CHAPTER – I

GENERAL INTRODUCTION
Diet, Nutrition and Cancer: An Overview

Nutrition’s vital role in cancer prevention and treatment

The extent of the published scientific evidence regarding the role of food and nutrition in cancer prevention and treatment is considerable. The initial database compiled for the Bristol Cancer Help Centre (BCHC, 1993) consisted of 5000 records during the previous decade alone! The database compiled for Positive Health numbers some 1000 references since 1993 and the World Cancer Research Fund’s epic tome (WCRF1997) cites more than 3000 references covering:

- Patterns of diet and cancer;
- diet and the cancer process, including the genetic and molecular processes of cancer initiation, promotion, and progression;
- types of scientific evidence published;
- 18 distinct types of cancer and how they are affected by food and nutrition;
- dietary constituents, i.e. carbohydrates, energy factors, fats, proteins, alcohol, vitamins, minerals;
- foods and drinks, i.e. grains, vegetables and fruits, pulses, nuts and seeds, meat, poultry, fish, eggs, milk, coffee, tea, and other drinks;
- food preparation, including contaminants, additives, processes such as curing, salting, and other cooking methods.

Some 15 distinguished international scientists assembled this massive amount of evidence which, in short, concluded the following with regard to cancer prevention:

- That 30%-40% of all cancers, representing the prevention of some 3-4 million cancer cases each year, could be prevented using appropriate diet, physical activity, and maintaining proper body weight;
- That diets with substantial and varied amounts of fruits and vegetables could prevent 20% or greater of all cancer cases;
- That if alcohol consumption were maintained within recommended limits, up to 20% of gastero-digestive tract, colon, rectal, and breast cancer cases could be prevented;
That appropriate diet could prevent most stomach cancers and that colon and rectal cancers are mainly preventable by diet, physical activity, and appropriate body weight.

Given the paltry attention and resources currently expended upon nutritional methods for the prevention and treatment of cancer, and considering the mammoth implications of the above projections, along with its respectable documentation of scientific literature, a great deal needs to change in order to accommodate nutritional methodologies into cancer care.

Diet is estimated to contribute to about one-third of preventable cancers. Maximum health and lifespan require metabolic harmony. It is commonly thought that the problem of how to ensure adequate intake of more than 40 essential micronutrients (vitamins, minerals and other biochemical’s that are required in small amounts) has been solved for most of the world’s population. Inadequate intake of essential vitamins and minerals might explain the epidemiological findings that people who eat only small amounts of fruits and vegetables have an increased risk of developing cancer. For example, the optimum amount of folic acid zinc that is required to minimize DNA damage and maximize a healthy lifespan is likely to be greater than the amount that is needed to prevent acute disease.

Deficiencies in one aspect of the metabolic network can cause repercussions in many systems. Recent experimental evidence indicates that vitamins (Folate, B₆, B₁₂ and C) and minerals (iron and zinc) deficiencies can lead to DNA damage (and cancer), promote neuronal decay (and cognitive dysfunction) or lead to mitochondrial disruption (accelerating ageing). The relationship between diet and cancer has, historically, been thought of in terms of exposure to potential carcinogens, such as alcohol or heterocyclic amines. Dietary deficiencies however, might be a much more important factor in cancer risk. Evidence for a link between various micronutrient deficiencies and DNA damage has been accumulating in recent years (Ames, 2001; Blount, et.al., 1997; MacGregor, 1990; Fraga, et.al., 1991).
The importance of nutrition in cancer development is actively studied and is controversial. Several studies have examined the association between diet and specific cancers, including breast (Willett, 2001) prostate (Meyer and Gillatt, 2002) and colorectal cancer (Kim, 2001). The association between cancer and specific dietary factors, such as meat (Truswell, 2002), fruits, vegetables (World Cancer Research Fund, 1997; Block, 1992; Steinmetz, and Potter, 1991; 1996), and specific nutrients such as vitamin D (Mehta, and Mehta, 2002; Plantz, 2000) and selenium, have been established. Whole grains are a better source of some vitamins or minerals (such as B6 and Mg) than fruits and vegetables (Chatenoud, 1998; Jacobs, et al., 1998). Increased consumption of whole grains has also been associated with a decreased risk of several cancers (Chatenoud, et al., 1998; Jacobs, et al., 1998). Reduced folate intake has been associated with cancer. Folate, B6 and B12 deficiencies cause the interference in incorporation deoxyuracil into DNA, leading to DNA breakage, and could promote tumorigenesis.

The mechanisms of action of dietary micronutrients are complex and are not fully understood. Micronutrients might function as antioxidants, antimitogens, anti-mutagens or in other ways (Patterson, et al., 2001; Steinmetz, and Potter, 1991; Murillo, and Mehta, 2001; Milner, 2002). Impending research by both bench scientists and epidemiologists on the many factors that are involved in the diet – cancer relationship, including gene – environment interactions, should begin to clarify the complex relationship between diet and cancer (Ames, 1999). Though DNA damage is a well-established risk factor for cancer caution, it should be emphasized that cell – division rates and other factors also contribute (Cairns, 1998; Henderson, and Feigelson, 2000; Tomlinson, and Bodmer, 1999).

The relationship of vitamin and mineral deficiencies and cancer is extremely complex. An integrated analysis of the findings from epidemiological, animal model, metabolic and intervention studies, as well as from genetic polymorphism research, is required. Approaches to eliminating micronutrient deficiencies include optimizing vitamins and mineral intake by encouraging supplements, and fortifying foods might therefore prevent cancer and other chronic diseases.
Vitamin D: What is it?

Vitamin D, calciferol, is fat–soluble vitamin, found in food. It is commonly referred to as the “Sunsine” vitamin because it can be made in the skin (body) after exposure to ultraviolet rays from the Sun (DeLuca, and Zierold, 1998; Reichel, et al., 1989). This fat soluble vitamin functions both as a hormone and a vitamin, occurs in two most prominent biologically active forms: ergocalciferol (VD$_2$) (Askew, et.al., 1931) and cholecalciferol (VD$_3$) (Windaus, et.al., 1936) as secosterols, derived from the photolytic cleavage of B rings of cholecalciferol found chiefly in fish liver oils, and ergocalciferol is found in irradiated yeast and plant steroid. Ergocalciferol is the form most commonly added to food and nutritional supplement. Both VD$_2$ and VD$_3$ are converted in the liver to 25-hydroxyvitamin D$_3$ [25 (OH) D$_3$] (Blunt, et.al., 1968) was found to be the major circulating metabolite of vitamin D, and then to 1,25-dihydroxyvitamin D$_3$ [1,25 (OH)$_2$ D$_3$] (Fraser, and Kodicek, 1970; Wasserman, and Fullmer, 1995; Lawson, et.al., 1971) in the kidney (van den Berg, 1997) and is now known to be the most active metabolite of vitamin D.

Vitamin D plays a critical role in regulating the metabolism of calcium and phosphorus (Institute of Medicine, Food and Nutrition Board, 1999) which are necessary for several body functions including normal growth and development of bones and teeth. Clinically, vitamin D is most useful for preventing and treating bone softening diseases like rickets (in children) osteomalacia (in adults) (Goldring, et.al., 1995; Favus, and Kchristakos, 1996) and osteoporosis (Le Boff, et.al., 1999). Vitamin D also necessary for maintaining the integrity of the nervous and musculoskeletal systems (Boland, 1986), normal heart function and normal blood clotting (Wu, et.al., 1995; 1996; Selles, et.al., 1997; Chapuy, et.al., 1992). Several studies have reported the possible therapeutic uses of vitamin D:

Osteoarthritis (MacLaughlin, and Hlick, 1985)
Rheumatoid arthritis (Holick, et al., 1989; Merlino, et al., 2004)
Atherosclerosis (Need, 1998)
Multiple sclerosis (Reid, 1998; Munger, et al., 2004)
Diabetes (Boucher, et al., 1995; Stene, et al., 2000; Hyppönen, et al., 2001)
Psoriasis (Yadhu N. Singh, 2004) and
Mental health (Alzheimer’s disease) (Vieth, et al., 2003)

Since most human cancers develop over long periods of time, it becomes difficult to perform reliable intervention studies on the association between vitamin D and cancer risks. However, there is sufficient evidence to conclude that enhanced sunlight exposure is associated with lower incidence of at least a dozen different cancers, in particular, colon, breast, prostate, ovary, uterus, bladder, esophagus, stomach, and rectal cancer (Guyton, et al., 2001). Laboratory, animal and epidemiological evidence suggest that vitamin D may be protective against some cancers such as colon cancer (Buchner, and Larson, 1987; Sato, et al., 1998) and prostate cancer (Holt, 1999).

Carcinogens and Mutagens in Foods

Naturally occurring food toxicants provide examples of both initiators and promoter, with up to 70 percent of cancer deaths being attributed to dietary factors (Doll, and Peto, 1981). Examples of likely dietary carcinogens as reviewed by the National Research Council (NRC, 1996) are provided in table 1. This list includes carcinogens derived from natural products both by commercial processing (e.g., alcohol) as well as biotransformation in the body (e.g., allylisothiocyanate and nitrosamines); and initiators (e.g., aflatoxins, furcocumars, pyrrolizidine alkaloids) as well as promoters (e.g., phorbol esters, fat, caffeine). In addition, residues of synthetic chemicals can be present in foods subsequent to their accidental contact or intentional use to increase production. An added dimension to diet is the formation of animal and possibly human carcinogens during cooking, including nitrosamines, aromatic hydrocarbons, amino acids pyrolysates, carbolines, imidazoquinolines, quinoxalines, and fat oxidation products including cancer of the liver, stomach, intestines, zymbal, and clitor sal glands, skin, and oral cavity and others (NRC, 1996). Caffeine, in addition to caffeine, is known to yield several animal carcinogens including caffeic acid, catechol, furfural, hydrogen peroxide, and hydroquinone during roasting and/or brewing (Ames and Gold, 1990). Carcinogenic natural pesticides are present in all classes of plant foods including fruits, vegetables, and spices (Ames and Gold, 1990).
Table 1. Naturally occurring carcinogens and potential carcinogens in the diet.

<table>
<thead>
<tr>
<th>Carcinogen / Mutagen</th>
<th>Major Foods Containing the Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Grains and fruits</td>
</tr>
<tr>
<td>Allylisothiocyanate</td>
<td>Cabbage, collard greens, Brussels sprouts, mustard (brown)</td>
</tr>
<tr>
<td>Caffeic acid, Caffeine, and Theobromine</td>
<td>Coffee, cocoa, fruits, and vegetables</td>
</tr>
<tr>
<td>Cyclopropene fatty acids</td>
<td>Cotton seed oil, kapok, and okra</td>
</tr>
<tr>
<td>Fat (unsaturated / cholestrol-cotaining)</td>
<td>Vegetable and animal fats</td>
</tr>
<tr>
<td>Flavonoids (Quercetin, etc.)</td>
<td>Vegetables, tea, coffee</td>
</tr>
<tr>
<td>Furocoumarins (psoralen)</td>
<td>Celery, figs, parsley, parsnips</td>
</tr>
<tr>
<td>Gossypol</td>
<td>Cottonseed oil</td>
</tr>
<tr>
<td>Hormones (estrogen, testosterone, progestins, and related)</td>
<td>Meats as residues, supplements</td>
</tr>
<tr>
<td>Hydrazines (agartine, gyromitrin)</td>
<td>Mushrooms</td>
</tr>
<tr>
<td>d-Limonine</td>
<td>Citrus juices</td>
</tr>
<tr>
<td>Methylazoxymethanol, cycasin</td>
<td>Cycads</td>
</tr>
<tr>
<td>Mycotoxins (aflatoxins, fumonisins, ochratoxin A, sterigmatocystin)</td>
<td>Corn, cottonseed, peanuts, wheat, and other grains</td>
</tr>
<tr>
<td>Nitrosamines</td>
<td>Beets, celery, spinach, meat preserved in nitrite</td>
</tr>
<tr>
<td>Phorbol esters</td>
<td>Croton oil, other Euphorbiaceae (herbal teas)</td>
</tr>
<tr>
<td>Polyphenols ( tannic acid)</td>
<td>Beverages (tea, cider, cocoa, red wine), fruits</td>
</tr>
<tr>
<td>Ptaquiliside</td>
<td>Bracken fern</td>
</tr>
<tr>
<td>Pyrrolizidine alkaloids</td>
<td>Herbs, herbal teas, honey</td>
</tr>
<tr>
<td>Safrole, estragole, methyleugenol, piperine, etc.</td>
<td>Nutmeg, other spices, black pepper</td>
</tr>
</tbody>
</table>

Balancing this bewildering array of toxins and carcinogens, in almost every food item, is another group of chemicals capable of antagonizing these effects. The dietary antimutagens and anticarcinogens, whose mechanisms of action are not always understood, belong to a wide variety of chemical structures. Important antimutagens and anticarcinogens naturally occurring in foods are given in Table-2. Interaction between antimutagens and anticarcinogens are complicated as indicated by the study of indole-3-carbinol, a component of cruciferous vegetables, known to inhibit mammary and forestomach neoplasia in rodents. When given as pretreatment, indole carbinol reduced the carcinogenicity of aflatoxin B1, while exposure resulted in an increase in aflatoxin carcinogenicity (Bailey, et al., 1985).
Table 2. Important antimutagens and anticarcinogens naturally occurring in foods.

<table>
<thead>
<tr>
<th>Class / subclass</th>
<th>Examples</th>
<th>Foods containing them</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Indole-3-carbinol, caffeine</td>
<td>Broccoli, cabbage, cauliflower, coffee.</td>
</tr>
<tr>
<td>Amino acids, arylyptanoids</td>
<td>Cysteine and tryptophan, curcumin</td>
<td>Many plants and animals, turmeric</td>
</tr>
<tr>
<td>Benzinoids</td>
<td>Gingerol, paradol</td>
<td>Ginger root and related plants</td>
</tr>
<tr>
<td>Cyclitols</td>
<td>Myoinositol, phytic acid</td>
<td>Wheat, other cereals, and nuts</td>
</tr>
<tr>
<td>Estrogens</td>
<td>Sitosteryl</td>
<td>Soybeans, alfalfa, etc.</td>
</tr>
<tr>
<td>Fatty acid derivatives</td>
<td>Conjugated linoleic and arachidonic acid</td>
<td>Vegetable oils</td>
</tr>
<tr>
<td>Fiber</td>
<td>Acid-soluble, neutral, etc.</td>
<td>Fruits and vegetables, cereal bran.</td>
</tr>
<tr>
<td>Minerals</td>
<td>Se, Ca++</td>
<td>Crops grown on Se containing soil, milk,</td>
</tr>
<tr>
<td>Phenolics, phenolic acids, phenyl propanoids</td>
<td>Gallic and protocatechuic acids, caffeic, cinnamic, chlorogenic and ferrulic acids, euginol, myristicin</td>
<td>Many fruits and vegetables, broccoli, other vegetables.</td>
</tr>
<tr>
<td>Flavones and isoflavones</td>
<td>Apigenin, myricetin, quercetin, robinetin, rutin, Biochanin A, genistein, daidzein, etc.</td>
<td>Fruits, herbs, and vegetables Soybeans and others</td>
</tr>
<tr>
<td>Polyphenos Lignans, Tannins</td>
<td>Sesamin, Ellagic and tannic acids, epigallocatechin-gallate</td>
<td>Sesame seed Chinese green tea, other teas, Cereals, legumes, and fruits</td>
</tr>
<tr>
<td>Protease inhibitors</td>
<td>Antipain, elastatinal, Chlorophyll, chlorophyllin, cytochrome C, hemin, hemoglobin, myoglobin</td>
<td>Green leafy vegetables, meats</td>
</tr>
<tr>
<td>Porphyrins</td>
<td>Benezyl isothiocyanate, cysteine, diallyl sulfide and disulfide, glutathione, isothiocyanate, phenethyl, sinigrin, sulforaphane</td>
<td>Broccoli, cabbage, cauliflower, garlic and onions.</td>
</tr>
<tr>
<td>Sulfur-containing compounds</td>
<td>Carveol, limonene, menthols</td>
<td>Citrus fruits, grapes, mint, other plants, wine</td>
</tr>
<tr>
<td>Terpenoids Monoterpenes</td>
<td>Cafestol, kahweol</td>
<td>Coffee, variety of plants, sponges, corals, etc.</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>Glycerrhetinic acid, its glycoside, limonin, oleanolic acid, and ursolic acid</td>
<td>Citrus fruits and a variety of medicinal plants</td>
</tr>
<tr>
<td>Triterpenes</td>
<td>Nerolidol</td>
<td>Medicinal plants and herbs</td>
</tr>
<tr>
<td>Sesquiterpenes</td>
<td>Canthaxanthin, α and β-carotene, fucoxanthin</td>
<td>Fresh green, leafy vegetables</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>Vitamins A, C, E and riboflavin</td>
<td>Fruits and vegetables (fresh), meats, fish.</td>
</tr>
</tbody>
</table>
Cancer and Carcinogenesis

Cancer, Terminology, Etiology and Occurrence: The term “cancer” derived from the Latin word “cancerum”. Both cancer and carcinoma appear to have the same origin. Cancer is one of the major public health problems and one of the leading causes of death, next to cardiovascular disease and it can result from exposure to exogenous chemicals (Weisburger, and Williams, 1995). The primary factors involved in the etiologic of cancer, one of the most common causes of death in the world, have not yet been fully elucidated. Accumulating epidemiological evidence suggests that a pronounced predisposition to develop cancer as a consequence of a mutation in a single gene is rare (approximately 1-5%) (Vineis, et.al., 2001). Environmental or nutritional factors probably account for up to 90% of human cancers. These factors include smoking, diet, and exposure to sunlight, chemicals, and drugs. Genetic, viral, and radiation factors may cause the rest.

Carcinogen as a ”substance” that causes a cell or group of normal cells, which would not otherwise have shown this property, to change its biological behavior and demonstrate progressive growth of a malignant character (Faccini, et.al., 1992).

Cancer has largely been viewed as a cellular disease and is marked by an uncontrolled division of cells. Anarchic division produces millions of cells which eventually constitute a tumor, also referred to as the neoplasm (in Greek neoplasm signifies “new growth”). The macroscopic tumor represents a large population of similar cells sharing a common set of genetic aberrations. The cells gradually lose their specialization until finally they spread from the tumor to the bloodstream and into the lymphatic system from where new growths may form at other body sites. The process is then termed “metastasis”.

Hanahan and Weinberg (2000) have argued that in connection with more than 100 distinct subtypes of cancer, malignant transformation required six functional alterations: self-sufficiency with respect to growth signals, insensitivity to growth-inhibitory signals, evasion of programmed cell death (apoptosis), the potential for unlimited replication, sustained angiogenesis, tissue invasion and metastasis. The exact
number of distinct stages involved may vary from tumor to tumor, since some of these acquired characteristics probably interact with other processes. Indeed, the heterogeneity of tumors, both with regards to morphology and pattern of gene expression, may even indicate the participation of many more sequential steps.

Types of Carcinogens

In the early 20th century, the etiology of cancer was thought to be either: 1) viral, 2) chemical or 3) genetic. Although each school of thought often predicted that “their mechanism” would be universal, each of these ideas has been proven to be correct. We now know that each factor can be important to human cancer incidence. The story of a chemical etiology for cancer began with work from the 18th and 19th century Europe where physicians had noted correlations between snuff use and mouth cancer and a high incidence of scrotal cancer in chimney sweeps. The story of the English physician Percival Pott is often recounted as a primary event in our understanding of chemical carcinogenesis. In addition, his observation led to an early scientific focus on the mechanism of action of “coal tar” carcinogens. The early coal tar connection led to the first chemical carcinogenesis experiment by Yamagiwa and Ichikwa who showed that rabbit ears painted with coal tar developed skin tumors. This observation led to the isolation and structural elucidation of the first carcinogens, dibenzanthracene and benzo(a)pyrene by Kennaway and Cook between the 1930s and 1950s (J.NIH Research 4:92-94).

Widely varied chemical structures have exhibited carcinogenic activity in rodents (Gold, and Zeiger, 1996). This reflects the multistep process of oncogenesis (fig.1) and influenced by chemicals in various ways, mainly involving either chemical reactivity in producing neoplastic transformation or neoplastic development. Thus, chemicals can give rise to increases in neoplasms through a variety of mechanisms, which have been broadly characterized as DNA reactive or Epigenetic (Williams, 1992). Genotoxic carcinogens include all direct-acting and primary carcinogens and many procarcinogens or secondary carcinogens. They function as electrophilic reactants and directly alter DNA, thus producing an abnormal cell (initiation). Epigenetic carcinogens are those for
which there is no evidence of genotoxicity. They include most identified drug carcinogens, asbestos and silica, many hormones and immunosuppressants, and cocarcinogens and promoters, which are not carcinogenic per se but potentiate the effects of a carcinogen. Epigenetic carcinogens may act by allowing latent tumor cells to proliferate. The types of chemicals that can be assigned to these categories are given in Table 3 & Table 4. Carcinogens are, of course, both naturally occurring and man-made.

**Fig. 1.** Sequences of oncogenesis.
Table 3: Biological Characteristics for Classification of Genotoxic and Epigenetic Carcinogens

**Genotoxic Carcinogens**
- Mutagenic
- Direct DNA reactivity
- Tumorigenicity is dose response
- Threshold??
- Can be complete carcinogens
- Irreversible
- Usually not strain or species specific
- Functions at initiation and progression stages of cancer process

**Epigenetic Carcinogens**
- Nondirectly DNA reactive
- Nonmutagenic
- Exhibits threshold
- Usually exhibits strain, species and tissue specificity
- Functions at the tumor promotion stage of the cancer process
- Reversible

Table 4: Selected Examples of Genotoxic and Epigenetic Hepatocarcinogens

**Selected Examples of Genotoxic Hepatocarcinogens**
- Nitrosamines (Diethylnitrosamine, Dimethylnitrosamine)
- Polycyclic Aromatic Hydrocarbons
- Mycotoxins (Aflatoxin B1)
- Aromatic amines (2-AAF, 2-Naphtylamine, 4 Aminobiphenyl)
- Nitrosoureas (Ethynitrosourea)

**Selected Examples of Epigenetic Hepatocarcinogens**
- Chlorinated compounds (Carbon tetrachloride, Chloroform)
- Organochlorine pesticides (Dieldrin, DDT, Chlordane)
- Peroxisome proliferators (DEHP, Clofibrate, Nafenopin)
- Other organochlorine compounds (TCDD, PCBs)
- Hormones (Estradiol, Diethylstilbestrol)
- Barbiturates (Phenobarbital, Sodium barbital).

**Overview of genotoxic and non-genotoxic effects of carcinogens**

When cells internalize chemical carcinogens, they are often metabolized, and the resulting metabolic products are either excreted or retained by the cell. Inside the cell, carcinogens or their metabolic products can either directly or indirectly affect the regulation and expression of genes involved in cell-cycle control, DNA repair, cell differentiation or apoptosis. Some carcinogens act by genotoxic mechanisms, such as
forming DNA adduct or inducing chromosome breakage, fusion, deletion, mis-segregation and non-disjunction. For example, carcinogenic ions or compounds of nickel, arsenic and cadmium can induce structural and numerical chromosome aberrations (Hughess, 2002; Beyersman and Hechtenberg, 1997; Kasprzak, et.al., 2003). Others act by non-genotoxic mechanisms such as induction of inflammation, immunosuppression, formation of reactive oxygen species, activation of receptors such as arylhydrocarbon receptor (AhR) or oestrogen receptor (ER), and epigenetic silencing. Together, these genotoxic and non-genotoxic mechanisms can alter signal-transduction pathways that finally result in hypermutability, genomic instability, loss of proliferation control, and resistance to apoptosis – some of the characteristic features of cancer cells fig.2.

**Fig. 2.** Multistage process of cancer. Cancer involves the formation of an altered cell that becomes a mutated initiated cell after a round of DNA synthesis. This initiated cell may clonally grow through either the induction of cell proliferation or the inhibition of apoptosis to a focal lesion. Subsequent additional DNA damage and genetic instability may allow selective focal lesions to progress to the neoplastic stage.

**Chemical Carcinogens – from past to present**

Epidemiological data on geographical and temporal variations in cancer incidence, as well as studies of migrant populations and their descendants that acquire the pattern of cancer risk of their new country, indicate that ‘environmental exposures’ make a substantial contribution to human cancers (Doll and Peto;1981, Kolonel, et.al.,2004).

1915 : Cancer was experimentally produced the first time by application of coal tar to the of rabbits.

1918: The mouse skin tumour bioassay was established.

1921: Induction of metastasizing skin cancer in mice through application of tar.
1922: Induction of skin cancer in mice by arsenic.

1930: First proof that single polycyclic aromatic hydrocarbons (PAHs) are capable of Inducing malignant skin tumours in mice.

1933: Isolation of benzo[a]pyrene (BP) from coal tar and proof of its carcinogenicity in mouse skin.

1935: The tricyclic aromatic hydrocarbon anthracene is hydroxylated in vivo.

1936: Butter Yellow (N,N-dimethyl-4-aminoazobenene) is found to be potent liver carcinogen.

1938: Production of bladder papillomas and carcinomas in dogs by 2-naphthylamine.

1944: Initiating and promoting effects of chemical carcinogens are distinguished.

1947: Two-stage mouse skin chemical carcinogenesis model established.

1948: Microsome-catalysed bio-transformation of N-N-diemethyl-4-aminoazobenene in a call free system.

1956: PAHs can induce metabolizing enzymes in rat liver in vivo.

1960: N-Hydroxylation of aromatic amines amides discovered.

1962: Discovery of cytochrome P450 in liver microsomes.

1964: Correlation between DNA-binding level and carcinogenicity of six PAHs.

1966: Binding of 2-acetylaminc fluorene to rate liver DNA discovered.


1974: Bp binds to DNA through its 7,8-diol-9,10-eboxide.

1976: Stereo selective enzymatic conversion of BP leads to the 7R,8S-diol-9S,10R-eboxide,(+) -ant-BP-diol-eboxide(BPDE).

Arylhydrocarbon hydroxylase is induced by 2,3,7,8-tetrachlorodibenzo-p-dioxin(TCDD) and carcinogenic PAHs through a cytosolic receptor protein.

1978: Binding of BP to DNA in vivo occurs predominantly through (+)-anti-BPDE.

1983: Induction of activating Hras mutations in mouse skin following exposure to PAHs.
1988: The exo-8,9-epoxide is the DNA binding metabolite of aflatoxin B,(REF,106)

1996: Arylhydrocarbon receptor-deficient mice are protected against TCDD-mediated carcinogenicity. DNA binding signature of BPDE in TP53 of lung epithelial cells corresponds to human lung cancer mutational hot spots.

2000: Arylhydrocarbon receptor-deficient mice are protected against PAHs-induced skin tumorigenesis.
Table 5 summarize the selected human chemical carcinogens.

<table>
<thead>
<tr>
<th>Compounds* type</th>
<th>Main sources/uses</th>
<th>Affected organs/cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aminoazo dyes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>o-Aminoazotoluene</td>
<td>Pigments; colouring oils; immunosuppressant</td>
<td>Liver, lung, bladder</td>
</tr>
<tr>
<td>N,N-dimethyl-4- aminoazobenzene</td>
<td>Colour polishes; waxes</td>
<td>Lung, liver</td>
</tr>
<tr>
<td><strong>Anticancer drugs</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melphalan</td>
<td>Chemotherapy</td>
<td>Leukaemia</td>
</tr>
<tr>
<td>Thiopeta</td>
<td>Chemotherapy (no longer in use)</td>
<td>Leukaemia</td>
</tr>
<tr>
<td>Aromatic amines/amides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-Naphthylamine</td>
<td>Dyes; antioxidant</td>
<td>Bladder</td>
</tr>
<tr>
<td>4-Aminobiphenyl</td>
<td>Dyes; antioxidant</td>
<td>Bladder</td>
</tr>
<tr>
<td>2-Acetylaminofluorene</td>
<td>Model compound; tested as a pesticide</td>
<td>Liver; bladder</td>
</tr>
<tr>
<td><strong>Aromatic hydrocarbons</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Benz[a]pyrene</td>
<td>Coal tar; roofing; cigarette smoke</td>
<td>Skin; lung, stomach</td>
</tr>
<tr>
<td>2,3,7,8-Tetrachlorodibenzo-p-dioxin</td>
<td>Tested as pesticide</td>
<td>Lung; lymphoma, liver</td>
</tr>
<tr>
<td>Polychlorinated biphenyls</td>
<td>Flame retardants; hydraulic fluids</td>
<td>Liver; skin</td>
</tr>
<tr>
<td><strong>Metals (and compounds)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arsenic</td>
<td>Natural ores; alloys; pharmaceutical agent</td>
<td>Skin, lung, liver</td>
</tr>
<tr>
<td>Cadmium</td>
<td>Natural ores; pigments; batteries; ceramics</td>
<td>Lung, prostate, kidney</td>
</tr>
<tr>
<td>Nickel</td>
<td>Natural ores; alloys; electrodes; catalysts</td>
<td>Lung, nasal cavity</td>
</tr>
<tr>
<td><strong>Natural carcinogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin-B</td>
<td>A mycotoxin (found in contaminated food)</td>
<td>Liver</td>
</tr>
<tr>
<td>Asbestos (fibrous silicates)</td>
<td>Thermal insulation; gaskets</td>
<td>Lung, mesothelioma</td>
</tr>
<tr>
<td><strong>N-nitroso compounds</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-nitrosodimethyamine</td>
<td>Polymers; batteries; nematocide</td>
<td>Liver, lung, kidney</td>
</tr>
<tr>
<td>4-(Methylnitrosoamino)-1-(3-pyridyl)-1-butane</td>
<td>Research tool; cigarette smoke</td>
<td>Lung, liver</td>
</tr>
<tr>
<td><strong>Olefines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethylene oxide</td>
<td>Glycol and polyester production; Sterilization</td>
<td>Leukaemia, lymphoma</td>
</tr>
<tr>
<td>Vinyl chloride</td>
<td>Plastics; co-polymers</td>
<td>Liver (angiosarcoma)</td>
</tr>
<tr>
<td>Trichloroethylene</td>
<td>Degreasing operations; Adhesives; lubricants</td>
<td>Liver, kidney</td>
</tr>
<tr>
<td><strong>Paraffines/ethers</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2-Dichloroethane</td>
<td>Vinyl chloride production; solvent; degreaser</td>
<td>Liver, lung, breast</td>
</tr>
<tr>
<td>Bis(chloromethyl)ether</td>
<td>Technical applications</td>
<td>Lung</td>
</tr>
<tr>
<td>Mustard gas (sulphur mustard)</td>
<td>Chemical warfare in First World War; research</td>
<td>Lung</td>
</tr>
<tr>
<td>Nitrogen mustard</td>
<td>Limited application as Antineoplastic agent</td>
<td>Lung, skin, lymphoma</td>
</tr>
</tbody>
</table>

*According to the National Toxicology Program 10th Report on Carcinogens, USA 2004, the compounds to be human carcinogens or reasonably anticipated to be human carcinogens.
Mechanisms of Carcinogenesis

Carcinogenesis is currently understood to be a multistage process that has been described as involving initiation, promotion, and progression of normal cells into neoplastic cells. Chemicals can act at one or more of these stages, and can act directly (e.g., Mutagen) or indirectly (e.g., Immune suppression).

Initiation is generally understood to be a permanent and irreversible event involving DNA mutation, and the first step in the process of carcinogenesis. Many genotoxic agents are considered to be initiators, thus having the potential to begin the transition from normal to cancer cells. In addition, studies investigating the number of preneoplastic focal lesions induced by an initiating agent did not find a measurable threshold. Ionizing radiation is an example of an initiating agent. In addition, certain chemicals (e.g., Aflatoxins and diethylnitrosamines, tobacco smoke) are considered to be complete carcinogens, capable of initiation, promotion, and progression. Potential factors modifying the efficiency of initiation include rates of cell division and DNA synthesis as well as the rate of metabolism of a chemical to its active form or rate of metabolic detoxification.

The promotion stage is characterized by clonal expansion of the initial cells. Promoting agents can act by various mechanisms to increase rates of cell proliferation or decrease rates of cell death. For example, cytotoxic agents or mitotic agents can induce cell proliferation. Interference with intercellular communication may also be responsible for clonal expansion of initiated cells. An important feature of this stage is its reversibility and, in some cases, the existence of a threshold for the effect. In many cancer model systems, withdrawal of the promoting agent halts the development of tumors. The promoting stage is also easily modulated by environmental factors including frequency of dosing, age of test animal, and diet. Promoting agents are generally thought to exhibit a threshold (or influention point) in the dose response curve. Examples of promoting agents include hormones, alcohol, and dietary fat.
Progression is an irreversible stage characterized by the development of malignant neoplasms, and is understood to require a second genetic mutation. Agents that act only during progression or advance a cell from promotion to progression have not yet been definitely characterized. It has been hypothesized that malignant neoplasms may all exhibit an abnormal expression of one or more proto- and cellular oncogenes (Pitot, and Dragan, 1991). In this scenario, initiation is defined by the first mutation event and progression as the second mutation, resulting in homozygosity at the anti-oncogene locus, and total loss of growth control (Moolgavkar, 1986). Multistage carcinogenesis mediated by the agents is given in the table 6.

**Table 6: Biological Characteristics of the Stages of Carcinogenesis**

**Initiation**
- Genotoxic event (mutation)
- Irreversible change
- Formation of preneoplastic cell
- Exhibits dose response properties
- Possible "spontaneous formation of initiated cell"
- Cell division necessary to "fix" mutation
- Apparent lack of threshold dose response

**Promotion**
- Nongenotoxic events
- Reversible
- Changes in gene expression
- Selective clonal expansion of preneoplastic cell population
- Clonal expansion dependant on constant exposure to agent
- Exhibits dose response properties
- Threshold apparent

**Progression**
- Irreversible change
- Karyotypic changes and instability
- Genotoxic event
- Demonstrable by formation of neoplastic lesions (adenoma and carcinoma)
- Changes promote preneoplastic cells to neoplastic cells
**Hepatocarcinogenesis**

**What is the liver?**

The liver is the largest solid organ of the body where it constitutes approximately 2.5% of total body weight and is located on the right side of the abdomen just beneath the right diaphragm. The liver controls many important functions in the body (Arias et.al. 1988; Parkinson 2001). It is responsible for:

- Filtering the blood to remove and process toxins
- Synthesizing and excreting bile, which is important in processing fat from our diet
- Regulate blood sugar (glucose) levels
- Producing factors that play an important role in blood clotting
- Lipid synthesis and metabolism
- Storage of vitamins and minerals
- Regulation of excess amino acids and ammonia
- Main organ for detoxification and metabolism of xenobiotics including clinical drugs

In the scientific literature, the functional unit is sometimes referred to as a liver lobule. The lobules are localized around the central vein and can be divided into three regions: the centrlobular (closest to the central vein), midzonal, and peripheral. Enzymes involved in the metabolism of xenobiotics have been found along the lobule. High amounts of the metabolizing enzymes cytochrome P450 (CYP 450) are predominantly located in the centrlobular region. In addition to hepatocytes, the liver is also having several other types of cells, including Kupfer cells, ITO (stellate) cells and endothelial cells. The hepatocytes are the main functional cells of the liver and represent about 300 million cells in the human liver, approximately 78% by volume, which exhibit a high degree of differentiation and slow rates of apoptosis and proliferation. Kupfer cells, ITO (stellate) cells and endothelial cells in liver constitute about 20% by total liver volume and are mainly located in the peripheral region (Oinonen and Lindros 1998; Parkinson 2001).
What is liver cancer?

Normally, cells in the body will grow and divide to replace old or damaged cells. This growth is highly regulated, and once enough cells are produced to replace the old ones, normal cells will stop dividing. Tumours occur when there is an error in this regulation and cells continue to grow uncontrolled. Tumours of the liver occur when there is an error in the regulation of growth of any of the cells in the liver, including the liver cells themselves (hepatocyte), the bile duct cells, or the blood vessels within the liver.

Tumours can either be benign or malignant. Although benign tumours grow uncontrolled, then do not break off and spread beyond where they started and do not invade into surrounding tissues. Malignant tumours, however, will invade and damage other tissues around them. They can also gain the ability to break off from where they started and spread to other parts of the body (metastasise), usually through the bloodstream or through the lymphatic system where the lymph nodes are located. Over time, the cells of a malignant tumour become more abnormal and appear less like normal cells. This change in the appearance of cancer cells is called the tumour grade, and cancer cells are described as being well differentiated, moderately differentiated, poorly differentiated, or undifferentiated. Well-differentiated cells are quite normal appearing and resemble the normal cells from which they originated. Undifferentiated cells are cells that have become so abnormal that, we cannot tell what types of cells they started from.

There are a number of benign liver tumours. Hemangiomas are the most common benign tumour of the liver, and occur when a benign, blood-filled tumour forms within the liver. Other benign tumours include adenomas (benign tumours of the hepatocyte) and focal nodular hyperplasia (a localized growth of several types of liver cells). Although these tumours do not invade surrounding tissues or metastasise, it is often difficult to tell the difference between benign and malignant tumours on radiographic imaging.
In addition to being a common site of metastasis for cancers from other sites in the body, primary liver cancers can arise from within the liver itself. Cancer arising from the hepatocyte is known as hepatocellular carcinoma (HCC). Globally, HCC is one of the most common type of primary liver cancer and accounts for around 70-85% of all liver cancers (Anthony, 2001; Thorgeirsson and Grisham, 2002; Parkin, et al., 2001; Parkin, 2001). Cancers that arise from the bile ducts within the liver are known as cholangiocarcinomas and represent 5-30% of all liver cancers. These cancers can arise from the bile ducts within the liver (known as intrahepatic cholangiocarcinomas) or from in the bile ducts as they lead away from the liver (known as extrahepatic cholangiocarcinomas). Other types of rare cancers can occur within the liver. These include hemangiosarcomas (malignant blood-filled tumours) and hepatoblastoma a rare cancer that develops in very young children, represents approximately 1% of childhood cancers.

**What causes liver cancer?**

Liver cancer is much more common in other areas of the world, particularly in sub-Saharan Africa and Southeast Asia. Worldwide, liver cancer is the fifth most common cancer with a half a million people afflicted each year. The number of people who develop liver cancer is increasing both abroad and in the United States. There are a number of risk factors that are associated with liver cancer. The most common cause of HCC development worldwide is chronic hepatitis B infection. However the increased incidence of HCC is due to chronic hepatitis C, cirrhosis (chronic alcohol use), Tobacco use, smoking, chemicals, and aflatoxin B1 exposure (Anthony, 2001; Wogan, 2000).

**Chemical hepatocarcinogenesis**

Chemical hepatocarcinogenesis is considered to be a multistep process. The first description of various stages in carcinogenesis by chemicals was reported for skin (Boutwell 1964; Siaga, 1981). Later, the liver became a common target organ for studies on chemical carcinogenesis (Farber 1973; Goldfarb 1973). From a toxicological
point of view, the liver is of particular interest, since in connection with lifetime bioassays of putative carcinogens in rodents, the liver is one of the organs most often affected (Maronpot, et.al., 1987) Hepatocytes exhibiting altered morphological and biochemical properties (referred to here as initiated hepatocytes) arise in response to exposure to hepatocarcinogens and the frequency of such initiated hepatocytes can be estimated employing simple immunohistochemical techniques. There is substantial evidence indicating that initiated hepatocytes are the precursors of HCC (Barlet, et.al., 2002; Sell, 2002; Farber, 1991). Experimentally chemical carcinogenesis can be classified into three separate steps termed initiation, promotion and progression (Farber 1984a, 1990).

The initiation step is considered to involve irreversible changes in the genome of the cells (mutational or epigenetic alterations). An initiator is a genotoxic agent possessing the capacity to induce genetic and heritable changes in the cells. The genetic change has to be fixed in the genome in order to remain after proliferation (Farber 1984b). Subsequently, the promotion phase is regarded to be reversible. Promoting agents are usually not genotoxic, but have the ability to select initiated cells to proliferate, whereas normal (not initiated) cell proliferation is inhibited. Initiated cells will not progress further into carcinogenesis unless a promoting agent to stimulate it and vice versa (Schulte-Hermann et al 2000; Pitot and Dragan 2001). Furthermore, some substances can act as both initiator and promoter. These substances are termed complete carcinogens and one example is 2-acetylaminofluorene, 2AAF. Only a few percent of the initiated clonally expanded cell lesions will grow into malignant lesions. During the progression phase, accumulations of mutations may occur. This probably drives the progression further and may eventually result in malignant cells. Many organs show similar progression of stepwise neoplastic alterations as in the liver, e.g. papillomas in skin and colon (Farber 1996; Williams 2001).
**Phenotypes of initiated cells**

The most extensively validated and used model for an limited carcinogenicity bioassay is rat liver (Dragan et al., 1991; Enzmann et al., 1998). This is a consequence of the extensive capability of chemical biotransformation in the liver and the availability of sensitive and reliable markers for preneoplastic lesions. The first altered cell in hepatocarcinogenesis is the “initiated hepatocyte”. Unfortunately, there are no accurate criteria to tag initiated hepatocytes. The most common approach has been to amplify them to form foci or nodules. Although one cannot be certain that every focus or nodule goes on to develop into hepatocellular carcinoma, the available evidence indicates that as a population they are one precursor lesion for hepatocellular carcinoma (Farber and Sarma 1987; Tatematsu et al., 1983). Many biochemical studies have been carried out to characterize the hepatocyte foci, early nodules, late nodules and hepatocellular carcinomas. Altered hepatocellular foci or nodules which show a specific biochemical / enzymatic phenotype (Farber and Sarma 1987) are enlisted in table 7.
Table 7: Biochemical and enzymatic properties that confer growth and survival advantages in the preneoplastic and neoplastic hepatocyte lesions.

**Alterations relatable to the resistance of cytotoxicity and/or mitoinhibition:**

- Decreased uptake
- Increased expression of Pgp
- Decreased Phase I system of microsomal xenobiotic metabolizing components (cytochromes p450, mixed function oxygenases)
- Increased Phase II system
  - (glutathione-S-transferase, γ-glutamyl transferase, UDP-glucuronyl transferase, epoxide hydrolase)
- Increased glutathione
- Decreased lipid peroxidation
- Increased expression of heat shock proteins
- Increased expression of N-acetyl glucosaminyl transferase III

**Alterations relatable to the growth advantage:**

- Increased expression of genes relatable to cell cycle
  - (c-fos, c-myc, c-Ha-ras, HMG CoA reductase, ribonucleotide reductase)
- Low threshold for growth stimuli
- Altered carbohydrate metabolism
  - (increased glucose-6-phosphate dehydrogenase, decreased glucose-6-phosphatase, increased pyruvate kinase-typeM2, etc.)
- Refractory to negative growth regulators

**Alterations relatable to survival advantage:**

- Mutated p53
Liver is the major organ that detoxifies and activates a wide variety of cytotoxic and/or mitoinhibitory xenobiotics. Therefore, it is not unreasonable to visualize that hepatocyte nodules have targeted their alterations in biochemical machinery to protect themselves from the cytotoxic and/or the mitoinhibitory effects of these Xenobiotics. For example, hepatic nodules exhibit: (a) a decreased net uptake of certain tumor promoters (Backway, et.al., 1994; Lea, et.al, 1990); (b) increased expression of P-glycoprotein (Pgp) (Fairchild, et.al., 1987; Thorgerisson, et.al., 1987; Bradley, et.al., 1992) involved in the efflux of certain classes of cytotoxic drugs; (c) decreased phase I microsomal drug metabolizing systems involved in the first step of metabolic activation of hydrophobic Xenobiotics (Cameron, et.al., 1976; Feo, et.al., 1978; Astrom, et.al., 1983; Roomi, et.al., 1985); (d) increased phase II drug metabolizing enzymes including glutathione-S-transferases, gammaglutamyl transferase, UDP-glucuronyl transferase involved in the conjugation and detoxification of xenobiotics oxidized or hydroxylated by phase I systems (Fiala, et.al., 1976; Sato, et.al., 1984; Bock, et.al., 1982); (e) increased cellular glutathione (Deni, and Oesterle, 1980; Ahluwalia, and Farber, 1984) a competing nucleophile which protects DNA from being attacked by the electrophilic derivatives of the xenobiotics and (f) increased levels of pyrimidine nucleoside phosphorylases (Backway, et.al., 1995; Yusuf, et.al., 1996) that degrade pyrimidine nucleosides which have the potential to inhibit DNA synthesis. In addition, hepatic nodules also exhibit increases N-acetylglucosaminyl transferase III (Narasimhan, et.al., 1988; Pascale, et.al., 1989) which has been implicated in resistance (Campbel, and Stanley, 1983). Further, nodules are also resistant to lipid peroxidation (Benedetti, et.al., 1984). This alteration is particularly interesting because oxidative damage is one of the major endogenous damages (Fraga, et.al., 1990) and membrane lipids are especially vulnerable for this type of damage. The resistance to lipid peroxidation may reflect certain alterations in the fatty acid composition of the membrane lipids. The hepatic nodules also exhibit increased expression of heat shock proteins (Carr, et.al., 1986). In addition to the above mentioned biochemical alterations, which can be relatable to survival advantage in an otherwise cytotoxic and mitoinhibitory environment created by xenobiotics as well as by endogenous processes.
Role of Oxidative Stress in Chemical Carcinogenesis

Oxidative stress occurs in a cell or tissue when the concentration of reactive oxygen species (ROS) generated exceeds the antioxidant capability of that cell (Sies, 1991). ROS can be produced both endogenously or exogenously. Endogenous oxidative stress can be the result of normal cellular metabolism and oxidative phosphorylation. The metabolism of substances by the P450 enzyme system generates oxygen free radicals through normal or futile cycling mechanisms (Parke, and Ioannides, 1990). Exogenous sources of ROS can also impact on the overall oxidative status of a cell. Drugs, hormones, and other xenobiotic chemicals can produce ROS by either direct or indirect mechanisms (Halliwell, 1996; Trush, and Kensler, 1991). Alternatively, oxidative stress can also occur when there is a decrease in the antioxidant capacity of a cell. Non enzymatic antioxidant levels (vitamin E, vitamin C, glutathione, etc.) and enzymatic antioxidant levels (superoxide dismutase, glutathione peroxidase, and catalase) in the cell can be decreased through modification in gene expression, decreased in their uptake in the diet, or can be overloaded in ROS production, which creates a net increase in the amount of oxygen free radicals present in the cell (Barber, and Harris, 1994; Vuillaume, 1987). Several human chronic disease states including cancer have been associated with oxidative stress produced through either an increased free radical generation and/or a decreased antioxidant level in the target cells and tissues (Trush, and Kensler, 1991; Rice-Evans, and Burdon, 1993). A role for reactive oxygen radicals in the etiology of cancer is supported by epidemiological studies. Specifically these epidemiological studies illustrated the protective role for antioxidants against cancer development (Ames, 1983; Willett, and MacMahon, 1984) and a correlation between tumor induction and the intake of high concentrations of transition metals such as iron, which facilitate the production of free radicals (Nelson, 1992; Stevens, and Nerishi, 1992).

Oxidative Damage in DNA, Lipid, and Protein

The formation of oxidative stress may result in damage to critical cellular macromolecules including DNA, lipids, and proteins. Oxidative DNA damage may participate in ROS-induced carcinogenesis (Breimer, 1990). A common form of
damage is the formation of hydroxylated bases of DNA, which are considered an important event in chemical carcinogenesis (Breimer, 1990; Chaudhary, et.al., 1994). This adduct formation interferes with normal cell growth by causing genetic mutations and altering normal gene transcription. Several different pathways by which oxidative DNA damage leads to mutations have been proposed, including chemical modification of nucleotide moieties in DNA causing alteration in their hydrogen bonding, exacerbation of polymerase-specific hot spots, conformational change in the DNA templates, and the induction of a DNA polymerase conformation that is error prone (Feig, et.al., 1994). Formation of 8-hydroxy-2'-deoxyguanosine (8-OHdG) [an oxidative modification of DNA produced by hydroxylation in the C-8 position of deoxyguanosine residues by the hydroxyl radical (Floyd, 1990)] has been used as a measurement of oxidative DNA damage.

Cellular fatty acids are readily oxidized by ROS to produce lipid peroxyl radicals and lipid hydro peroxides (Rice-Evans, and Burdon, 1993). Lipid peroxyl radicals can subsequently propagate into malondialdehyde (MDA). The formation of lipid damage (lipid per oxidation) may result in several possible sequelae including protein oxidation (Rice-Evans, and Burdon, 1993). These lipid radicals can diffuse through membranes, thus modifying the structure and function of the membrane and resulting in a loss of cell homeostasis. In addition, lipid peroxides may result in the interaction with cellular DNA and cause the formation of DNA-MDA adducts (Chaudhary, et.al., 1994).

Proteins are also easily attacked by ROS directly or indirectly through lipid per oxidation. Protein radicals can be rapidly transferred to other sites within the protein infrastructure. This can result in further modification of enzyme activity (stimulation or inhibition) (White, et.al., 1976; Bellomo, et.al., 1983). In addition to enzymes, damage to the membrane transport proteins may produce cellular ionic homeostasis and lead to alterations in intercellular calcium and potassium that will trigger a series of changes in cells (Kerr, et.al., 1992). Changes to receptor proteins and gap junction proteins may also modify signal transfer in cells. In selective cases alteration of protein structure may allow the target protein to be further attacked by proteinases (Davies, 1986). Thus protein oxidative damage can result in the modifications in structure, enzyme activity, and signalling pathways.
Other Targets of Oxidative Stress

Activation of transcription factors is an important signalling pathway for the regulation of gene transcription by ROS (Storz, and Polla, 1996). Transcription factors are low-molecular-weight proteins that can bind with the promoter region of a gene. Transcription factors regulate the transcription of genes involved in the development, growth, and aging of cells (Vellanoweth, 1994). The regulation of subcellular localization from cytoplasm to nuclear is first step of transcription factor activity (Whiteside and Goodbourn, 1993). Oxidative stress is believed to be involved in this process. Nuclear factor kappa B and AP-1, by direct oxidation and phosphorylation, are two transcription factors that are modulated by oxidative stress (Schenk, 1994). The AP-1 transcription factor is a dimer of a protein complex joined by c-fos, c-jun, jun-B and jun-D. AP-1 controls genes required for cell growth and compounds that induce cellular proliferation increase its activity. ROS can cause activation of AP-1 as well as new synthesis of AP-1 (Kerr, et.al.,1992). Oxidative stress can also induce the immediate early proto-oncogenes c-fos, jun-B, c-jun, and jun-D, and thus increase AP-1 transcription factor activity. Therefore ROS may play a central role in signal transfer system. High levels of ROS may alter signal pathways by oxidative damage of the cell membrane, changes in enzyme activity, and/or the activation of transcription factors. These alterations may be important links between xenobiotic exposure and tumorigenesis. The effects of ROS in stimulating cell growth have been seen in vitro. ROS regulate genes via protein kinase C (PKC) activation, oxidative damage, and/or ROS direct activation of transcription factors (Feig,et.al., 1994; Storz, and Polla, 1996; Brawn,1995). The mediation of ROS on gene transcription may also inhibit normal cell apoptosis by modulation of myc, bcl-2 and p53 expression and result in an increase in cell number.

Oxidative Stress in the Cancer Process

Chemically induced cancer is a multistage process definable by at least three steps or stages: initiation, promotion, and progression (Fig.2). The tumour promotion stage involves the selective clonal expansion of the initiated cell population through either increased cell division and/or decrease cell death (apoptosis) (Ames, and Gold,1992; Schulte-Hermann,et.al., 1990). The final stage (progression) involves the development
Initiation involves a nonlethal and inheritable mutation in cells by interaction of a chemical with DNA. This mutation confers a growth advantage to that cell. For the mutation to be set a round of DNA synthesis must occur to lock in the mutation. The activation of the carcinogen to an electrophilic DNA-damaging moiety is a necessary step for this stage. ROS are believed to mediate the activation of such carcinogens through hydroperoxide-dependent oxidation that can be mediated by peroxyl radicals (Trush, and Kensler, 1991). This occurs with aflatoxin B₁, aromatic amines, and polycyclic aromatic hydrocarbon dihydrodiols (Trush, and Kensler, 1991). ROS or their by-product of lipid peroxidation, MDA, can also directly react with DNA to form oxidative DNA adducts (Chaudhary, et.al., 1994). The presence of carcinogen-DNA adducts and oxidative DNA adducts generated by chemical carcinogens suggest an interactive role of ROS in initiation. ROS, therefore, can have multiple effects in the initiation stage of carcinogenesis by mediating carcinogen activation, causing DNA damage, and interfering with the repair of the DNA damage (Fig. 3).
Oxidative stress interacts with all three stages of the cancer process. During the initiation stage oxidative DNA damage may produce gene mutations and structural alterations of the DNA, resulting in a heritable mutation. During the promotion stage ROS and oxidative stress can contribute to abnormal gene expression, blockage of cell-to-cell communication, and modification of second messenger systems, resulting in an increase in cell proliferation or a decrease in apoptosis in the initiated cell population. This results in the clonal expansion of the initiated cells to preneoplastic focal lesions. Oxidative stress may also participate in the progression stage of the cancer process by imparting further DNA alterations to the initiated cell population. These changes may result in changes in enzyme activity and make the lesions resistant to normal growth control. Abbreviation: GJIC, gap junctional intercellular communication.
Promotion involves the selective clonal expansion of the initiated cell population through either increased cellular proliferation and/or inhibition of cell death (apoptosis). Pathologically this results in the formation of the preneoplastic lesion (foci from the initiated cell). ROS are specifically generated in initiated cell populations such as preneoplastic foci in liver. Because ROS generation is related to P450 enzyme activity, oxidative stress may have an important role in the clonal expansion of these initiated cells. In fact, higher levels of ROS have been found in neoplastic nodules of rat liver than in the surrounding normal tissue; Phenobarbital treatment enhanced this formation by increasing the mono-oxygenase system in the nodules (Scholz, et.al., 1990). Another suggested source of ROS is from the oxidation of glutathione by glutymyltranspeptidase in preneoplastic foci (Stark, 1991). Extracellular sources of ROS may come from inflammatory cells. The accumulation of neutrophils following topical application of both phorbol and nonphorbol tumour promoters in skin has been reported (Cerutti, and Trump, 1991). These multiple sources of ROS may contribute to a persistent oxidative stress environment that results in pathophysiologic changes and allows for the selective growth of preneoplastic initiated cells.

Tumour progression results in the development of malignant growth from benign lesions. In this stage oxidative stress may play a direct role in the development of cancer characteristics such as uncontrolled growth, genomic instability, chemotherapy resistance, and invasion and metastasis. Tumour cells continually undergo high and persistent oxidative stress, as was shown by the measurement of higher 8-OHdG levels in human carcinoma cells than in surrounding normal cells (Toyokuni, et.al., 1995). This persistent oxidative stress does not appear large enough to induce cell death because tumour cells have a decreased cell sensitivity to oxidative stress (Toyokuni, et.al., 1995; Palozza, et.al., 1994). Cancer cells emerging from the multistep carcinogenic process with inactivated or deleted tumour-suppressor genes and/or activated oncogenes are much less dependent than normal cells on external growth factors because they can manufacture their own factors. High antioxidants induced by persistent oxidative stress in cancer cells increase the chemotherapy resistance of the cells. Increased protein oxidative damage on certain protease inhibitors facilitates tumour invasion (Toyokuni, et.al., 1995).
2-AAF in Hepatocarcinogenesis

2-Acetaminofluorene (2-AAF) is a commonly used carcinogen, is considered to have distinguishable tumour-initiating and tumour-promoting properties that make it as a complete carcinogen in experimental rat liver carcinogenesis. During tumour promotion 2-AAF inhibits the growth of normal hepatocytes, whereas initiated hepatocytes are resistant to mitoinhibitory effect (Tatematsu, et.al., 1988) and expand clonally to form enzyme altered foci and, later, nodules (Solt and Farber, 1976; Farber, 1990). The possible contribution of non-genotoxic stress to the promoting capacity of 2-AAF is not known. Hydroxylation of 2-AAF was consider as a prerequisite for its carcinogenic activity (Solt and Farber, 1976). Moreover it has been reported that metabolic activation of hydroxylated 2-AAF through for example sulphonation is required to form ultimate carcinogens / mutagens in vivo (van Den goorberg et al., 1985; Michejda and Kroeger koepke, 1994). Metabolites of 2-AAF react with multiple components of cells including DNA, and may there by cause cellular stress and adaptive response which can affect cell proliferation and survival. Where as 2-AAF mediated initiation is related to levels of DNA adduct formation, the relation between DNA adducts and chromosomal uncertain (Heflich and Neft, 1994). Continuous exposure to moderate doses of 2-AAF efficiently induce tumour in rat liver, but does not induce over liver toxicity as judged by oxygen radical formation or liver morphology (Neumann et al 1990; Wilson et al., 1941).

Cancer Chemoprevention

Most importantly, knowledge of carcinogenesis has provided new promising opportunities to prevent cancer – that is, to treat precancer or inhibit carcinogenesis (a process often involving 20-30 years in human epithelial cancers) rather than waiting to treat the cancer. Sporn, and Newton (1979) coined the term chemoprevention to describe this discipline in oncology: use drugs, biological, or nutrients that can be applied at any time in the process before invasive disease to inhibit, delay, or reverse
carcinogenesis. Since that time, remarkable progress has been made in developing chemoprevention strategies, started by Sporn, and Newton (1979) and Wattenberg’s (1978;1985) research on mechanisms of chemo preventive drugs and assays for evaluating these drugs in animal models, and Hong’s early clinical studies on prevention of head and neck carcinogenesis (Hong, W.K., et.al., 1986;1990). In the early 1980s, the US National cancer Institute (NCI), recognizing the promise of chemo prevention, established a chemo prevention drug development program that has grown to incorporate and support mechanistic research on potential chemo preventive agents, in vitro and animal efficacy screening, efficacy modeling of human cancer biomarkers as potential surrogate endpoints, preclinical toxicology and pharmacology, clinical safety and pharmacology, and clinical efficacy studies. In the mid-1990s NCI and FDA scientists worked together to develop guidance for developing and obtaining marketing approval for chemo prevention drugs (Kelloff, G.J., et.al., 1995). The chemo preventive agent development program has been complemented by world wide research efforts in screening and early diagnosis, epidemiology of cancer prevention, mechanisms of carcinogenesis, and agent discovery. The 1990s was the first fruits of chemo preventive agent development –FDA approvals for tamoxifen in prevention of breast cancer (Fisher, B., et.al., 1995) and celecoxib in treatment of colorectal precancers (Steinbach, G., et.al., 2000).

Cellular control mechanisms are of great interest for cancer therapy, and molecules on signal transduction pathways that mediate these mechanisms are potentially good targets for cancer drugs. Because many of these molecular targets are over expressed, amplified, or mutated in precancers. Signal transduction pathways are also interest as mechanisms for chemoprevention. The rationale and potential strategies for chemoprevention at some of these targets: EGFR, ODC, ras, raf, cyclic-GMP phosphodiesterase, Hsp90, and molecules involved in cell cycle control. Because signal transduction pathways are also critical to normal cell function, chemoprevention strategies involving these pathways are designed to minimize effects on normal cells. For example, potential chemo prevention agents inhibit targets expressed or depleted only in rapidly proliferating cells or focus on targets at points on the pathways that allow normal cell to function via alternative routs.
Dietary antioxidants (e.g., tea, polyphenols, flavonoids) and modulators of fat metabolism (e.g., 4-3 fatty acids, conjugated linoleic acid), vitamins and their analogs (e.g., carotenoids, vitamin C, folic acid), vitamin antioxidants (e.g., lycopene, vitamin E) and minerals (e.g., calcium and selenium) have demonstrated chemo preventive efficacy in animal and in some cases, clinical and epidemiological cancer settings.

**Cancer chemoprevention of Vitamin D₃**

Vitamin D and its homologues have been investigated as dietary anticarcinogens for decades, particularly as they relate to colon, rectum, prostate, liver and breast cancer (Platz and Giovannucci, 1999). Numerous *in vitro* and *in vivo* studies have shown that vitamin D potentially inhibits cell proliferation in wide range of cell types, including carcinomas of the breast, prostate, colon, skin and brain, myeloid leukemia cells, and others (Guyton, et al., 2003; Johnson, et al., 2002). The active form, 1,25-hydroxy cholecalciferol [1, 25(OH)₂D], binds intracellularly to cytoplasmic vitamin D receptor (VDR). This receptor is found widely in many non-neoplastic peripheral tissues in the body, especially in the intestines, bone, pancreas, breast, prostate, pituitary, gonads, brain, mononuclear cells, Lymph nodes, activated T-lymphocytes, Keratinocytes and skin, suggesting a role for vitamin D in the regulation of normal cellular growth at a local level (Zehhder et al., 2000; Lehmann, et al., 1998).

**Vitamin D – Mediated Signaling**

Vitamin D and its analogues exert their activity through both genomic and non-genomic pathways to inhibit carcinogenesis. The classic genomic response is mediated through the VDR, a member of the steroid hormone super family (Mangelsdorf, et al., 1995). VDR are present in more than 30 tissues, including intestine, kidney, bone, brain stomach, heart, pancreas, skin, activated T and B prostate (Berger et al., 1988; Holick, 2003). The resulting 1, 25(OH)₂D-VDR complex is recognized to interact with nuclear Vitamin D-responsive DNA elements (VDRE) and, thereby, initiates or represses nuclear transcription of various target genes (Giovannucci, 1998) VDR is a ligand-
activated transcription factor that, in combination with the retinoid-X receptor (RXR) and in some cases the retinoid A receptor (RAR), binds to the Vitamin D response element (VDRE) in the promoters of target genes (Kilewer, et al., 1992).

The High-affinity VDR/RXR receptor heterodimer interacts with co activator complexes that link VDR to the RNA polymerase complex and initiate transcription. A number of genes are recognized to contain functional vitamin D response elements. These include several bone-related genes [osteocalcin, osteopentin, bone sialoprotein, the calcium binding proteins calbindin-D28k and D9k, fructose 1.6-bisphosphatase, parathyroid hormone, parathyroid hormone-related protein (Christakos, et al., 2003; Osborne, et al., 2002), human growth hormone (Seoane, et al., 2002), and receptor activator of Nf-κB ligand (RANKL) (Kitazawa, 2002)], as well as the cell cycle regulator p21 (Liu, et al., 1996), the insulin receptor (Maestro, et al., 2003), 25(OH)2D3 24-hydroxylase (Zierold, et al., 1994), GADD45 (Jiang et al., 2003), tumor necrosis factor α (Hakim, and Bar-Shavit, 2003), CYP3A4 (Thompson et al., 2002), urokinase plasminogen activator, protein lipase Cγ (PLCγ), transforming growth factor β2, fibronectin, β3 integrin (Osborne and Hutchinson, 2002), and involucrin (Bikle et al., 2002).

In addition to the classic genomic effects of VDR, vitamin D regulates a number of cytoplasmic signaling pathways through protein kinase C (de Boland, et al., 1994, 1996; Beno, et al., 1995) ras and mitogen-activated protein kinase (MAPK) (Beno, et al., 1995; Gniadecki, 1996; Park, et al., 2000), protein lipases A and prostaglandin’s (Vazquez, et al., 1995; Bellido, et al., 1993), cyclic AMP and protein kinase A (Santillan and Boland, 1998) phosphatidyl inositol-3, the ceramide pathway (Bektas et al., 2000), and ca2+-regulated voltage –sensitive(VSCC) or insensitive(VICC) channels (de Boland and Norman, 1990). Activation of the cytoplasmic signaling pathways often results in rapid changes in intracellular calcium and the activation or deactivation of proteins such as bcl-2 and c-jun. A number of these pathways ultimately affect cellular growth, differentiation, and apoptosis and may cooperate with the classical pathway to transactivate the VDR.
Antineplastic Activity in Preclinical Models

In 1981, Abe et al. were first to demonstrate the potential of VDR ligands to treat cancer. They reported that mouse myeloid leukemia cells possessed VDR and their exposure to vitamin D led to terminal differentiation. Since then, antineoplastic activity of VDR ligands has been demonstrated in both in vitro and/or in vivo models of carcinoma of the bladder (Konety, et.al.,2001) breast (Colston, et.al.,1992), colon (Cross, et.al.,1991) endometrium (Yabushita,et.al.,1996), kidney (Nagakura, et.al.,1986; Fujioka et.al.,1998), lung (Higashimoto, et.al.,1996) pancreas (Zugmaier,et.al.,1996), prostate (Skowronski,et.al.,1993; Peehl, et.al.,1994; Schwartz et.al.,1994; Skowronski, et.al.,1995; Hedlund, et.al.,1996; Zhuang et.al.,1997; Getzenberg, et.al.,1997), sarcomas of the soft tissues (Shabahang, et.al.,1996) and bone (Tokuumi, 1995; Hara et.al.,2001), neuroblastoma (Veenstra, et.al.,1997; Celli, et.al.,1999) glioma (Naveilhan, et.al.,1994), melanoma (Colston, et.al.,1981), squamous cell carcinoma(SCC) (Hershberger, et.al.,1999; McGuire, et.al.,2001) and others.

Inhibition of proliferation and differentiation

Inhibition of proliferation, in some tumor models associated with induction of differentiation, is the most extensively studied mechanism of 1,25(OH)2D3’s antineoplastic activity. 1,25(OH)2D3 induces arrest in the G1 phase of the cell cycle in numerous cell lines(McGuire, et.al.,1996; Liu et.al.,1996; Sheikh, et.al.,1995; Zhuang and Burnstein,1998; Cambell and Koeffler,1997). In several tumor models, including HL-60 leukemia cells and U937 myelomonocytic cells, transcriptional activation of cyclin dependent Kinase(CDK) inhibition P27KIP1 and P21Waf1, respectively, has been implicated as the mechanism responsible for cell cycle arrest in response to vitamin D (McGuire, et.al.,1996; Liu et.al.,1996).

Other mitogenic signals may also be inhibited by vitamin D, including signaling through the mitogenic ERK/mitogen-activated protein kinase pathway (Park, et.al.,2000). 1,25(OH)2D3 may also inhibit proliferation by interfering with growth factor signaling. In some models, 1,25(OH)2D3 decreases the expression of epidermal
growth factor receptors (EGFRs) (Tong, et.al., 1998) induces transforming growth factors β(MD) (Haugen, et.al., 1996; Wu et.al., 1997) and alters components of the insulin like growth factor (IGF) system (Vink-van Wijngaarden, et.al., 1996; Drivdahl, et.al., 1995; Scharla et.al., 1991).

Vitamin D, through the action of VDR, also has been shown to be immunosuppressive by repression of interlackin-2 cytokine gene transcription (Towers, et.al., 1999). VDR binds to the DNA adjacent to the jun-fos gene complex, inactivating jun, which is an activating gene for transcription (Towers, et.al., 1999). This results in the repression of normal cell transcription and may be a mechanism for vitamin D inhibition of cell proliferation.

Another molecular target for vitamin D is the c-myc protooncogene, which induces proliferation and tumor growth. In human Caco-2 colon and HL60 leukemic cancer cells treated with vitamin D, VDR binding is increased and c-myc expression is down regulated which suppresses cell division and induces differentiation (Cross, et.al., 1991; Reitsma, et.al., 1983; Matsumoto, et.al., 1990).

**Apoptosis**

Vitamin D induces apoptosis in several tumor models, including carcinomas of the breast, colon, prostate as well as myeloma, and (β-cell chromic) lymphocytic leukemia (Park et al., 2000; Simboli-Campbell, et al., 1996; Sergeev et al., 1997; Vandewalle et al., 1995; Guzey et al., 2002; Elstner et al., 1996; Modzelewski et al., 1999; Pepper et al., 2003). The anti-apoptotic protein Bcl-2, which is over expressed in many tumors, is down regulated by 1,25(OH)_{2}D_{3} or its analogues in several prostate cancer cell lines (Guzey et al., 2002), as well as in β-cell chronic lymphocytic leukemia cells (Pepper et al., 2003), MCF-7 breast cancer cells and retinoblastoma cells undergoing apoptosis in response to vitamin D treatment (James et al., 1996; Wagner et al., 2003). In invasive breast cancer cells (SUM-159PT cells), the reduction in Bcl-2 protein is accompanied by an increase in the pro-apoptotic protein Bax and release of cytochrome c from the mitochondria followed by PARP cleavage (Flanagan et al., 2003). In Vitro studies of
MCF-7 breast cancer cells, LNCap prostate cells, β-cell chronic lymphocytic leukemia cells indicate that vitamin D-mediated apoptosis in these cell lines is independent of P53 status (Mathiasen et al., 1999; Polek et al., 2003).

In the prostate cancer cell lines LNCaP and ALVA-31, as well as in the MCF-7 breast cancer cells, vitamin D simulates cytochrome c release from mitochondria by a caspase independent mechanism. Other suggested mechanisms for the apoptotic effects of vitamin D include down-regulation of the anti-apoptotic insulin-like growth factor receptor, up-regulation of the pro-apoptotic signaling molecule MEK kinase-1, activation of the sphingomyelin-ceramide-ganglioside GD3 signaling pathway, reduced expression of Akt, a kinase which regulates an important survival pathway, increased activity of the pro-apoptotic agent tumors necrosis factor α, and increased are mobilization of cytosolic calcium.

**Invasiveness and Angiogenesis**

1,25(OH)2D3 may also affect invasion and metastasis. In vitro assays show that 1,25(OH)2D3 is able to inhibit the invasiveness of breast (Hansen et al., 1994), lung (Young et al., 1995), and prostate carcinoma cells (Schwartz et al., 1997; Sung and Feldman, 2000). In vivo inhibition of tumour metastasis has also been demonstrated in several rodent tumor models including prostate cancer (Getzenberg et al., 1997), melanoma (Yudoh et al., 1999), and bladder cancer (Konety et al., 2001). Proposed mechanisms for the anti-invasive effects of 1,25(OH)2D3 include inhibition of serine proteinases (such as components of the plasminogen activator system) and decreases in the activity of metalloproteinases (Schwartz et al., 1997; Koli and Keski-Oja,2000), as well as decreased expression of α6 and β4 integrins (Sung and Feldman, 2000), increased expression of E-cadherin, a tumor suppressor associated with the metastatic potential of cells (Campbell et al., 1997), and inhibition of tenascin-C, an extracellular matrix protein which promotes growth, invasion, and angiogenesis and is up-regulated in many cell types during tumorigenesis (Gonzalez-Sancho et al., 1998). Inhibition of angiogenesis may also contribute to the observed anti-metastatic activity of 1,25(OH)2D3. In vitro, 1,25(OH)2D3 inhibits the proliferation of some tumor-derived
endothelial cells (Bernardi et al., 2002) and inhibits sprouting and elongation of endothelial cells induced by vascular endothelial growth factor (Mantell et al., 2000). 1,25(OH)$_2$D$_3$ also has been shown to inhibit tumor-induced angiogenesis in mice (Mantell et al., 2000; Majewski et al., 1996). Calcitriol exerts potent antineoplastic activity in a broad range of tumor models. Several mechanisms of activity have been proposed. Growth inhibition and accumulation in G$_0$-G$_1$, associated with transcriptional activation of CDK inhibitors p27$^{kip1}$ and/or p21Waf1 has been the most extensively studied mechanism; however, effects on other mitogenic signals, induction of apoptosis, and inhibition of invasiveness and angiogenesis have also been reported (Table 8. Summarize the VitaminD-mediated regulation of genes in different cancers)
Table 8. Vitamin D-mediated regulation of genes in different cancers.

<table>
<thead>
<tr>
<th>Types of cancer</th>
<th>Direction of gene regulations by VD (References)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Down regulation</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>Estrogen receptor (Swami et al., 2000)</td>
</tr>
<tr>
<td></td>
<td><em>c</em>-myc, Cyclin A, and E (Jensen et al., 2001)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Colon cancer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukemia</td>
<td><em>c</em>-myc (Caligo et al., 1996)</td>
</tr>
<tr>
<td></td>
<td>IL-1β (Peleg et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>PA inhibitor-2 (Kole et al., 1991)</td>
</tr>
<tr>
<td></td>
<td>VDR (Song, 1996)</td>
</tr>
<tr>
<td></td>
<td>VLA-4 (Kaneko et al., 1999)</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>Androgen receptor (Zhao et al., 2000)</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The objectives of the present study

The present study is an attempt to understand the cellular and molecular actions of the 1α,25-dihydroxyvitamin D₃ as a chemopreventive agent against 2-acetylaminofluorene induced rat hepatocarcinogenesis. A brief outline of the essential objectives taken into consideration include-

**First**: to deduce the potentiating effect of VD if any inhibiting rat liver preneoplastic transformation in vivo. Since the process of carcinogenesis is a multi-stage and intricate phenomenon, it would be justifiable to differentiate the process primarily in to initiation, long term and promotional phases and to identify out the most effective and pronounced phase[s] at which VD is mostly effective alleviating the processes of rat hepatocarcinogenesis.

**Second**: to identify the basic biochemical mechanism of action that VD could elicit during the coexistence with a strong hepatocarcinogen like 2-AAF in vivo. Major emphasis should be paid on the most important biochemical milieu that happens to alter during the early carcinogenic in the rat liver.

**Third**: to achieve to meaningful correlation between the primary pathology and the overtly aggressive hepatic nodulogenesis and the changed hepatic enzymology during different phases of carcinogenesis.

**Finally**: to make a conclusive confirmation in relation to VD’s potential anticancer ability in inhibiting chemical rat hepatocarcinogenesis, certain strong liver specific immunohistochemical detection of protein expression and early molecular makers that alter at the level of genomic DNA would be done.

The observations embedded in this thesis particularly focuses defined actions of VD₃ in limiting neoplastic transformation against the carcinogenic regimen induced by a potent hepatocarcinogen 2-acetylaminofluorene in liver. The study is a clear indication of the interactions of a dietary micronutrient VD₃ to combat the process of hepatocarcinogenesis and may rank a unique attempt for therapeutics in near future.