Chapter 2

Review of Literature
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It was in the second half of the eighteenth century, reports connecting involvement of chemical agents in the etiology of cancer begins. Knowledge of risks associated with tobacco and nasal cancer, soot and scrotal cancer were the major achievements in the studies of cancer of that time\textsuperscript{116,117}. Important occupational cancers noted in the nineteenth century include development of skin cancer associated with contact of skin with tar and paraffin and bladder cancer among aniline dye workers\textsuperscript{118,119}. After that, many decades elapsed before evidence was available to indicate that polycyclic hydrocarbons\textsuperscript{120}, radioactive substances\textsuperscript{121}, aromatic amines \textsuperscript{122} and nitro compounds\textsuperscript{123} are carcinogens which explained some of the early findings. The epidemiological studies, which revealed the risk associated with the specific environmental exposure and host factors leading to cancer, also provided the basis for instituting preventive measures. These studies support the concept that carcinogenesis is a lengthy multistage process that is affected by wide varieties of factors, in which some are accelerators while others are inhibitors\textsuperscript{124}.

Since carcinogenesis is a multistage process that encompasses a prolonged accumulation of injuries at different biological levels and include both genetic and biochemical changes in the cell\textsuperscript{125}, the idea of chemoprevention arises as an attempt to use natural as well as synthetic compounds to intervene in any of the early precancerous stages of carcinogenesis before the invasive disease begins.

The great interest in chemical carcinogenesis among both scientists and lay person is based in part on the conclusion of epidemiologists, starting with Higginson in 1969, that human cancers have important environmental factors in their etiology\textsuperscript{126}. Due to lack of definite data on the role of infectious virus in the
causation of human cancers, environmental chemicals has been placed on the major factors in the causation of cancer other than skin cancer caused by UV radiation at that time.

It was in 1930, Hieger revealed the fluorescence spectra of a product from tar and of synthetic benz(a)anthracene derivatives are similar\(^{127}\). This observation lead to the demonstration by Kennaway and Hieger, of dibenz(a,h)anthracene as the first synthetic carcinogen\(^{128}\). Soon after a carcinogenic hydrocarbon isolated from coal tar called benzo(a)pyrene was identified by Cook\(^{129}\). Extensive studies by Kennaway, Shear, Fieser and their associates soon lead to a large literature on the chemical factors required for the carcinogenicity of the polycyclic aromatic hydrocarbons\(^{130}\). Of the carcinogenic polycyclic aromatic hydrocarbons studied during this early period, benzo(a)pyrene, 3-methylcholanthrene, dibenz(a,h)anthracene and 7,12 dimethyl benz(a)anthracene have been most widely used in subsequent experimental studies. Carcinogenic activities of new classes of chemicals revealed include 2-acetylaminofluorene, carbon tetrachloride, thiourea, alkalating agents, dialkynitrosamines etc\(^{131}\).

Chemical carcinogens are not chemically reactive per se, but most undergo metabolic activation to form an electrophilic reactant\(^{132}\). These reactive groups can interact with nucleophilic groups in DNA to induce point mutation and other genetic lesions. The importance of metabolic activation in carcinogenesis is highlighted by the fact that target organ specificities and even species susceptibilities can be determined through the presence or absence of metabolic activation pathways\(^{133}\).

The elucidation of specific pathways involved in carcinogen metabolism and the identification of covalently bound adduct have been major accomplishments in the field of chemical carcinogenesis\(^{134}\). Precise delineation of
the metabolic activation of aromatic amines\textsuperscript{13}, nitrosamines\textsuperscript{136}, aflatoxins\textsuperscript{137} and other compounds in animal models comprised a large portion of the chemical carcinogenesis research effort during the past several decades. Many of these compounds are metabolized initially by the complex mixed function oxidase system of microsomal enzymes to a proximate carcinogen\textsuperscript{138}. These enzymes, and subsequent enzymatic pathways which further modify metabolic products of MFO are ultimately responsible for the generation of DNA bound moieties\textsuperscript{139,140}. Additional pathways produce carcinogenic metabolites which may be directly removed from the cells and excreted\textsuperscript{141} or covalently bind to proteins\textsuperscript{142}.

However, it is becoming increasingly apparent that electron oxidation or reduction of procarcinogens yielding a radical intermediate having an odd or spin unpaired electron in its outer orbital may also play critical roles in the activation of carcinogens to DNA damaging species. If this DNA damage is not repaired, cell replication serves to fix the lesions in DNA and completes the process of initiation. It is generally accepted that the stages of initiation and promotion are present during complete carcinogenesis\textsuperscript{143}. But that they may occur by distinct mechanisms. During the initiation stage, carcinogens are metabolically activated to form ultimate carcinogens, which react with DNA at critical target genes such as c-Ha-ras protooncogenes resulting in mutation\textsuperscript{144}. Since initiation stage is irreversible, inhibition of the formation of DNA-carcinogen adducts, should reduce the development of tumours.

The initiated cell as such is a dormant tumour cell in which the potential neoplastic phenotype remains unexpressed in the absence of subsequent exposure to tumour promoters\textsuperscript{145}. Tumour promotion stage characterized by the use of chemical agents, which are not themselves carcinogenic, but which modulate phenotypic expression in both initiated and non-initiated cells. In some
circumstances, tumour promoters may modulate gene expression to result in the proliferation rather than the differentiation of initiated cells. Although tumour promoters typically do not produce damage to DNA and are considered to be epigenetic in action, their effects are often ultimately on the genome. The molecular mechanism by which tumour promoters affect gene expression and cell proliferation are incompletely resolved, but it clearly involves the signal transduction cascades employed by the growth factors and other regulators of cellular homeostasis. One component of the action of tumour promoters on tissues involves the elaboration of free radicals.46

Free radicals are very much involved in the process related to radiation and chemical carcinogenesis.45,147-149 Reactive oxygen species generated chemically or enzymatically are capable of oxidatively modifying DNA both in vitro and in vivo. There is extensive evidence supporting the role of free radical in the promotion process.150,151

The appearance of a tumour affects the metabolism of the host at two levels.52,53 First it produces changes in the metabolism and the hormonal environment of the host as a result of the successful competition of the tumour with the normal tissues for important metabolites, both building blocks and energetic substrates, trophic factors. Second, it influences some host tissues by decreasing differentiation, changing their enzyme characteristics, their sensitivity to hormones and disturbing the negative feedback systems which co-ordinate the activities of central and peripheral endocrine glands.

The metabolic activation of carcinogenesis is highly dependent upon the presence of appropriate activating enzymes. The best characterized enzyme system identified in the bioactivation of precarcinogens is the mixed function oxidase system.154 Within this system, hydroxidation and epoxidation reactions
are exclusively catalysed by one of the many cytochrome P450s found in the endoplasmic reticulum. The Mixed function oxidase system also generates superoxide (O\(^{-}\)), hydrogen peroxide (H\(_2\)O\(_2\)) singlet oxygen (\('O_2\)) and a hydroxyl-like radical which can participate in xenobiotic activation mechanisms\(^{155}\). Besides this, prostaglandin H synthase, a number of tissue specific peroxidases and a cytoplasmic enzyme xanthine oxidase also participate in the metabolic activation of carcinogens\(^{156-158}\). There are reports on the elevation of liver enzymes and antioxidant enzymes and reduced levels of antioxidants during cancer and other liver damage\(^{159}\).

The importance of diet and nutrition in the etiology of many cancers has gained wide acceptance. It has been estimated that as many as 60% of cancers in women and 40% of cancers in men are directly influenced by diet and nutrition\(^{160}\).

Several studies, particularly in 1970s and 1980s, have suggested a protective effect of vitamin A in humans. These studies demonstrated that low intake of dietary vitamin A had higher risk of cancer than with higher intakes\(^{161,162}\).

Several authors reported that vitamin deficient people and rats are prone to more severe infections and have a higher risk of mortality rate than vitamin A sufficient rats\(^{163,164}\). According to Sinha and Rothman well done cooking of meats and fish provide significant exposure to heterocyclic amines, formed from pyrolysis of creatine and amino acids. These compounds are suspected to be colon and breast carcinogens\(^{165,166}\).

As early as 1950 Boyland suggested that the series of metabolic phenols and dihydrodiols which might be secondary products of metabolically formed epoxides might be involved in tumour induction\(^{167}\). Studies by Grover showed that
usually K-region epoxides were more active than the parent hydrocarbons for the transformation of mouse fibroblasts in culture158.

In 1971 Selkirk et al. also showed that formation by liver microsomes of unidentified epoxides from benz(a)anthracene and dibenz(a,h)anthracenes. In the second half of the century the attention has been focussed on the nature of ultimate carcinogenic metabolites of Benz(a)pyrene and on the induction of its nucleic acid bound derivatives159.

The electrophilic ultimate carcinogen can react with a number of nucleophilic sites in DNA, RNA and proteins. The strong electrophilic nature of ultimate carcinogen is consistent with both genetic and epigenetic mechanism of carcinogenesis132. Covalent DNA adducts of many carcinogens have been synthesised in vitro170 and characterised in different tissues of animal species171. Although Wolbach and Howe noted that there were historical resemblance between vitamin A deficient organ and neoplastic tissues, but they were not able to provide any mechanistic evidence of this important analysis172. Most notable was the introduction of organ culture methodology by Lasnitzki, who showed that the premalignant phenotype of mouse prostate glands treated with the carcinogen 3-methylcholantherene could be cultured by retinoids173. In prostate organ culture she showed that retinoid treatment caused disappearance of atypical epithelial cells that had been induced by carcinogen with replacement by cells with more normal morphology.

Most of the experimental evidence regarding chemoprevention of skin cancer by retinoids has been derived from studies utilising mouse skin carcinogenesis models174. Davis was the first to show that papilloma formation in mice could be modified by retinoids175. In 1971 Shamberger showed the natural retinoids to be effective chemopreventive agents for mouse skin when applied
It was shown that the introduction of epidermal ornithine decarboxylase by tumour promoting agents is an important and possibly obligatory step in the process of skin tumour promotion. β-carotene, which is metabolised to retinaldehyde and retinol, effectively inhibit UV induced skin carcinogenesis when given intraperitoneally and in the diet. More over in another experimental model, the dietary administration of retinyl acetate was found to inhibit the induction of rat skin tumours by mono energetic electrons. It has been reported that high dietary retinoic acid is effective in inhibiting papilloma and carcinoma formation.

The vitamin A derived retinoids play an important role in regulating cellular growth and differentiation. In cell culture, retinoids, in particular all-trans retinoic acid are potent inhibitors of proliferation in a variety of normal and malignant cell types and suppress the transformed phenotype. Retinoids have well established inhibitory effects on chemically induced cancers in experimental animals and exhibit different degrees of efficacy in prevention and therapy of some human malignancies.

Vitamin A seems to have an exceptional ability in rat liver microsomes to suppress metabolic activation and modulate DNA-B adduct formation. Firozi suggests that this protective effect towards the mutagenic activation of AFB1 was due to competitive inhibition of retinol with microsomic enzymes. Vitamin A deficiency increase the liver microsomal activity for AFB1 activation.

Retinol mobilisation and delivery are highly regulated processes that are particularly controlled by processes that regulate the rates of Retinol Binding Protein (RBP) synthesis and secretion by the liver. In addition, delivery of retinol to peripheral target tissues may involve specific cell surface receptors that
recognize Retinol Binding Protein. One factor that specifically influence RBP secretion from liver is the nutritional retinol status of the animal. Thus retinol deficiency specifically blocks the secretion of RBP, so that plasma RBP levels falls and liver RBP level rise. RBP metabolism in the liver is also under endocrine control.

Sporn et al. were the first to demonstrate the efficacy of retinoids in the inhibition of bladder carcinogenesis in experimental animals. They found that 13-cis-retinoic acid not only inhibited the incidence but also reduced the severity of bladder neoplasm induced by the intravesical administration of N-methylnitrosourea.

Studies of the inhibition of chemically induced tumorigenesis of the respiratory tract by retinoids have been equivocal and in some cases, contradictory. Saffiotti et al. were the first to describe an inhibitory effect of retinoids on respiratory carcinogenesis. In 1976, Nettesheim et al. found that increased dose of retinyl acetate inhibited development of preneoplastic lung nodules in rats. Some of the most convincing evidence for the chemoprevention of cancer by retinoids comes from chemical carcinogenesis studies of mammary gland. The earliest study to report an inhibition of mammary carcinogenesis was that of Moon et al. who found retinyl acetate reduce the incidence of mammary carcinogenesis. Subsequent studies confirmed the suppressive effect of retinyl acetate in DMBA and MNU induced carcinogenesis.

The nuclear vitamin A content is proportionally correlated with vitamin A dietary intake. It represents 30% of the total cellular fraction under normal conditions then on important physiological role of this vitamin is expected in the nucleus. It has been postulated that retinol can be transferred to the cell nucleus and interact directly with genomic material. Ferrari and Vidali
reported that the binding of retinol to chromatin causes deep structural and metabolic changes\textsuperscript{156}.

Epidemiological study on the relation ship of vitamin A and cancer have not produced entirely consistent results. Interaction of ethanol, retinol and zinc deficiency have been postulated as probable factors responsible for inconsistent results observed with retinol\textsuperscript{197}.

It was in 1989, Bertram’s group discovered that retinoids, stimulate communication between cells through gap junctions, and they were able to correlate enhanced gap junctional communication with decreased growth of transformed cells\textsuperscript{198}. Communication between cells is an important means for growth control tissue homeostasis. Yamasaki distinguishes between two different types of cell-cell communication. One depends on secretion of growth factors and other requires cell-cell contact and takes place across the gap junction between cells\textsuperscript{199}.

Vitamin A deficiency has been associated with a higher incidence of cancer and increased susceptibility to carcinogens\textsuperscript{163}. Vitamin A and related retinoids may increase immunity to tumours by several mechanisms, including enhancement of T cytotoxic lymphocyte activity, NK cell activity, apoptosis. Macrophages isolated from vitamin A supplemented rats had enhanced phagocytosis and tumoricidal activity compared with that of control rats\textsuperscript{200}. Up regulation of HLA-DR expression by all trans-retinoic acid has also been described in human breast cancer cells which also suggests the potential of retinoids, to induce tumour immunity\textsuperscript{201}.

The use of retinoic acid and retinyl esters in cancer prevention is severely limited by their toxicity when administered in pharmacological doses. But modification of the basic vitamin A structure has produced retinoids with
increased target specificity. These compounds maintain antitumorigenic efficacy while toxicity is reduced. Retinyl acetate, retinyl methyl ether, axerophthenic, N-(4-hydroxy phenyl) all-trans-retinamide and N-(4-hydroxy phenyl) 13-cis-retinamide all showed significant inhibitory activity in experimental models for breast cancer.

Temporal relationships of carcinogen and retinoid administration have been explored in other experiments. The treatment with retinyl acetate earlier than carcinogen administration appears to impart a permanent inhibition on tumorigenesis. Retinyl acetate also retards the increased synthesis of DNA in mammary glands during tumour development. It is clear that the retinoid derivatives chosen for use, as cancer preventives must cause minimal toxicity. It is possible that synergistic combination of retinoids with other chemopreventive agents will diminish toxicity and increase therapeutic activity. Combined action of retinyl acetate and selenium is an example for this. Retinoidal cancer prevention of epithelia of digestive tract has been disappointing when compared to the positive results obtained with the skin, breast and urinary bladder cancers.

Chedid et al. reported that hormones such as adrenocorticotropin, cortisol and corticosterone decreased the toxicity of aflatoxin B1. Carcinogenic potency of aflatoxin B1 has been correlated with the extent of covalent binding to DNA. There are reports on the formation of AFB1-DNA adducts which can lead to mutation of protooncogenes and tumour suppressor genes.

Since covalent binding of aflatoxin B1 with hepatic DNA is a critical step in hepatocarcinogenesis, this is depend on various endogenous factors and concurrent exposure to other environmental agents. Age and habits of smoking and drinking alcohol were also found to be associated with a higher percentage
of AFB₁-N²-guanine in total metabolic excretion, indicating an increased activation of aflatoxin B₁.

Allameh suggested that BHT inhibited aflatoxin induced liver tumour formation by the induction of liver glutathione-S-transferase²¹⁰. The aflatoxin B₁ conjugate formation to liver glutathione, catalysed by various hepatic cytosolic glutathione-S-transferase, is one of the detoxification pathway of ultimate carcinogen, aflatoxin B₁ epoxide. Cytosolic glutathione-S-transferase play an important role in the inhibition of aflatoxin B₁ DNA binding in vitro and in vivo²¹¹.

Soni et al. reported that food additives such as turmeric and active ingredient curcumin, asafoetida, garlic, butylated hydroxy anisole, butylated hydroxy toluene and ellagic acid were found to be inhibited the mutagenesis induced by aflatoxin B₁. In their study dietary administration of these additives significantly reduced the γ-glutamyl transpeptidase positive foci induced by aflatoxin B₁,³⁷ which is considered as the precursor of hepatocellular carcinoma.

Several investigators have reported that the adducts formed between aflatoxin B₁ and DNA in human colon are quantitatively similar to adducts formed in the rat liver²¹². The major adduct has been identified as 2,3, dihydro-2 (N-7-guanyl) 3-hydroxy aflatoxin B₁. Adduct formation with DNA induces a positive charge in the imidazole ring of guanine, which possibly alters the tertiary structure of DNA in the vicinity of the adduct. This substitution also weakens the covalent bond between the adduct and the DNA backbone which may facilitate its rapid chemical and/or biochemical removal²¹³. Repair of DNA in human cells after aflatoxin exposure has been reported conversely²¹⁴. Aflatoxin B₁ exposure may affect the rate of removal or repair of DNA.
Lalitha and Selvam reported that the decreased antioxidant enzyme activities of superoxide dismutase, catalase, glutathione peroxidase, glutathione-S-transferase and concentrations of reduced glutathione and ascorbate nearly normalised with a turmeric antioxidant protein isolated from aqueous extract of turmeric in carbon tetrachloride treated liver injured rats. Nishimura et al. observed that chronic alcohol consumption to rats resulted in an enhancement of γ-glutamyl transpeptidase. It has also been reported that treatment with mouse skin with tumour promoters leads to a fall in superoxide dismutase activity. This might tend to increased oxidative damage to DNA in vivo. Indeed mitochondria from several malignant animal tumours, and from tumour cell in culture, are deficient in superoxide dismutase activity when compared to mitochondria from normal tissue.

 Mg Bodile et al. has shown that reduced glutathione (GSH) levels in rats render the animals more sensitive to the toxic effect of aflatoxin B1. Similarly Hoffman and Campbell demonstrated that binding of aflatoxin B1 metabolites to DNA is inversely related to hepatic glutathione levels. The mechanism whether caused by the rate of aflatoxin B1 adducts removal, by DNA repair or by free radical scavenging, is not clear.

 Tumour cell lines are widely used as interesting models for cancer research, because of its usefulness in pre-clinical system for evaluating new or known drugs in the treatment of various cancers. Human acute promyelocytic leukaemia ascites model in SCID mice, because of the presence of a specific PML-RAR alpha fusion gene, is associated with the clinical response to retinoic acid differentiation therapy.

 Kuttan et al. reported that activation of peritoneal macrophages by Viscum album extract could inhibit the tumour growth in mice bearing Ehrlich
ascites tumour and increased the lifespan\textsuperscript{222}. Administration of flavanoid quercetin has shown to be chemopreventive property in Sarcoma 180 induced ascites tumour\textsuperscript{223}.

Toxicity has been the main concern with the retinoid supplementation. Vitamin A has a long biological half-life and accumulates in the liver and fatty tissues. The available data do not permit identification of an upper safe limit of intake. An intake of 10,000 IU/day may be low enough to avoid toxicity and an intake of 25,000 IU/day carries some risk of toxicity\textsuperscript{224}. Several studies have used natural analogues of vitamin A such as retinyl acetate and retinyl palmitate at dose ranging from 100,000 to 300,000 IU per week for periods varying from six months to five years in chemoprevention settings\textsuperscript{225}. No significant toxicity has been reported by these studies. However vitamin in doses ranging from 150,000 to 300,000 IU per day for one to three years was associated with significant toxicity\textsuperscript{226}.

The possible cellular actions of retinoids such as induction of apoptosis and epithelial cell maturation, and limited evidence available from studies of their effects in human cancers indicate that their chemopreventive efficacy should be evaluated further particularly in the upper aero-digestive cancers even though toxicity is a major problem. Natural analogues of vitamin A, such as retinyl acetate and retinyl palmitate have demonstrated as acceptable and favourable toxicity profile and activity comparable to that of synthetic analogues. So they seem to be the candidate agents for further evaluation.