possible and the aim is for local disease control or symptomatic relief). The side effects that accompany radiotherapy include fatigue, skin irritation, temporary change in skin color, fibrosis, dryness and temporary or permanent loss of hair in the area being treated, infertility and lymphedema. The body may be rendered susceptible to infections because of the fall in number of WBCs after radiotherapy (Symonds, 2001; Symonds and Foweraker, 2006). Since radiation itself is a cause of cancer, therefore the risk of developing secondary malignancies also increases after radiotherapy. Secondary malignancies usually occur near the treated area within 20-30 years post radiotherapy although some hematological malignancies may develop within 5 - 10 years. The nature, severity, and longevity of side effects depends on the organs that receive the radiation, the treatment itself (type of radiation, dose, fractionation, concurrent chemotherapy), and the patient.

Hormonal therapy is used for the treatment of cancers derived from hormonally responsive tissues, such as breast, prostate, endometrium and adrenal cortex. It involves the manipulation of the endocrine system through exogenous administration of specific hormones, particularly steroid hormones, or drugs which inhibit the production or activity of such hormones. Certain steroid hormones like estrogen, progesterone regulate the expression of genes involved in cancer formation. Therefore, changing the levels or activity of certain hormones can affect the survival and development of hormone responsive tumors. Like other treatment modalities, hormonal therapy is also not devoid of side effects. Fatigue, fluid retention, weight gain, nausea, vomiting, hot flashes,
change in appetite etc are some of the side effects of hormonal therapy. In pre-menopausal women, bone loss occurs after hormonal therapy (Shapiro, 2004; Yeh, 2007). Tamoxifen, raloxifene, and tibolone used to treat breast cancer significantly reduce invasive breast cancer in midlife and older women, but also increase the risk of adverse side effects (Oncogenetics.org, 2009). Use of tamoxifen increases the risk of developing endometrial cancer (Machado et al., 2005).

Chemotherapy involves the use of chemical substances for the treatment of disease. Anticancer drugs are cytotoxic substances which not only inhibit the proliferation of cancer cells but also kill the cancer cells. Other anticancer drugs interfere with the survival and growth of cancer cells by various other mechanisms such as inhibition of enzymes that promote the growth of cancer cells. Anticancer drugs are used for the treatment of cancers that have spread from their primary site and also are used as adjuncts to other treatment modalities like surgery and radiotherapy for localized cancers. A few examples of anticancer drugs used commonly are cyclophosphamide, methotrexate, 5-fluoro-uracil, vincristine, vinblastine, adriamycin, cisplatin, mitomycin C etc. Some anticancer drugs are adequate in themselves for treatment of certain types of cancer. However, considering the multi-pronged nature and complexity of this disease it is considered that a combination treatment would be more effective since each of the drugs would act differently and yield a synergistic effect. Unfortunately, this treatment modality is also not devoid of side effects. These cytotoxic drugs have deleterious effects on normal healthy cells of the body causing damage to bone marrow, skin, stomach lining, kidney etc. The severity of side effects that accompany the use of cancer chemotherapeutic drugs depend upon the drug being used and its treatment regime. The commonly experienced adverse effects are weakness, loss of appetite, nausea, vomiting, sore mouth, diarrhea and hair loss, low RBC and WBC count causing anemia and depressed immune system. The use of some anticancer drugs can also affect the nervous system and cause problems like
fuzzy thinking and difficulty in concentration (Chabner and Longo, 2001; Symonds and Foweraker, 2006).

**Chemoprevention of Cancer**

It is abundantly clear that treatment of cancer poses enough health risks to the patients disrupting their life in more than one ways and often, the patients run the risk of relapse of the disease even after the treatment is over. Also, the economical burden of the treatment process, at times makes the treatment inaccessible to the economically weaker sections of the society. Unfortunately, cancer patients suffer a lot and lead miserable lives owing to the deadly nature of the disease. Many a times, especially in advanced stages of the disease, pain management and palliative care are the only means of mitigating the suffering.

The International Agency for Research on Cancer (2008) has projected cancer as the leading cause of death in the coming years. Cases of cancer doubled globally between 1975 and 2000, will double again by 2020, and will nearly triple by 2030. There were an estimated 12 million new cancer diagnoses and more than 7 million deaths worldwide this year. The projected numbers for 2030 are 20 to 26 million new diagnoses and 13 to 17 million deaths. Several billions of dollars are spent worldwide for research and treatment of cancer. However, the outcome of this expenditure does not equal the input that is involved. Apart from causing deaths, this disease also affects the countries economically.

Although cancer is a devastating and debilitating disease, it is largely preventable. It is known that about 80% of cancers are caused by environmental causes such as use of tobacco, alcohol consumption, consumption of high fat diet, exposure to carcinogens at work place, exposure to carcinogens in vehicle exhaust, radiation exposure, viral infections etc (Pitot and Dragon, 2001; Abraham et al., 2004). Thus, avoiding exposure to carcinogens and adopting certain life style modifications is a rational approach.
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to prevent cancer. Preventive measures, such as tobacco control, reduction in alcohol consumption, consumption of low fat diet, increased physical activity, vaccinations for hepatitis B and human papilloma virus, and screening and awareness, could have a great impact on reducing the global cancer burden. Primary prevention of cancer attempts to eliminate the disease by protecting the individuals from the causative agents, mainly by abolishing contact with the carcinogen. However, this is not possible, since we are constantly exposed to carcinogens present in our environment and complete isolation is not feasible. Secondary prevention aims at correcting those pathological conditions which have already been produced and if allowed to continue, may progress to cancer.

Chemoprevention by natural or synthetic agents is an effective means of controlling cancer and is fast gaining importance as a preventive strategy (Wattenberg, 1997; Pitot and Dragon, 2001; Surh, 2003). The success obtained in pre-clinical studies involving chemoprevention of cancer provides a strong mandate for adopting this approach for cancer prevention in humans (Pitot and Dragon, 2001; Surh, 2003). Cancer chemoprevention can be described as the prevention, inhibition, or reversal of carcinogenesis by administration of one or more chemical entities, either as individual drugs or as naturally occurring constituents of the diet (Wattenberg, 1992; Wattenberg, 1997). Because of the pharmacological safety of natural chemopreventive agents, they can be used in combination with chemotherapeutic agents to enhance the effect at lower doses and thus minimize chemotherapy induced toxicity.

There is a dire need for discovery of new agents which are safe and effective. Another matter of concern is the development of new dose scheduling paradigms that will allow their beneficial use over extended periods of time (but not constantly) in a manner which is free of undesirable effects.
Azadirachta indica

Azadirachta indica A. Juss (Melia Azadirachta) commonly known as ‘Neem’ is well known in India and the neighbouring countries for more than 2000 years. Neem exhibits a wide range of biological activity and has immense and versatile medicinal value. Its taxonomic position is as follows: Order: Rutales; Suborder: Rutinae, Family: Meliaceae, Subfamily: Meliodeae, Tribe: Melieae, Genus: Azadirachta, Species: Indica

A indica A. Juss and M. azedarach are two closely related species of Meliaceae. The former is popularly known as Indian neem (margosa tree) or Indian lilac and the latter is known as the Persian lilac. The latinized name of neem ‘Azadirachta indica’ (in Persian, Azadi meaning free, diracht meaning tree, indica meaning India) means ‘free tree of India’. It has been named so because it is free from insect and diseases. The neem tree is considered as a ‘sarvaroga nivarini’ (the panacea for all diseases), ‘village dispensary’ and ‘nature’s drugstore’ (Puri, 1999).

Neem is an evergreen large tree that may attain a height upto 20 m. The leaves are alternate and the leaflets contain 8-19 leaves that may appear in March-April (Figure: 7). The leaves are bitter in taste and have a characteristic smell (Puri, 1999). Neem has been extensively used in ayurveda, unani and homeopathic medicine and is now serving as the cynosure of modern medicine as well. For a very long time, neem has been used for the treatment of various human ailments, such that it is still regarded as ‘village dispensary’ in India (Chopra et al., 1956; Chopra et al., 1958).
All parts of the neem tree – leaves, flowers, seeds, roots and bark have medicinal value and have been used as household remedies against various human ailments. Table 1 lists the pharmacological of different parts of neem. However, neem leaf has been used more often than other parts of this plant and the medicinal utilities of neem leaf have been elucidated very well (Akhila and Rani, 1999; Brahmachari, 2004). In traditional medicine, neem leaves have been used extensively because of their easy availability throughout the year and the ease of extracting compounds (Keher and Negi, 1949; Puri, 1999). The neem leaf is a very rich source of organic compounds and contains 0.13% essential oils which is responsible for the characteristic smell of the leaves (Puri, 1999). Table 2 shows the principle constituents of neem leaves.

Chemical investigations on the constituents of the neem tree started in the twentieth century and the first bitter compound ‘Nimbin’ was isolated from neem oil in 1942 by Siddiqui (Siddiqui, 1942). Since then more than 140 chemically diverse and structurally complex active substances have been isolated from various parts of the neem tree. These isolated compounds have
been divided into two major classes: isoprenoids and nonisoprenoids. The isoprenoids include diterpenoids, triterpenoids containing protomeliacins, limonoids, azadirone and its derivates, gedunin and its derivatives vilasinin type of compounds, limonoids and its derivatives, and C-secomeliacins such as nimbin, salanin and azadirachtin. The nonisoprenoids include proteins, polysaccharides, sulphurous compounds, polyphenolics such as flavonoids and their glycosides, dihydrochalone, coumarins, tannins and their aliphatic compounds (Keher and Negi, 1949; Devakumar and Dev, 1993). Some of the phytochemicals present in neem leaf are listed in Table 3.

<table>
<thead>
<tr>
<th>Part</th>
<th>Biological activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>• Antifungal&lt;br&gt;• Antibacterial&lt;br&gt;• Antimalarial&lt;br&gt;• Antifertility&lt;br&gt;• Antipyretic&lt;br&gt;• Analgesic&lt;br&gt;• Antilucericogenic&lt;br&gt;• Antihyperglycaemic&lt;br&gt;• Neuropharmacological&lt;br&gt;• Antidermatophytic&lt;br&gt;• Ovodeal protective&lt;br&gt;• Hepatoprotective&lt;br&gt;• Immunostimulant&lt;br&gt;• Antioxidant&lt;br&gt;• Anticancer</td>
</tr>
<tr>
<td>Bark</td>
<td>• Antibacterial&lt;br&gt;• Antimalarial&lt;br&gt;• Antiflammatory&lt;br&gt;• Antilucer effect&lt;br&gt;• Hepatoprotective&lt;br&gt;• Immunostimulant&lt;br&gt;• Anticancer</td>
</tr>
<tr>
<td>Flower</td>
<td>• Antioxidant&lt;br&gt;• Anticancer</td>
</tr>
<tr>
<td>Seed</td>
<td>• Antimalarial&lt;br&gt;• Antifertility&lt;br&gt;• Antioxidant&lt;br&gt;• Anticancer</td>
</tr>
<tr>
<td>Oil</td>
<td>• Antifungal&lt;br&gt;• Antifertility&lt;br&gt;• Antipyretic&lt;br&gt;• Antihyperglycaemic&lt;br&gt;• Immunostimulant</td>
</tr>
</tbody>
</table>

Table 1: Pharmacological activities of different parts of Neem (Subapriya and Nagini, 2005)
### Content (on dry matter basis)

<table>
<thead>
<tr>
<th>Component</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate*</td>
<td>47.46 - 51.2</td>
</tr>
<tr>
<td>Crude protein*</td>
<td>14.01 - 18.82</td>
</tr>
<tr>
<td>Crude fiber*</td>
<td>11.20 - 23.80</td>
</tr>
<tr>
<td>Fat*</td>
<td>2.31 - 6.93</td>
</tr>
<tr>
<td>Ash</td>
<td>7.73 - 8.52</td>
</tr>
<tr>
<td>Moisture (g/100g)</td>
<td>59.49</td>
</tr>
</tbody>
</table>

#### Amino acids (mg/100g)

- Glutamic acid: 73.3
- Tyrosine: 31.5
- Aspartic acid: 15.5
- Alanine: 6.4
- Proline: 4.0
- Glutamine: 1.0

#### Minerals (mg/100g)

- Calcium: 3.4
- Iron: 510.0
- Phosphorus: 0.13 - 0.24
- Thiamine: 90.0
- Niacin: 17.1
- Vitamin C: 0.04
- Carotene: 1.4

#### Caloric value (Kcal/100g)

- 129.0

* - percent/100g

### Table 2: Principle Constituents of Neem leaf (Keher and Negi, 1949; Devakumar and Dev, 1993)

- 3-Acetyl-7-ugloyl-lactone-vilasinen
- 3-Desacetyl-3-cinnamoyl-azadirachtin
- 3-Desacetyl-salann
- 4α, 6α-dihydroxy-A-homo-azadiradione
- 6-desacylnimbine
- Azadiractam
- Azadiractasin-A
- Beta-sitosterol
- Hypeoside
- Isoazadirholide
- Nimbathione
- Nimbendol
- Nimbene
- Nimbolide
- Quercetin
- Quercitin
- Rutin
- Vilasin

### Table 3: Phytochemical constituents in Neem leaf (Keher and Negi, 1949; Devakumar and Dev, 1993; Biswas et al., 2002)
Pharmacological actions of *Azadirachta indica*

There is extensive literature on the pharmacological activities and medicinal applications of the various parts of neem. These biological activities have been reported with crude extracts, active principles and different fractions from leaf, bark, root, seed and oil. In traditional ayurvedic medicine, crude extracts of different parts of neem have been used (Varma, 1976). The medicinal applications have been very well investigated for leaf, fruit and bark (Thakur et al., 1981). Table 4 lists some medicinal uses of Neem as described in the Ayurvedic texts. Based on modern scientific investigations, several important biological activities of neem have been studied which have relevance to human ailments and their prevention and cure.

<table>
<thead>
<tr>
<th>Part</th>
<th>Medicinal use</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf</td>
<td>Leprosy, eye problem, epistaxis, intestinal worms, anorexia, biliousness, skin ulcers.</td>
</tr>
<tr>
<td>Bark</td>
<td>Analgesic, alternative and curative of fever.</td>
</tr>
<tr>
<td>Flower</td>
<td>Bile suppression, elimination of intestinal worms and phlegm.</td>
</tr>
<tr>
<td>Fruit</td>
<td>Relieves piles, intestinal worms, urinary disorder, epistaxis, phlegm, eye problem, diabetes, wounds and leprosy.</td>
</tr>
<tr>
<td>Twig</td>
<td>Relieves cough, asthma, piles, phantom tumour, intestinal worms, spermatoorrhoea, obstinate urinary disorder, diabetes.</td>
</tr>
<tr>
<td>Gum</td>
<td>Effective against skin diseases like ringworms, scabies, wounds and ulcers.</td>
</tr>
<tr>
<td>Seed pulp</td>
<td>Leprosy and intestinal worms.</td>
</tr>
<tr>
<td>Oil</td>
<td>Leprosy and intestinal worms.</td>
</tr>
<tr>
<td>Root, bark, leaf, flower and fruit together</td>
<td>Blood morbidity, biliary affictions, itching, skin ulcer, burning sensation and leprosy.</td>
</tr>
</tbody>
</table>

Table 4: Some medicinal uses of Neem as mentioned in Ayurveda (Biswas et al., 2002)

*Azadirachta indica as an Anticancer Agent*

There is abundant literature highlighting the anticancer potential of *Azadirachta indica* in several animal models and cell cultures. There are
several reports from our laboratory and others delineating the plausible mechanism of its anticancer action. In studies carried out on murine forestomach tumorigenesis, it was observed that Aqueous *Azadirachta indica* leaf extract (AAILE) exerts chemopreventive action against B(a)P induced forestomach tumorigenesis (Gangar et al., 2006a) as revealed by the decrease in tumor incidence, tumor burden and tumor multiplicity in AAILE + B(a)P group when compared to the animals treated with B(a)P only. Administration of AAILE exhibited preventive effects on B(a)P-DNA adduct formation in murine forestomach and hepatic tissues (Gangar et al., 2006) which could be related to its anti-initiating ability. Further, it was observed that AAILE modulated initiation phase of murine fore-stomach tumorigenesis by altering the activity of carcinogen biotransformation enzymes in liver and forestomach (Gangar and Koul, 2007). Intervention with *Azadirachta indica* during the forestomach tumorigenesis process mediated the induction of apoptosis (Gangar and Koul, 2008b) which was accompanied by an increase in lipid peroxidation (LPO) levels in tumors of animals treated with AAILE and B(a)P.

Studies carried out on skin tumorigenesis have revealed that AAILE administration to tumor bearing mice exhibited a significant reduction in the mean tumor burden and tumor volume in comparison to the animals treated with DMBA only (Koul et al., 2006b). The skin tumors of AAILE + DMBA treated group exhibited enhanced peroxidation levels in comparison to the tumors of DMBA treated group. Another report from Dasgupta et al., (2004) had demonstrated the anticancer action of neem leaf extract in murine skin carcinogenesis model. Administration of aqueous neem leaf extract significantly reduced tumor burden and tumor incidence in both B(a)P-induced forestomach tumors and DMBA-induced skin papillomagenesis. The results of their study provide evidence that neem leaf extract exerts its chemopreventive effect by inducing phase-II enzyme activities associated with carcinogen detoxification, as well as by enhancing the antioxidant status in the liver.

Several researchers have demonstrated the chemopreventive potential of neem leaf extracts on MNNG-induced forestomach tumors and 4-nitroquinoline 1-oxide induced oral carcinogenesis (Manoharan et al., 1996; Arivazhagan et al.,
1999; Arivazhagan et al., 2000; Arivazhagan et al., 2001; Subapriya and Nagini, 2003). Studies indicate that aqueous as well as alcoholic extracts of neem leaf effectively suppressed DMBA-induced HBP carcinogenesis by modulating the cellular redox status as well as carcinogen-metabolizing enzymes in the target organ, as well as in host liver and blood (Balasenthil et al., 1999; Subapriya et al., 2003; Subapriya et al., 2005c). Administration of ethanolic neem leaf extract (ENLE) significantly inhibited the development of HBP carcinomas as revealed by decreased expression of PCNA, mutant p53 and bcl-2 and overexpression of cytokeatin (Subapriya et al., 2006). Inhibition of HBP by ENLE was accompanied by the induction of pro-apoptotic proteins like bim, caspase 3, caspase 8 and inhibition of anti-apoptotic proteins like bcl-2 (Subapriya et al., 2005b). Neem offers protection against diethylnitrosamine and acetyl-aminofluorene-induced hepatocellular carcinoma by boosting the cellular antioxidant defense and detoxification systems (Hanachi et al., 2004). Neem inhibits the synthesis of prostaglandins and other essential metabolites involved in tumor promotion (Okpako, 1977).

Neem leaf contains a number of potent antioxidants and anticarcinogens like flavonoids, limonoids, polyphenols etc that have been documented to retard carcinogenesis at initiation as well as promotion stages of carcinogenesis by virtue of their radical scavenging properties (Makita et al., 1996; Rice-Evans et al., 1996). A significant reduction in the growth of Ehrlich carcinoma and B16 melanoma cells was observed upon treatment with neem leaf extract (Baral and Chattopadhyay, 2004). Nimboide, a limonoid present in various parts of Azadirachta indica exhibited cytotoxic effects on leukemic cell lines (Roy et al., 2007). Azadirone 1, a limonoid constituent of Azadirachta indica has been found to possess cytotoxic activity against breast, melanoma and prostate cancer cell lines (Nanduri et al., 2003). Akudugu et al (2001) have reported the cytotoxicity of azadirachthin A in human glioblastoma cell lines. Nimbroide and 28-deoxonimboide have been identified as cytotoxic constituents of neem leaves (Kigodi et al., 1989).

Quercetin, a bioflavonoid isolated from neem is attracting a lot of attention as an anticancer agent. It has been documented that quercetin retards
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carcinogenesis at initiation, as well as promotion phases of carcinogenesis by virtue of their radical scavenging properties (Balasubramanian and Govindasamy, 1996; Makita et al., 1996; Rice-Evans et al., 1996). The anti-proliferative effects of quercetin have been documented in experimental animal models, humans and a number of malignant cell lines (Castillo et al., 1989; Larocca et al., 1991; Lamson and Brignall, 2000). Quercetin has also been found to enhance the therapeutic efficacy of radiation, as well as chemotherapeutic drugs (Hoffman et al., 1990; van Rijn and van den Berg, 1997). Quercetin causes inhibition in phase I carcinogen metabolising enzymes (Buening et al., 1981), expression of mutant p53 protein, as well as p21-ras oncogene (Avila et al., 1994; Ranelletti et al., 1999) and protein kinase C and/or tyrosine kinase activity (Lamson and Brignall, 2000). Quercetin has also been reported to down regulate signal transduction pathways in human breast carcinoma cells (Singhal et al., 1995) and it also induces type II estrogen receptor (ER II) expression in estrogen receptor-negative human breast cancer cells, thereby inhibiting their growth (Scambia et al., 1993).

Limonin 17β-D-glucopyranoside, a limonoid found in neem, has been shown to inhibit DMBA induced oral carcinogenesis (Miller et al., 1992). Nimboide exhibited anti-proliferative and apoptosis inducing effects on human choriocarcinoma (BeWo) cells. ROS and Bel-2/Bax was involved in mitochondrial mediated apoptosis in BeWo cells in response to nimboide treatment (Kumar et al., 2009). ENLE inhibited the growth of PC-3 cells which was attributable to apoptosis induced cell loss and decrease in cell proliferation (Kumar et al., 2006). Neem leaf preparation (NLP) caused prophylactic growth inhibition of murine Ehrlich carcinoma and B16 melanoma (Baral and Chattopadhyay, 2004). NLP was nontoxic, hematostimulaory and immunostimulatory (Haque et al., 2006). It was observed that when spleen cells from NLP treated mice were co-administered with Ehrlich carcinoma (EC) cells and maintained in vitro, a significant inhibition in tumor growth was noted in comparison to mice co-administered with normal spleen cells and EC cells. It was suggested that NLP activates murine immunocompetent cells to
restrict tumor growth. This was mediated by activation of NK cells and NK-T cells by NLP (Haque and Baral, 2006).

Neem has the potential to prevent DNA damage in cells in response to carcinogenic and mutagenic agents. Koul et al., (2007) have reported that that neem can protect hepatic tissue from DNA damage in response to intraperitoneal administration of DMBA. This was accompanied by the alterations in liver marker enzymes. Aqueous neem leaf extract was found to reduce the incidence of rat bone marrow micronuclei and chromosomal aberrations induced by MNNG (Arivazhagan et al., 2003). Pretreatment with ENLE exerted significant protective effects against the genotoxic effects of the carcinogens MNNG and DMBA in mice and hamsters respectively (Subapriya et al., 2003; Subapriya et al., 2004). This protection offered against mutagens and carcinogens can have positive implications in the chemoprevention of cancer using neem.

**Rationale for Adopting Azadirachta indica as a Chemopreventive Agent**

The use of Neem has been recommended by practitioners of traditional medicine. It is extensively used in Ayurveda, Unani and homeopathic medicine and is becoming the cynosure of modern medicine as well. *Azadirachta indica* contains multiple active compounds that work simultaneously via different mechanisms. This characteristic explains its effectiveness as a pesticide, spermicide, fungicide etc and hence appears to be responsible for its potent impact on several types of cancers as well. It must be known that no other plant or tree in the world has been so extensively researched or used, in all possible capacities so far. Although extracts from various parts of neem have medicinal applications from time immemorial, modern treatment/preventive strategies can be developed after extensive investigation of its bioactivity, mechanism of action, pharmacotherapeutics, toxicity and after proper standardization and clinical trials. Extensive investigation is needed to exploit its therapeutic utility to combat diseases. As the global scenario is now changing towards the use of nontoxic plant products having traditional medicinal use, development of prevention/treatment strategies from neem should be emphasized for the control of various diseases.
In fact, time has come to make good use of centuries-old knowledge on neem through modern approaches of drug development. Over the last two decades, researchers over the world have investigated the anticancer activity of neem based preparations and the mechanisms involved. Even though substantial amount of data exists reiterating the anticancer potential of *Azadirachta indica* but still it cannot be used for human consumption presently. Extensive work needs to be done to determine the most suitable formulation/preparation, dose, route etc of neem administration before it can be used to cure human ailments. Further, toxicity studies need to be done to determine its safety in human consumption. Still, there is a need of carrying out mechanistic studies for *Azadirachta indica*’s chemopreventive action against different carcinogens and the same would be helpful in strengthening the rationale behind use of *Azadirachta indica* leaf preparations.

Seeking, enough confidence from the safety and effectiveness conferred by neem based products against skin diseases and other ailments, it is hoped that studies unraveling the medicinal utilities of plants like *Azadirachta indica* could help in their better economic and therapeutic utilization. *Azadirachta indica* has shown to exert profound benefits against several skin ailments such as acne, psoriasis, eczema, ringworm and even stubborn warts, which fail to respond with classical treatments. It has been observed that when neem leaf preparation is applied locally, it can cure these dermatological conditions within 3-4 days in acute stage or a fortnight in chronic cases. Synthetic chemicals used to treat these conditions can produce side effects such as rashes, allergic reactions and redness. Considering the immense potency and safety of neem based products in treating skin diseases, and reports available from literature that suggest the anticancer activity of *Azadirachta indica* against several forms of cancer including skin cancer. Therefore, this study has been designed with the idea of evaluating the effectiveness of *Azadirachta indica* against murine skin cancer and delineating the mechanism involved.