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1.1. Introduction

Cancer is a clonal disorder and is genetically thought to be the results of multiple genetic events. Cancer can largely be conceived as a consequence of genetic catastrophes resulting in genetic events that disturb the physiological function of a normal cell. Cancer cells possess a multitude of gross genomic aberrations reflected as defects in chromosomal number and integrity that would necessarily lead to the direct as well as indirect disruption of several genes. So these disruptions in the structure of genes cause abnormalities in the functions that lead to uncontrolled cellular proliferation and unrestricted growth which characterizes a malignant cell. Thus cancer can be considered as a genetic disease, characterized by the accumulation of multiple somatic mutations in a population of cells undergoing neoplastic transformation. The origin of cancer is multifactorial in nature and cancer cells display an increased rate of mutations, chromosome gain or loss in contrast to normal cells. They are more autonomous than their normal counter parts (Perantoni, 1998; Felsher, 2004; Coleman and Tsongalis, 2006).

The primary classification of cancer is benign and malignant. Benign tumors are slow growing and localized to one position. They are variously classified, some based on their cells of origin, others on microscopic architecture and some on their macroscopic patterns. They generally do not cause death except for some rare exceptions. Generally they cause the host little or no damage. However by virtue of their positions near a vital centre or by the ability to synthesize large amount of biologically active molecules may destroy their hosts. Malignant tumors possess an intrinsic capacity to kill the host. These types of tumors carry several types of mutations and abnormal karyotypes. Cells are generally pleomorphic in size and shape. As compared to their benign counterpart malignant tumors are less differentiated and often associated with large areas of necrosis (Pierce, 1998).

1.2. Hallmarks of cancer cell: Normal cells require mitogenic growth signals for their transition from a quiescent state to an active proliferative state. But tumor cells acquire a self sufficiency in growth signals. Most of the growth signaling pathways are deregulated in tumors
and abnormalities in growth factor signaling is strongly connected with a variety of chronic diseases. Tumors also show an over expression of growth factor receptors. The human epidermal growth factor receptor (c-erbB) is up regulated in stomach, brain and breast cancers (Favoni and Cupis, 2000).

In normal cells there are multiple anti-proliferative signals that operate to maintain cellular quiescence and tissue homeostasis. But tumor cells are insensitive to anti-growth signals. The Rb-E2F pathway which controls the entry of the cells into S phase is often mutated in cancer cells. In some virus mediated cancers the oncoprotein of the virus may bind and inactivate Rb (Doorbar, 2005).

Apoptosis or programmed cell death is the mechanism by which organisms control their cell number and eliminate the unwanted cells. In response to abnormalities like DNA damage, oncogene activation, hypoxia or survival factor insufficiency, apoptosis is initiated. Tumor cells acquire resistance to apoptosis. The tumor suppressor gene p53 is an initiator of apoptosis in response to different stimuli. p53 is mutated in nearly 50% of cancers (Morris, 2002). The antiapoptotic survival pathway mediated by Akt is activated in many tumors (Kaufmann and Hengartner, 2001). This pathway also activates factors like IGF1 and 2 (Pollak et al, 2004).

In normal human cells, telomeres shorten with successive rounds of cell division which define the replicative potential of the cell. The loss of telomeres leads to end-to-end chromosomal fusions, facilitates increased genetic recombination, and triggers cell death through apoptosis. There are several observations which suggest that human cancer cells achieve immortalization in large part through the illegitimate activation of telomerase expression. Telomere maintenance is evident in virtually all types of malignant cells and is the key component of the capability for unlimited replication (Masutomi et al, 2003).

The tumor cells possess an intrinsic ability to promote angiogenesis, the process of formation of new blood vessels. Tumors appear to activate the angiogenic switch controlled by angiogenesis inducers and inhibitors by shifting the equilibrium towards the favor of inducers. VEGF plays an important role in sustained angiogenesis (Carmeliet, 2005).
The malignant tumors also acquire capacity to invade and metastasize into other distant organs through blood flow. Metastasis is a cause of nearly 90% of human cancer death. During metastasis a cell or group of cells leave the primary tumor, enter circulation, extravasate the extracellular matrix and enter to another organ and proliferate into a secondary colony. Collagen is the major component of extracellular matrix and a group of enzymes that degrades collagen termed as matrix metalloproteases play an active role in the process of invasion. The acquired capabilities of cancer cells are given in Figure 1.1.

1.3. Concept of carcinogenesis:

Carcinogenesis is a multistep process in which genetic and epigenetic events determine the transition from a normal to a malignant cellular state. An agent that initiates the process of carcinogenesis can be termed as a carcinogen. Our environment is often described as a “sea of carcinogens” because of the presence of a variety of chemicals with oncogenic viruses and high energy radiation, all of which may contribute significantly to the incidence of cancer.

1.3.1. Types of Carcinogenesis:

The major types of carcinogenesis are (i) Physical; (ii) Chemical; (iii) Hormonal; (iv) Diet and (v) Viral carcinogenesis.

1.3.1.1. Physical carcinogenesis:

The major types of physical carcinogens are radiation, ultraviolet (UV) radiation and asbestos.

Radiation:

Radiation is considered as a universal carcinogen which relates to its specific characteristics that differentiate radiation from chemical carcinogens and other physical carcinogens which are usually tissue specific in their action. Radiation is unaffected by the usual cellular barrier presented to the chemical carcinogens and all the cells in the body are therefore susceptible for radiation induced damages. Epidemiological studies demonstrated that increased risk of breast cancer in women exposed to as little as 1Gy as a result of atomic bomb, therapeutic or diagnostic radiation exposure (Hoff, 2005).
**Mechanism of radiation induced malignancy:** Radiation can induce a broad spectrum of lesions and principle lesions of importance is in DNA which include damage to nucleotide bases, single and double strand breaks in DNA and unreparable double strand breaks. Radiation can induce a wide variety of stable chromosomal aberrations and reciprocal translocations. Amplification and rearrangement of c-myc was found in a small percentage of radiation induced murine sarcomas. Mutations were also found in ras family proteins, p53 and MDM2 (Little, 1997; 2000).

**UV radiation:**

UV radiation forms a part of the electromagnetic spectrum with wavelengths between 200 nm and 400 nm. It is divided into three categories dependent on wavelength, long wave UVA (320–400 nm), medium wave UVB (280–320 nm), and short wave UVC (200–280 nm) (Pattison and Davies, 2006). Epidemiological, clinical and laboratory studies have implicated solar ultraviolet (UV) radiation as a tumor initiator, tumor promoter and complete carcinogen, and excessive exposure of mammalian skin to UV radiation induces a number of biological responses, including development of erythema, edema, sunburn cell formation, hyperplasia, immune suppression, DNA damage, photoaging and melanogenesis. These alterations are directly or indirectly involved in the development of keratinocyte-derived skin cancers and cutaneous malignant melanoma (Melnikova and Ananthaswamy, 2005; Baliga and Katiyar, 2006; Wolnicka-Glubisz and Noonan, 2006).

**Mechanism of UV radiation induced malignancy:** Direct UVB absorption by DNA leads to dimers of nucleic acid bases including cyclobutane pyrimidine species and pyrimidine (6-4) pyrimidone compounds. These classes of dimers are implicated in the mutagenicity of UV radiation, which is typified by a high level of CC-->TT and C-->T transversions (Pattison and Davies, 2006). UV exposure to the skin results in generation of reactive oxygen species. The majority of UV-induced protein damage appears to be mediated by $^1\text{O}_2$, which reacts preferentially with Trp, His, Tyr, Met, Cys and side chains of cystine (Davies, 2003). Excess of free radicals results in a cascade of events mediating progressive deterioration of cellular structure and function,
and this can lead to a loss of cellular integrity by modification of DNA and abnormal expression of cellular genes. Mutations are frequently observed in the ras proto-oncogene and p53 tumor suppressor gene in human skin cancers of sun-exposed area and in UV-induced mouse skin cancers (Nishigori, 2006). UV-generated ROS affect mitogen-activated protein kinase (MAPK) signaling cascades. These events have been shown to activate NF-κB as well as c-Jun N-terminal and p38 MAP kinases followed by activation of transcription factor AP-1 (Einspahr, 2003; Bachelor and Bowden, 2004). Persistent oxidative stress in cancer may also cause activation of transcription factors and protooncogenes such as c-fos and c-jun as well as genetic instability (Nishigori, 2006).

**Asbestos:**

Asbestos is a naturally occurring fiber with industrial and commercial relevance. Lynch and Smith (1935) described a case of lung carcinoma with asbestosis for the first time. The carcinogenic effects of fibers of asbestos in various organs are well established now. The most common type of cancer is mesothelioma but the chances of developing gastrointestinal, kidney and bronchogenic cancer are not rare. Mesothelioma has an unusual molecular pathology with loss of tumour suppressor genes being the predominant pattern of lesions, especially the \( P^{16\text{INK4A}} \), and \( P^{14\text{ARF}} \), and NF2 genes, rather than the more common p53 and Rb tumour suppressor genes (Robinson et al, 2005).

**Mechanism of asbestos induced malignancy:** Asbestos fibers possess both genotoxic as well as cytotoxic properties. Fibers have been shown to induce DNA damage, single and double strand breaks, mutations and chromosomal damage. Experimental data indicated that fibers can cause aneuploidy by impairing mitosis and chromosomal segregation. Fibers also tend to induce inflammatory response which results in the generation of cytokines and the cytokines so produced can facilitate the growth, selection and expansion of initiated cells (Ullrich, 2001). Several reports show that SV40 act as co-carcinogen along with asbestos in the development of mesothelioma (Carbone and Rodan, 2004). SV40 enhances the risk of malignant mesothelioma among people exposed to asbestos (Cristaudo et al, 2005). How exactly SV40 works along with asbestos in the pathogenesis of mesothelioma is not yet fully understood.
SV40, by inhibiting the synthesis of NO, could favor the survival of transformed and potentially neoplastic cells (Aldieri et al, 2004).

1.3.1.2. Chemical carcinogenesis:

It was in 1775 when Percivall Pott, an eminent English physician and surgeon described that soot, an environmental agent, was responsible for tumor induction in Chimney sweepers in London. This was followed by the work of two Japanese pathologists Yamagiwa and Ichikawa who were the first to produce malignant epithelial tumors by application of coal tar to the ears of rabbits. Finally in 1933 Cook et al successfully isolated the substance responsible for the carcinogenicity of coal tar in rabbit skin and the compound was Benzo(a)pyrene [B(a)P], a member of a class of carcinogens called polycyclic aromatic hydrocarbons (PAHs). B(a)P is now recognized as reputed human and rodent carcinogen capable of inducing tumors in a variety of organs and tissues. After the discovery of B(a)P several hundreds of occupational and chemical carcinogens were identified. In spite of their diverse chemical natures, more than 95% of the known carcinogenic chemicals are fall into one of the following three classes. The three classes are

(A) Alkylating agents: Chemicals that transfer alkyl groups (mainly methyl or ethyl) to nucleotides to form adducts in the DNA. Eg. Nitrosamines, aflatoxins.

(B) Aralkylating agents: Chemicals that transfer aromatic or multiringed compounds to a nucleotide to form adducts. Eg. PAHs

(C) Arylhydroxylamines: Chemicals that transfer aromatic amines to nucleotides to form adducts. Eg. Aniline dyes, 2-napthylamines.

Mechanism of chemical carcinogens induced malignancy: Most of the known carcinogens are metabolized/detoxified in the body for better excretion from the living system. More often the final metabolized product act as the carcinogen. The metabolism of carcinogens are mediated by Phase I enzymes of which the Cytochrome P450 (CYP450) class is of utmost interest. CYP450 is a major superfamily of enzymes and are microsomal heme containing monoxygenases located in the endoplasmic reticulum. The final product after detoxification binds with DNA and forms adduct in the DNA. The DNA adduct is potential mutagenic lesion and can cause irregularities in replication and
transcription. As shown in Figure 1.2, B(a)P is metabolized into BP-7,8-dihydriodiol-9,10-epoxide by a series of reaction catalyzed by arylhydrocarbon hydroxylase (AHH) and epoxide hydratase (EH). BP-7, 8-dihydriodiol-9,10-epoxide is a highly reactive species and forms multiple adduct with DNA and cause mutations (Perantoni, 1998). Similarly DMBA is oxidized by CYP 450 enzymes into 3, 4-epoxide, which is hydrolysed by microsomal epoxide hydrolase (mEH) to the proximate carcinogenic metabolite, DMBA-3,4-diol, and finally by CYP P450 to the ultimate carcinogenic metabolite, DMBA-3,4-diol-1,2-epoxide, capable of producing DNA adducts (Sugiyama et al, 2002) (Figure 1.3). Some other known examples are the activation of aflatoxin by CYP450 enzymes (CYP1A2 and CYP3A4) to a reactive intermediate (Aflatoxin-B1-8,9-epoxide) which further act on DNA (mainly with the guanine base) and forms an adduct namely 8,9-dihydro-8-(N7-guanyl)-9-hydroxyaflatoxin B1 (Essigmann et al,1982).

1.3.1.4. Hormonal carcinogenesis:

Hormone-related cancers namely breast, endometrium, ovary, prostate, testis, thyroid and osteosarcoma share a unique mechanism of carcinogenesis. Endogenous and exogenous hormones can trigger cell proliferation and thus there is an opportunity for the accumulation of genetic errors. Available experimental, clinical and epidemiological evidences strongly suggest a causative role of hormones in human cancer (Henderson et al, 1982). No specific initiator is required for hormonal carcinogenesis. Mechanism of hormones induced malignancy: Estrogens play role in the etiology of breast cancer. Animal studies proved that estrogens promote mammary tumors in rodents. The major established factors for the cause of breast cancer is due to the over exposure of hormones during early menarche, late menopause, postmenopausal obesity and hormone replacement therapy. Estrogens have been suspected as etiologic factors of ovarian cancer. Ovarian tissue estrogen levels are at least 100-fold higher than circulating levels and those in the follicular fluid of ovulatory follicles are even higher (Lindgren et al, 2002). The role of other hormones other than estrogen is less clear. The role of elevated levels of
progesterone in breast cancer is still controversial (Henderson et al, 1997; Henderson and Feigelson, 2000).

1.3.1.5. Diet and Cancer:

Diet may play a substantial role in the development of many cancers and it has been estimated that ~35% of all cancers are due to dietary factors. It is now estimated that an average of 35% of human cancer mortality is attributable to the dietary habits (Doll and Pro, 1981). A wide variety of substances derived from the diet have been found to stimulate the growth and development of tumors in experimental animals and they also possess the capacity to transform normal cells to malignant cells. The recent dose response meta-analysis of epidemiological studies by Norat et al (2002) suggests that red meat and processed meat intakes are associated with increased risks of colorectal cancer. Salt-preserved foods and dietary nitrite found in preserved meats are potentially carcinogenic. Intake of salted food may increase the risk of Helicobacter pylori infection and act synergistically to promote the development of gastric cancer.

One of the best examples of a genotoxic component is heterocyclic amines (HCAs) which are produced as a result of cooking proteinaseous food like meat and fish. Since HCAs are naturally occurring, complete avoidance of exposure is not practically possible (Nagao et al, 1977). HCAs are shown to induce tumors of breast, colon, skin, ear ducts, liver and prostate which are increasing in developed countries (Sugimura, 2000). Epidemiological studies draw a positive correlation between cancer incidence and consumption of heavily cooked food. HCAs have been assigned to the group 2 category in the classification of the human carcinogens by IARC. Other examples of micro components are mycotoxins, pyrrolizidine alkaloids from plants and flavonoids and related compounds from plants (Sugimura, 2000).

Mechanism of malignancy: HCAs are converted to their hydroxyamino derivatives by cytochrome P450s especially by CYP1A2 subtype and the process is further activated by esterification by acetyltransferase and sulfotransferase. The reactive forms produce adduct with guanine at C8 position resulting in change in DNA sequence by base substitution, deletion and insertion (Sugimura, 1997). HCAs also produce genomic

There is a positive correlation between excess of fat intake and development of breast and prostate cancer and colon carcinogenesis (Fisher, 2000) and sodium chloride act as a promoter for gastric carcinogenesis (Hirayama, 1984). Excess of caloric intake results in fat deposits. Digestion, absorption, metabolism and excretion of excess of nutrients require metabolism and that can produce more reactive oxygen species which may lead to oxidative DNA damage. The ω6 PUFA such as arachidonic acid act as a substrate for COX enzyme which results in the production of various prostanoids and that are seems to be involved in colon carcinogenesis (Ulrich et al, 2006). Sodium chloride has been act as promoter for MNNG induced gastric carcinoma in rats. High salt concentration leads the disruption of mucin layer of gastric epithelium and prolonged damage results in atrophic gastritis and intestinal metaplasia, both of them are now considered as precursor lesions for gastric cancer (Takahashi et al, 1994). Humans are exposed to MNNG-like carcinogenic N-nitroso compounds through luminal nitrosylation of guanidine compounds, which are naturally produced by dietary nitrite in the presence of acid in the stomach (Bartsch et al, 1992).

1.4. Cellular and molecular mechanisms of multistage carcinogenesis:

The process of carcinogenesis is divided into 4 steps; initiation, promotion, progression and malignant conversion.

1.4.1. Initiation:

This process occurs intracellularly by either spontaneously or by the action of chemical, physical or biological agent that alters the structure of genome resulting in a cell having the potential of developing into a clone of neoplastic cells. Generally the changes are irreversible but all the initiated cells may not be converted into a cancerous cell. Initiation involves an alteration in the signal transduction pathways that regulate cellular responses to extracellular signals. Initiation generally occurs when the balance between the activation of proto-oncogenes and
inactivation of tumor suppressor genes are getting disturbed. Molecular analysis during initiation revealed that point mutations occur during initiation.

The major biological nature of initiated cells are (a) increased lifespan; (b) increased proliferation capabilities; (c) resistance towards apoptotic stimuli; (d) resistant to inhibition of cellular proliferation; (e) altered dependence of growth factors and hormones and (f) alterations in the control of cell proliferation and differentiation.

1.4.2. Promotion:

It is the clonal expansion of initiated cells and agents that can cause promotion are known as promoters. During promotion stage reversible expansion of initiated cell population and alterations in the gene expression profiles are occurred. Promotion stage can be affected by various factors which include diet, age, hormonal balance and sex. Agents which can cause both initiation and promotion are termed as complete carcinogen. Reactive oxygen species (ROSs) play an important role in the process of progression. ROSs can directly produce single- or double-stranded DNA breaks, deoxyribose modifications of the purines or pyrimidines and DNA cross-links. Persistent DNA damage can result in either arrest or induction of transcription, induction of signal transduction pathways, replication errors, and genomic instability, all of which are seen in carcinogenesis. 12-O-tetradecanoylphorbol-13-acetate (TPA) acts as a promoter for skin carcinogenesis initiated by DMBA in rodent skin and phenobarbitol act as a promoter for hepatocellular carcinoma in rodents. The major characteristics of promotion stage are (a) the clonal expansion of initiated cell population; (b) alteration in the genetic expression profile and stimulation for cellular proliferation; (c) activation of cellular receptors; (d) mutation and clastogenicity; (e) activation of protein kinase C and (f) inhibition of apoptosis.

1.4.3. Progression:

Progression can be defined as that stage of carcinogenesis exhibiting measurable and/or morphological karyotypic changes on the structure of the genome. It is an irreversible process. Cells at this stage shows considerable heterogeneity and become aneuploid. Genome is highly unstable causing chromosomal alterations with increasing
frequency during progression. The major characteristics of progression stage are (a) irreversible alteration in the genome; (b) increased growth rate; (c) gene amplification; (d) tendency for invasion and metastasis and (e) several types of karyotypic abnormalities and instability.

1.4.4. Malignant conversion:

The clonal populations in the progression stage acquire more mutations as a result of additional exposure of carcinogens resulting in genomic instability and errors in replication. As a result of genetic alteration cells acquire tumorigenecity and loss of regulation of growth and ability to invade and metastasize into other distant organs. The major characters of malignant conversion stage are (a) unregulated growth; (b) capacity for local invasion and metastasis; (c) resistance towards apoptosis and (d) angiogenic tendency.

1.5. Role of oncogenes and tumor suppressor genes:

Proto-oncogenes and suppressor genes are normal counterparts of the body. Due to mutation the function of a proto-oncogene is altered then it becomes an oncogene with a gain of function. In the case of suppressor genes, mutations result in loss of functions. Mutations in these two groups of genes are common in cancer. The major proto-oncogenes are src, ras, cyclin, c-myc, NF-kB etc and the tumor suppressor genes are p53, Rb, PTEN, BRCA, LKB1 etc.

Mutations in ras gene are very common in cancer. Ras proteins have been found mutated in about one-third of human tumors. The three types of ras genes are H-ras, K-ras and N-ras. K-ras mutation is seen in sporadic colorectal adenomas (Einspahr et al, 2006). N-Ras oncogenes are frequently mutated in thyroid carcinomas. K-ras is the most frequently mutated gene in pancreatic cancer; reported rates range from 70% to 90% (Marchese et al, 2006). Oncogenic ras expression occurs in up to 40% of multiple myeloma cases and correlates with aggressiveness of the disease (Hoang et al, 2006). Mutated ras pathway has been implicated as a key component of the proliferative drive in AML (Bowen et al, 2005). K-ras is frequently mutated in lung adenocarcinomas. Mutant K-ras might lead to generation of reactive oxygen species (ROS) and DNA damage, contributing to malignant transformation (Maciag et al, 2004). H-ras oncogene mutation is
associated with the progression of papillomavirus induced lesions of uterine cervix (Alonio et al, 2003). Nearly 20% of non-small cell lung cancers have mutations in ras. BRAF is a cytoplasmic serine/threonine kinase in the MAPK pathway that transduces signals from ras family members to MEK1/2. Mutations in the BRAF gene have been described in the majority of cutaneous melanomas, papillary thyroid carcinoma and non-Hodgkin's lymphoma (Jarry et al, 2004). Both ras and BRAF mutations contribute to the pathogenesis of stomach cancer (Lee et al, 2003).

The Src family of protein tyrosine kinases (SFKs) plays key roles in regulating signal transduction by a diverse set of cell surface receptors in the context of a variety of cellular environments. Src is mutated and truncated in retroviruses; truncated in colon cancer; overexpressed in mammary, pancreatic and other cancers. Another member of this family ‘yes’ is overexpressed in colon, malignant melanoma and other cancers (Parsons and Parsons, 2004). The introduction of v-Src can lead to the disruption of intercellular adhesion and induction of in vitro invasion. The over expression of activated c-Src in pancreatic cancer cells and hepatocellular carcinoma resulted in E-cadherin down-regulation and stimulates both cell proliferation and migration. c-Src family kinases are activated in ErbB-2/neu-induced mammary tumors. c-Src activity had been demonstrated to have a role in the regulation of expression of several factors responsible for angiogenesis which include VEGF, FGF and angiopoietin. (Irby and Yeatman, 2000; Alper and Bowden, 2005).

The tumor suppressor gene p53 is mutated in nearly 50% of the tumors. More than 20,000 mutations in the p53 gene have been accrued by IARC and it is readily available for public use (www-p53.iarc.fr/index.html). p53 predominantly shows missense mutations, most of which accumulate in the DNA binding domain of the protein. Li-Fraumeni syndrome is a rare autosomal disorder characterized by a familial clustering of tumors and is mainly caused by germ-line mutations in p53 (Olivier et al, 2003; Eeles, 2003). There are reports of frequent mutations of p53 in lung cancer, esophageal squamous cell carcinoma, breast cancer, melanoma, ovarian cancer, colorectal and pancreas cancer (Olivier et al, 2002; Hussain and Harris, 2006).
1.6. Apoptosis: A program for cell death:

Apoptosis or programmed cell death is a genetically controlled program involved in the regulation of homeostasis, tissue development and the immune system by eliminating cells that are no longer useful. Apoptosis is also involved in the elimination of aberrant cells that are created by damage to DNA or infected with viral pathogens.

The Greek word apoptosis literally means “to fall away from”. Vogt (1842) described this phenomenon in the developing neurons of the toad. This was followed by the classical studies of Kerr et al (1972) which provided evidence for the first time that cell may undergo at least two distinct types of cell death: The first type is known as necrosis, and another type is called as apoptosis. Necrosis is a spectrum of morphological changes that affecting extensive cell populations, characterized by cytoplasm swelling, destruction of organelles and disruption of the plasma membrane, leading to the release of intracellular contents. This may elicit inflammation also. By electron microscopy, necrotic cells are characterized by overt discontinuities in plasma and organelle membrane, marked dilation of mitochondria, intracytoplasmic myelin figures and aggregates of fluffy material which probably representing denatured protein (Kumar et al, 2004). Apoptosis is a remarkably distinct type of cell death. It is identified in single cells usually surrounded by healthy looking neighbors, and characterized by cell shrinkage, blebbing of the plasma membrane, maintenance of organelle integrity, and condensation and fragmentation of DNA, followed by ordered removal through a process known as phagocytosis (Guimaraes and Linden, 2004).

Apoptosis is a complex process and occurs through two distinct mechanisms.

(a) The extrinsic pathway or the cytoplasmic pathway triggered through the Fas death receptor, a member of TNF super family.

(b) The intrinsic pathway or the mitochondrial pathway, where stimulation leads to the release of cytochrome C from mitochondria and activation of death signal.

Both the pathways converged at a final common pathway involving the activation of a cascade of proteases namely caspases.
(cysteine aspartases). Caspases are the engines of cellular destruction which cleave the regulatory as well as structural molecules culminating in the death of the cell (Danial and Korsmeyer, 2004). Schematic representation apoptotic pathways are given in Figure 1.4.

1.6.1. The extrinsic/cytoplasmic/death receptor pathway:

The receptors triggering this pathway are located in the plasma membrane of the cell which undergoes apoptosis and they are activated by extracellular ligands. This pathway constitutes several proteins including the death receptors; the membrane bound Fas ligand, the Fas complexes, FADD and caspases 8 and 10 which activate the down stream caspases resulting in cell death. Activation of the extrinsic pathway is initiated with the ligation of cell surface receptors called death receptors (DRs). Fas is a member of the tumor necrosis factor receptor super family and are also called Apo-1 or CD95. Other TNF receptors include TNF R1 (CD120a), DR3 (Apo 2), DR4 (tumor necrosis factor related apoptosis-inducing ligand receptor 1-TRAIL R1), DR5 (TRAIL R2), and DR6. CD 95 ligand (CD95L) binds to Fas and TNF and lymphotoxin-α bind to TNFR1. Fas signaling plays an important role in immune surveillance of transformed or virus infected cells and in the removal of self-reactive lymphocytes. Therefore, defects in this pathway have been implicated in many malignancies and autoimmune diseases (Ashkenazi and Dixit, 1998; Fulda and Debatin, 2004; Ghobrial et al, 2005). The Fas-associated death domain protein (FADD) was discovered as a protein that interacts with the Fas receptor. In Fas signaling, when the FasL binds to Fas, it leads to the trimerization of receptor. Adaptor proteins via their death domains (Fas-associated death domain protein, FADD) then bind to the cytosolic death domains (DD) of Fas. FADD in addition contains a death effector domain (DED), to which the DED of pro-caspase-8 can interact. The complex of Fas, FasL, FADD and pro-caspase-8 is termed as the DISC (death inducing signaling complex). The pro-caspase-8 molecules are brought into close proximity in the DISC, so that they can transactivate one another. Active caspase-8 then can directly cleave caspase-3 or other executioner caspases, eventually leading to the apoptotic outcome (Ashkenazi, 2002; Lawen, 2003). In some cells, the activation of caspase 8 may be the only requirement to
execute death, but there are instances where caspases 8 activation is not sufficient to induce apoptosis. In that case caspase 8 interacts with the intrinsic apoptotic pathway by cleaving Bid (a proapoptotic member of the Bcl-2 family), leading to the subsequent release of cytochrome-c (Ming, 2000; Wajant, 2002).

1.6.2. The intrinsic/mitochondria pathway: The major regulators of the pathway are Bcl-2 family members. The Bcl-2 family includes proapoptotic members such as Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, and antiapoptotic members such as Bcl-2, Bcl-XL, Bcl-W, Bfl-1, and Mcl-1. The balances between these two groups of members are an important factor for normal cell survival (Adams and Cory, 1998; Cory and Adams, 2002).

The diverse signaling pathways can promote mitochondrial membrane permeabilization which results in the release of caspase activating factors. The release of cytochrome C is the committing step. The other factors which release from mitochondria to cytosol are Smac/Diablo, and Omi/htra2. Once released into the cytosol, cytochrome C activates Apaf-1 which together with pro-caspase-9 forms a complex namely apoptosome. Caspase-9 then activates pro-caspase-3 which subsequently activates the rest of the caspase cascade and leads to apoptosis. Smac/Diablo, and Omi/htra2 complexes bind to IAPs and prevent their action (Ferraro et al, 2003; Ghobrial et al, 2005; Vermeulen et al, 2005).

1.6.3. Caspases: The final pathway that leads to execution of the death signal is the activation of a series of proteases termed caspases. Caspases are synthesized in the normal cells as inactive pro-enzymes and can rapidly be activated by autoproteolytic cleavage or cleavage by other caspases as specific Asp residue. The roles of caspases in the process of apoptosis were implicated when CED-3 was discovered which was required for apoptosis in the nematode Caenorhabditis elegans. Later the human counterpart of CED-3 was identified as mammalian interleukin 1β-converting enzyme (ICE) or caspases-1 (Thornberry and Lazebnik, 1998). Currently 14 members of the family have been identified out which 7 mediate apoptosis. They are caspases 2, 3, 6, 7, 8, 9 and 10. Rests of the caspases are involved in processing pro-
inflammatory cytokines and in mediating inflammatory responses (Earnshaw et al, 1999; Riedl and Shi, 2004; Philchenkov, 2004).

All the caspases are not involved in cell death. Both intrinsic and extrinsic apoptotic pathways merge at the site of caspase-3, which cleaves the inhibitor of the caspase-activated deoxyribonuclease, which cleaves the DNA. The downstream caspases induce cleavage of (i) several protein kinases there by inhibiting the signaling pathways; (ii) cytoskeletal proteins which results in the alteration of cytoskeletal structures; (iii) DNA repair proteins thereby affecting the cell cycle, (iv) inhibitory subunits of endonucleases and (v) destruction of “housekeeping” cellular functions thus preparing the cell for execution. All these events ultimately leading to the morphologic manifestations of apoptosis, such as DNA condensation, fragmentation and membrane blebbing. Finally the apoptotic bodies formed are engulfed by macrophages, immature dendritic cells, fibroblasts and endothelial cells (Hengartner 2000; Okada and Mak, 2004; Vermeulen et al, 2005).

1.6.4. Role of mitochondria in apoptosis:

The mitochondria, plays an important role in apoptosis. Several studies conclude that different signals may converge on mitochondria to trigger or inhibit the release of cytochrome C into the cytosol. Mitochondrial outer membrane permeabilization (MOMP) is considered the ‘point of no return’ as this event is responsible for engaging the apoptotic cascade in numerous cell death pathways. The pro-apoptotic signals changes the mitochondrial membrane potential and induces the release of cytochrome C. These changes are mainly mediated by the members of Bcl-2 family. Especially Bax and Bad are the crucial molecules that trigger collapse of mitochondrial membrane potential (MMP). Furthermore increase in calcium levels and arachidonic acid causes mitochondrial damage in Bax/Bad independent manner. Several other apoptotic mediators are released from mitochondria during apoptosis. There are reports that intramitochondrial pool of pro-caspase-3 participates in the apoptosis of some primary cells. AIF is another factor released from mitochondria. Once released it translocates to nucleus where it induces a caspases independent type of chromatin condensation in a yet unidentified mechanism. AIF also initiates DNA fragmentation.
Emerging evidences suggest that AIF may serve as a safe-guard death executioner in some cancer cells with faulty caspases activation in vitro (Jaattela, 2004; Modjtahedi et al, 2006). Mitochondria is the major source of superoxide anion production in cells and the levels of superoxides are increased during apoptosis.

1.6.5. Other regulators of apoptosis: p53 is a regulator of DNA repair, cell cycle and apoptosis. When irreparable damage of DNA occurs, p53 triggers apoptosis in that cell. The action of p53 is mediated by transcriptional activation dependent and independent pathways. p53 induces the transcription of Bax as a result of DNA damage. CD95 receptor is also under the control of p53. p53 can contribute to apoptosis by direct signaling to mitochondria. p53 translocates into mitochondria and trigger the release of cytochrome C. The loss of Rb also triggers p53 mediated apoptosis (Bellamy et al, 1997; Kam and Ferch, 2000; Lowe and Lin, 2000).

The action of p53 is mediated by transcriptional independent pathways as seen in human vascular smooth muscle cells where CD95 expression was transiently increased by p53 by transport from the Golgi complex. This cell surface redistribution of CD95 sensitizes the cell towards apoptosis without any apparent synthesis of new mRNA (Zoring et al, 2001).

NF-kB is a nuclear transcription factor that regulates expression of a large number of genes involved in the regulation of apoptosis and it has been shown to have both anti and proapoptotic functions that may be determined by the nature of the death stimulus rather than by the origin of the tissue. Under normal physiological conditions, the activation of NF-kB induces resistance to apoptotic stimuli through the activation of many complex proteins including TNF receptor-associated factor, IAP etc. However, in response to certain stimuli especially with the activation of some other pro-apoptotic members like c-myc, p53, and caspases, NF-kB activation may lead to apoptosis (Reed and Pellecchia, 2005). In case of viral infections the virus mediated apoptosis is mediated by NF-kB activation.

IAPs block apoptosis either by binding and inhibiting caspases or through caspases-independent mechanisms. So far eight human IAPs
were discovered, out of which XIAP, c-IAP1 and c-IAP2 and survivin directly bind and inhibit caspases 3, 7 and 9. The caspases independent mechanism is mainly mediated via MAPK/JNK1 pathway where NF-kB also plays an important role. Some of the IAPs showed a marked increase in tumors. For example high level of survivin in colorectal cancer correlated with its clinical status. Survivin also found to be high in soft tissue sarcoma and esophageal cancer (Kawasaki et al, 2001; Nachmias et al, 2004).

1.6.6. Caspase independent apoptosis: Mainly in tumor cells the classical apoptotic pathways are altered. It is mainly because of the mutation in any of the participating molecule in the entire cascade. Often there are viral proteins which are powerful inhibitors of apoptosis. Many of the tumor cells can unexpectedly survive the activation of caspases. Mutated oncogenes in the body also act as inhibitors of apoptosis. Caspase independent death mechanisms are now gaining considerable interest (Leist and Jaattela, 2001; Mathiasen and Jaattela, 2002).

Apoptosis-like PCD is a type of cell death independent of apoptosis and the term Apoptosis-like PCD is used to describe the forms of PCD with chromatin condensation that is less compact/complete than in apoptosis (geometrically more complex and lumpier shapes), and with the display of phagocytosis-recognition molecules before lysis of the plasma membrane. Any degree and combination of other apoptotic features can be found (Leist and Jaattela, 2001). Like the classical apoptosis, this PCD also depend on proteases and MMP. The major proteases involved are cathepsins B, D and L, calpains and granzyme A and B. Hsp 70 can also induce apoptosis like PCD. Intracellular calcium levels, NO, arachidonic acid and gangliosides are the major messengers which alter the MMP during apoptosis like PCD.

1.7. Viral carcinogenesis:

The role of viruses in the etiology of cancer is well established now and viruses play a role in the substantial fraction of human malignancies. Human leukemias and lymphomas are the prime tumor types for possible viral involvement (Butel, 2000). The field started with the discovery of Ellerman and Bang (1908) who were doing research on leukemia in chickens provided important evidence that malignant tumors
in animals could be caused by some “filterable agents”. This was followed by the observation of Peyton Rous that chicken sarcomas could be transmitted with cell free filtrate (Rous, 1911). This was followed by the discovery of MMTV by Bittner (1936), murine leukemia virus by Gross (1951) and polyoma virus by Stewart et al (1958). The viral infections are linked to at least 15% of all malignant tumors in humans. Both RNA and DNA viruses are associated with various forms of malignancies and some important ones are described in Table 1.1.

1.7.1. DNA viruses:

1.7.1.1. Human Papilloma Virus (HPV): It is a non-enveloped DNA virus and in humans HPV is mainly associated with cervical cancers (Snijders et al, 2006). So far 118 types of HPV are completely described and out of which HPV 16 and HPV 18 are closely associated with human malignancies and are termed as high-risk HPV genotypes (de Villers et al, 2004) and to lesser extent HP V31 also is implicated (Zheng and Baker, 2006). The initial infection of HPV requires the availability of epidermal or mucosal epithelial cells that are still able to proliferate. Three of the HPV proteins namely E5, E6 and E7 modulate the transformation process. E1 and E2, the two regulatory proteins, modulate the transcription and replication while C1 and C2 constitute the structural proteins which compose the viral capsid. The E1, E2, L1 and L2 ORFs are well conserved among all members of HPV family (Burd, 2003).

Mechanism of HPV induced malignancy: E5, E6 and E7 genes has the proliferation stimulating capacity and are the early transcribing viral genes. E5 stimulate the growth by forming complex with EGFR, PDGFR and CSFR-1. E5 also has been shown to inhibit the apoptosis following DNA damage (Zhang et al, 2002). The E6 and E7 proteins are the key players involved the process of transformation and associated malignancy.

E6 protein is basic protein with two C-terminal zinc binding motifs. E6 initially binds to p53 and trigger its degradation via ubiquitin mediated pathway. E6 of HPV16 also activates telomerase, a ribonucleoprotein complex that synthesizes telomere repeat sequence and is linked to cell immortalization. E6 also degrade a pro-apoptotic
protein BAK which results in resistance to apoptosis and an increase in chromosomal instability (zur Hausen, 2001; 2002).

E7 protein is an acidic protein with one zinc binding motif at the C-terminal. E7 bind to pRb which is a negative regulator of the cell cycle that normally prevents S-phase entry by associating with the E2F family of transcription factors. E7 binding to pRb displaces E2F, irrespective of the presence of external growth factors, and leads to the expression of proteins necessary for DNA replication (Doorbar, 2005). E7 stimulates the S-phase genes cyclin A & E and blocks the function of cyclin dependent kinase inhibitors WAF1 and KIP1 (Kubbutat and Vousden, 1996; Hoppe-Seyler and Butz, 1999; zur Hausen, 2002). Though both E6 and E7 can independently transform cells their combined action results in a marked elevation of transformed activity.

1.7.1.2. Epstein - Barr virus (EBV): It is a B-cell lymphotropic virus belonging to the herpes virus subfamily gamma herpesviridae and is the most highly transforming known virus (Klein, 2002). Primary EBV infection results in infectious mononucleosis. Now it is well established that EBV play a role in the causation of several type of cancers (Kutok and Wang, 2006) as described in Table 1.

A number of EBV genes that are expressed in EBV associated malignancies have activities which may contribute to the deregulation of normal course of cell growth and induces oncogenesis (Hoppe-Seyler and Butz, 1999).

Mechanism of EBV induced malignancy: LMP1 (latent membrane protein) which codes for an integral membrane protein can morphologically transform epithelial and rodent cell lines (Fahraeus et al, 1990). Experimentally, expression of LMP1 has multiple effects. It increases the expression of intracellular adhesion molecules (ICAM-1 and LFA-1), various B-cell activation markers (CD21, CD23 and CD40) and induces anti-apoptotic proteins such as Bcl-2 and Mcl-1. A number of signaling pathways including nuclear factor-kappa-B (NFkB), c-Jun NH\textsubscript{2}-terminal kinase (JNK)/AP-1, and p38/mitogen-activated protein kinase (MAPK) are implicated in the function of LMP1 (Tao et al, 2006). LMP1 functions in a paracrine manner in epithelial cells to augment transformation (Damanaia, 2004). Another gene LMP2 encodes two proteins possessing
structural similarity namely LMP2A and LMP2B. LMP2A activate src family of tyrosine kinases and activate EBV lytic cycle in B-cells. LMP2A also activate β-catenin signaling pathway as well as Akt pathway and both these pathways have been linked to cell survival and cell proliferation (Morrison et al, 2003). LMP2B mainly modulates the function of LMP2A and combined action of both LMP2A and 2B can influence squamous epithelial cell behavior such as cell adhesion, motility and invasion, leading to increased capacity of epithelial cells to spread and migrate on extracellular matrix (Tao et al, 2006).

Another set of proteins belonging to the class of EBNA (EBV encoded nuclear antigen) plays a vital role in the EBV associated cell transformation and oncogenesis. EBNA1 is constitutively expressed in all EBV positive malignancies. EBNA1 can act as a DNA binding protein as well as a transcription activator and induces the expression of RAG 1 and RAG 2 \textit{in vitro}, which are implicated in chromosomal translocations in B-cell tumors (Srinivas and Sixbey, 1995). EBNA-LP can interact with both pRb and p53. EBV also prevents the apoptosis of infected cells (Clemens, 2006). The combined actions of these proteins are responsible for the oncogenic nature of EBV (Hoppe-Seyler and Butz, 1999; Howley et al, 2001).

1.7.1.3. Hepatitis B Virus (HBV): It is an enveloped DNA virus with a strong tropism for hepatocytes. Persistence infection with HBV is a major health problem world wide as it can lead to cirrhosis and primary hepatocellular carcinoma (HCC) (Huang et al, 2006). Based on prospective epidemiological studies chronically HBV infected individuals exhibit a 100-fold increased risk for developing HCC and it is estimated that approximately 60-80% all HCCs are linked to chronic HBV infection (Hoppe-Seyler and Butz, 1999; Liu et al, 2001).

\textit{Mechanism of HBV induced malignancy:} The role of HBV in tumour formation appears to be complex and may involve both direct and indirect mechanisms. HBV positive HCCs typically contain viral DNA integrated apparently on non-specific chromosomal sites. Integration often leads to deletions and rearrangements within viral DNA. The compact genome is organized into four overlapping ORFs that encode viral polymerase, the core (HBcAg) and precore proteins, three envelope
proteins of HBsAg protein and viral X protein. The precore protein is secreted into serum as HBeAg (Lewin et al, 2002). The viral X protein has transforming activities and X protein activates the expression of a wide array of cellular genes such as c-myc and c-jun which could result in the deregulation of cellular genes involved in normal growth and cell cycle and contributes to cellular transformation. X protein also binds and inactivate p53 and inhibits p53 mediated apoptosis. The X protein also activate ras-raf-MAP kinase pathway (Koike and Takada, 1995). In addition, HBx activates NFkB and its entry into the nucleus. HBx enhances HBV replication 5- to 10-fold in transfected HepG2 cells by triggering the cytosolic Ca\(^{2+}\) release, which in turn activates the proline-rich tyrosine kinase 2 (Pyk2). The activated Pyk2 activates Src kinase which in turn activates several types of signal transduction cascade in the down stream direction (Ryu, 2003). Another possibility is that integration of viral DNA can disrupt or alter growth regulatory genes such as cyclin A. Prolonged expression of the viral regulatory protein HBx and the large envelope protein LHBs may contribute in deregulating the cellular transcription program and proliferation control, and sensitizes liver cells to carcinogenic factors (Cougot et al, 2005). Moreover HBV has a higher mutation rate than any other known DNA viruses (2 × 10\(^{-4}\) base substitutions per site per year) (Mimms, 1995). Since the chronic proliferation, regeneration and inflammation during persistent HBV infection of the liver is associated with higher risk of mutations in the cellular DNA, we could expect that these mutations will affect the normal function of cellular oncogenes and tumor suppressor genes eventually results in hepatocytes with tumorigenic growth behavior (Wong et al, 2006).

1.7.1.4. Human Herpes Virus-8 (HHV-8): It is a type of herpes virus which was initially isolated from Kaposi sarcoma (KS) and the virus is alternatively called as Kaposi’s sarcoma associated herpes virus (KSHV) (Chang et al, 1994). The virus shows sequence homologies to EBV. KS is the major cancer in AIDS patients and developing KS was found to be 20000 fold higher in HIV patients than the uninfected population (Beral et al, 1990). But the occurrence of HIV-negative cases of KS indicated that HIV cannot be solely responsible for KS development. Human
herpesvirus-8 seems able to transform human endothelial cells and its genome includes a number of potential oncogenes (Stebbing et al, 2006; Ganem, 2006).

**Mechanism of HHV-8 induced malignancy:** Complete sequencing of HHV-8 genome revealed that it contains nearly 81 ORF with striking homology to human cellular genes. At present, we have no animal model of KS, and no experimental animal can sustain a clinically evident infection following KSHV inoculation. Despite these limitations, much has been learned about the individual gene products that have been expressed in KS. Out of the six proteins known to be expressed in latently infected KS cells three of them [LANA, v-cyclin, and v-FLIP] are encoded from one transcription unit.

LANA is multifunctional marker often served as a marker of KSHV infection and localized in the nucleus. The best-characterized function of LANA is its involvement in the establishment and maintenance of the latent viral episome in the nucleus. It is observed that in transfected cells, LANA binds p53, blocking its ability to act as a transcriptional activator and conferring increased resistance to p53-dependent apoptosis (Friborg, 1999). LANA has also been reported to bind to the tumor suppressor Rb in transfected cells and binding is associated with loss of Rb function which leads to enhanced expression of E2F-dependent reporter genes (Radkov et al, 2000). LANA also stimulates the cellular accumulation of β-catenin by preventing its ubiquitin mediated degradation there by activating the wnt pathway (Fujimuro et al, 2003; Hayward and Fujimuro, 2006).

v-cyclin is a homolog of human cellular cyclin D and is able to inactivate Rb. v-cyclin over expression induces S-phase entry in quiescent 3T3 cells and also overcomes the Rb-mediated growth arrest induced by cdk inhibitors and loss of p53 allows cells to survive in the presence of elevated levels of v-cyclin (Chang et al, 1996; Cathomas, 2003).

v-FLIP is the third coding region expressed from the LANA promoter and protect cells from Fas-induced apoptosis in vitro, promote tumour growth in vivo, and activate the NF-κB pathway (Thome et al, 1997; Keller et al, 2000; Brown et al, 2003; Ganem, 2006). These are the
major events responsible for the induction of KS by HHV-8. Schematic representation of events mediated by different viral onco-proteins of DNA viruses are shown in Figure 1.5.

1.7.2. RNA viruses

1.7.2.1. Hepatitis C Virus (HCV): HCV is a RNA virus belonging to the family of hepacivirus. HCV infected an estimated 200 million persons worldwide and HCV infection varies throughout the world (Shepard et al, 2005; Stauber and Stadlbauer, 2006). HCV now has 6 genotypes and nearly 80 subtypes because of genetic heterogeneity (Hoppe-Seyler and Butz, 1999; Poduri, 2001). Chronic HCV infection is accompanied by variable degrees of hepatic inflammation, damage and fibrosis with an increased risk of developing liver cirrhosis and hepatocellular carcinoma (Otero et al, 2006).

Mechanism of HCV induced malignancy: The genomic organization of HCV is given in Figure 1.6. HCV encodes a single polypeptide of 3011 amino acids which is then processed into 10 mature structural and regulatory proteins. The virus mutated rapidly because of the presence of a RNA dependent RNA polymerase lacking “proof-reading” function thereby escaping from the immune surveillance (Lauer and Walker, 2001).

![Figure 1.6: Genomic organization of HCV RNA](image)

How exactly the virus and viral proteins are involved in the neoplastic process is still a matter of question. Four HCV proteins, core, NS3, NS4B and NS5A, have been shown to be able to transform cells either alone or in combination with each other. The core protein mainly functions in the nucleus where it controls the expression of certain host genes. It also prevents the apoptosis of infected cells by interacting with members of TNF family for eg; lymphotoxin-b receptor (LTR) and LTR is known to be involved in apoptotic signaling. This strongly suggests that core may have an immunomodulatory function and play a critical role in
the establishment of persistence and in disease pathogenesis (Matsumoto et al, 1997; Clarke, 1997). The envelope glycoprotein 2 (E2) represents the most variable region of the HCV genome and the variation is assumed to be caused by random mutation and selection of mutants capable of escaping from neutralizing antibodies produced in the host. There are reports that core protein may interact with p53 and inhibits its activity. The HCV core transactivates a number of cellular promoters, including c-myc, and activates NF-kB and AP-1 and their interaction with c-JNK, ERK and MAPK signaling. HCV also blocks the induction of apoptosis in infected cells and NF-kB has been shown to play a major role in the antiapoptotic action of HCV proteins (Marusawa et al, 1999; Bantel and Schulze-Osthoff, 2003; Lindenbach and Rice, 2005).

Prolonged infection with HCV lead to chronic inflammatory state, associated with over production of nitric oxide (NO). NO is a mutagen as well as a vasodilator. The constant prolonged exposure of NO to the cells lead to genomic instability. NO often damages mitochondria, leading to induction of double-stranded DNA breaks and accumulation of oxidative DNA damage (Machida et al, 2004). HCV core protein is shown to transactivate iNOS gene promoter through NF-kB activation (de Lucas et al, 2003). Oxidative stress has been indicated as one of the possible mechanism of HCV-induced hepatocarcinogenesis. HCV core protein is able to enter into the mitochondria and induces a marked increase in reactive oxygen species (ROS) levels in hepatoma cells. Also three of the HCV proteins core, E1, and NS3 involved in the activation of STAT3 (Machida et al, 2006). All these sited events eventually progress into HCC.

1.7.3. Animal models of viral carcinogenesis:

The value of animal models of viral carcinogenesis is very important especially with respect to the investigation of mechanism of transformation. There are several examples of their contribution in the understanding of viral pathogenesis and mechanism of transformation. One of the major drawbacks of animal models is that it will not exactly mimic the pathogenesis of viral carcinogenesis in humans. The collection of cancer causing retroviruses recovered from spontaneous tumors in mice and chickens provided the tools for dissecting the biological and
molecular mechanism of carcinogenesis. The oncogenic properties of several DNA viruses had been determined by their injection into susceptible hosts. These models revealed the existence of viral oncoproteins and also pave away for analyzing viral transformation functions (Butel, 2000). The polyoma virus, SV40, Friend leukemia virus etc, are the commonly used viruses to create viral cancer models in animals.

1.7.3.1. *Friend leukemia virus (FMuLv) induced erythroleukemia in mice:*

The erythroleukemia induced by FMuLv is one of the best animal models available to study the stepwise leukemia progression due to the reproducibility of the sequential genetic mutations leading to transformation of infected erythroblasts. In 1957, Charlotte Friend described a virus preparation derived from passing Ehrlich Ascites cells through newborn Swiss mice that caused acute erythroid hyperplasia when injected into adult mice. The virus preparation was termed as Friend virus (FV) complex. Two separate isolates of Friend virus termed as FV-A and FV-P have been identified. Both are complexes of two distinct viral species, namely a unique replication defective spleen focus forming virus (SFFV-A and SFFV-P respectively) and a common replication competent Friend murine leukemia virus (FMuLv) (Ruscetti, 1999; Lee et al, 2003).

The leukemias induced by both FV-P and FV-A are multistage malignancies which commence within days of viral inoculation. The virus is injected to newborn BALB/c mice less than 24hr old through intraperitoneal route. The initial stage of the disease is characterized by massive splenic proliferation of non-tumorogenic erythroid progenitor cells. Within 1-2 days of infection infected erythroblasts infiltrate into spleen. By 12-15 days of post viral infection the spleens of the infected animals shows massive enlargement nearly 10-12 times than their normal size. At this stage infected cells are designated as preleukemic. In the late of the disease, cells are both tumorigenic *in vivo* and capable of forming spleen colonies (Lee et al, 2003).

The FMuLv cannot induce erythroleukemia in adult mice but when injected to newborn animals it causes anemia and splenomegaly (Ben-David and Bernstein, 1991)
Mechanism of FMuLv induced malignancy: Transformation towards malignancy is dependent upon the activation of Spi-1 gene by retroviral insertional mutagenesis. Spi-1 was found to be rearranged in 95% of erythroleukemias (Gachelin et al, 1988). The upregulation of antiapoptotic Bcl-2 was noticed in most of the erythroleukemias. This has provide an evidence that upregulation of Bcl-2 confers a selective growth advantage during the progression of leukemia (Howard et al, 2001). The tumor suppressor gene p53 bear mutations or deletions making it inactive, which accelerates the growth and survival of transformed cells (Wong et al, 1999). P45\textsuperscript{NFE2} is a part of NFE2 (nuclear factor erythroid) complex which plays an active role in the regulation of erythroid specific gene expression. This nuclear factor binds to several AP-1 like consensus binding sites of several enhancers and promoters of various erythroid and megakaryocytic specific genes. It contain two subunits, p45\textsuperscript{NFE2} (highly tissue specific conferred to erythroid cells only) and p18\textsuperscript{NFE2} which is widely expressed in tissues. Several studies revealed that p45\textsuperscript{NFE2} functions as an inhibitor of erythroid cell growth and perturbation of its expression contributes to the progression of leukemia (Ney and D’Andrea, 2000; Li et al, 2001). The erythropoietin (Epo) gene is also over expressed in leukemias. The activation of Epo in majority of FMuLv induce leukemias provide an autocrine loop resulting in the constitutive activation of Epo receptor mediated signal transduction cascade thereby conferring growth and survival advantage to proliferating cells (Howard et al, 1996; Ruscetti, 1999). The Raf-1/MAPK pathway is a growth factor activated signal transduction cascade that transduces signals from the cell surface to the nucleus. This pathway is over expressed in erythroleukemia to achieve maximum proliferation of erythroid cells (Muszynski et al, 1998). The animals generally die because of splenic rupture.

1.7.3.2. SV40 induced tumors in hamsters:

Since its discovery Simian virus 40 (SV40) has been one of the most intensely studied animal viruses. Sweet and Hilleman in 1960 described an agent that induced cytopathic effects and vacuole formation in monkey cells and has been termed as SV40. It belongs to polyomaviruses. It is a major controversy even today regarding the
suspected role of SV40 in human cancers. Still the virus has not been linked directly to cancer development in humans. The sequence of SV40 DNA like has been isolated from some tumor samples like osteosarcoma and bone tumors. SV40 has been implicated in 40-60% cases of mesothelioma and in nearly 40% of malignant lymphomas (Nakatsuka et al, 2003)

SV40 induces tumors when injected to 21-day old Syrian golden hamsters and the type of tumors developed will depend on the route of inoculation. The hamsters generally survived for 9 months (Cicala et al, 1993; Vilchez et al, 2004). SV40 induces lymphomas, brain tumors, osteosarcomas and mesotheliomas in hamsters.

*Mechanism of SV40 induced malignancy:*

SV40 undergoes a lytic replication cycle, when it infects its natural host. The early viral gene encodes the tumor (T) antigens namely large T antigen (LT), small t antigen (ST) and 17KT antigen (tiny T) (Garcea and Imperiale, 2003). LT plays a dominant role in infection. It acts as an initiator of viral DNA replication. The function of LT resembles that of a helicase enzyme (Stahl et al, 1986). LT can alone immortalize the human cells. LT binds pRb, p107 and p130 resulting in the release of E2F from Rb which lead to the stimulation of genes involved in the S-phase. LT also binds to p53 and inhibits its function thereby preventing the apoptosis mediated cell death of infected cells (Butel and Lednickly, 1999). Less is known about the role of ST in transformation. ST known to bind and inhibit PP2A, a cellular phosphatase resulting in the continuous activation of signal transduction pathways (Conzen and Cole, 1994). ST is also known to cause chromosomal instability. These are major events which lead to cellular transformation.

1.7.3.3. Polyomavirus induced tumors in mice:

Polyomavirus is one of the smallest oncogenic viruses known. It belongs to Papoviridae. The virus was initially characterized by Gross (1953) and Stewart et al (1958). Neonatal BALB/c and C3H/BiDa strains of mice are highly susceptible for Polyomavirus. The virus in injected either intraperitoneally or subcutaneously to the mice. The kidneys and salivary glands were identified as major targets of replication and persistence while tumors occur in a much wider spectrum of organs and
in particular tumors were developed largely in the mammary gland, bones and skin (Fluck and Haslam, 1996; Wirth et al, 1997; Benjamin, 2001).

**Mechanism of Polyomavirus induced malignancy:**

The viral genome is a small double stranded circular DNA and 3 types of tumor antigens are produced when Polyomavirus infects a host and they are named as large T (LT), middle T (MT) and small T antigens (ST). LT have the potential for altering cell growth controls. It initiates synthesis of viral DNA. LT binds to the members of Rb family and promotes G\(_{1}\)-S transition and inhibits the functions of p53 (Doherty and Freund, 1997). In vitro studies confirmed the immortalization capacity of LT. MT is the major transforming protein and MT interacts with members of c-src family. It also activates ras pathway constitutively. MT also triggers mitogenesis. (Dilworth, 1995). ST activates MAP kinase pathway and inhibits the function of a cellular phosphatase PP2A. All these events finally lead to initiation and progression of tumors (Benjamin, 2001).

**1.8. Concept of radioprotectors and chemoprotectors as adjuvant in cancer therapy:**

Radiotherapy is one among three main strategies used in cancer therapy. Radiotherapy is a regional therapy and is mostly toxic to proliferating cells. Mammalian cells are more sensitive to radiation induced damage during the late G\(_{2}\) and M phase of the cell cycle. Chemotherapeutic regime uses either a single drug or a combination of different drugs and is frequently used in several types of advanced solid tumors and hematological malignancies.

Both radiotherapy as well as chemotherapy possesses several side effects of its own. The damage to normal cells along with the tumor cells is the major toxicity associated with both the types of therapies. In both radiotherapy and chemotherapy, protection of normal tissues is as important as the destruction of cancer cells. A judicious balance between total radiation delivered and threshold limit of the surrounding normal tissue is required for getting optimum results. Therefore the role of radioprotectors is very important in clinical radiotherapy. Several compounds have been shown to act as radioprotectors. In clinic [S-2-(3-
aminopropyl-amino) ethyl phosphorothioic acid] which is commonly known as WR-2721 or amifostine is the only approved drug. It is highly expensive and associated with side effects of its own. The radioprotectors act via any of the following mechanism. (a) By suppressing the formation of reactive species; (b) detoxification of radiation induced species; (c) target stabilization and (d) enhancing the repair and recovery process (Nair et al, 2001; Arora et al, 2005).

Several natural compounds are now known as radioprotectors which include antioxidants like vitamin A, E and C, cytoprotective agents like MESNA, lipopolysaccharides and prostaglandins (Nair et al, 2001). The plants which reported to have radioprotective activity include *Aegle marmelos*, *Allium sativum*, *Glycyrrhiza glabra*, *Mentha arvensis*, *Syzygium cumini*, *Centella asiatica*, *Mentha piperita*, *Zingiber officinale*, *Ocimum sanctum*, *Terminalia chebula*, *Podophyllum hexandrum*, *Embiica officinalis* etc (Samarth and Kumar, 2003; Arora et al, 2005; Gandhi and Nair, 2005; Maurya et al, 2006). Some of the herbal formulations like Brahma rasayana, Chynaprasha and Triphala also possess radioprotective potential (Praveen et al, 1996; Jeena and Kuttan 1998; Rekha et al, 2000).

Chemoprotectors are agents which can be administered along with chemotherapy to prevent the damages of the normal tissues without interfering the therapeutic outcome of chemotherapy. Chemoprotectors can improve the efficiency of chemotherapy. Several compounds have been screened as chemoprotective agents which include several plant extracts and isolated compounds which include garlic extracts and its isolated sulfur compounds like diallyl disulfide, diallyl sulfide, plants like *Tinospora cordifolia*, *Andrographis paniculata* and poly herbal formulations like Rasayanas and Chynaprasha (Unnikrishnan et al, 1990; Mathew and Kuttan, 1997; Praveen et al, 2002; Manesh and Kuttan, 2002; Sheeja and Kuttan, 2006).

**1.9. Chemoprevention:**

Cancer chemoprevention is defined as the use of specific agents, which can be natural, synthetic or biological agents to reverse or prevent the process of carcinogenesis thereby to prevent the development of cancers. The term chemoprevention was coined by Sporn (1976). It has
been now estimated that more than two-thirds of the human cancers could be prevented through appropriate lifestyle modification. It is now well established that a group of non-nutritive components present in the diet especially from plant products have significant anticarcinogenic and antimutagenic activity. Vegetables and fruits are excellent source of cancer chemopreventive substances. As per WHO report 2002, there at least 2.7 million deaths globally per year, which are primarily attributable to low fruit and vegetable intake.

Wattenberg (1985) classified chemopreventive agents into two broad categories; blocking agents and suppressing agents. Blocking agents prevent carcinogens reaching the target sites and prevent its interaction with crucial cellular macromolecules like DNA, RNA and proteins. Suppressing agents inhibit malignant transformation of initiated cells in either the promotion stage or the progression stage.

1.2.8.1. Role of plants and phytochemicals in cancer therapy and prevention:

Despite recent advances in our understanding of the biological processes leading to the development of cancer, there is still a need for new and effective agents to bring cancer under control. The plants have been selected as an excellent source for new phytochemicals. Plants and plants based medicines have been used since the dawn of the civilization to maintain health and to treat a variety of diseases. Even though we enter the new century with its exciting prospect of gene therapy, herbal medicines remains one of the common forms of therapy available to much of the world population. The field of chemoprevention is an active area of research and several molecules are identified as cancer chemopreventive agents. They include Curcumin, resveratrol, sulfur compounds, epigallocatechin gallate, silibinin, gingerol, capsaicin etc (Surh, 2003; Kundu and Surh, 2005; Aggarwal et al, 2006).

In the present study we have tested a herbal extract (Phyllanthus amarus) and 3 natural products (Curcumin, Berberine and Picroliv) for their biological activity against cancer. All these compounds possess antiviral activity and hence we decided to evaluate their possible role in the inhibition of viral carcinogenesis. So far no study has been reported on their role in virally induced cancers.
1. *Phyllanthus amarus* Schumach and Thonn.

This plant belongs to the family Euphorbiaceae and is widely distributed in most tropical and subtropical countries and it grows abundantly during monsoon season (Figure 1.7). The plant is used in indigenous system of medicine such as Ayurveda and Unani to mainly combat the liver disorders. *P. amarus* has been demonstrated to have a wide array of pharmacological activities (Calixto et al, 1998). It has significant antioxidant activity in vitro (Joy and Kuttan, 1995). It also possesses significant anticarcinogenic activity against several cancers induced by chemicals. The NDEA induced hepatocellular carcinoma (HCC) was significantly inhibited by *P. amarus* with significant increase in the lifespan of animals harboring HCC (Joy and Kuttan, 1998; Rajeshkumar and Kuttan, 2000). *P. amarus* also inhibited the MNNG induced glandular stomach carcinogenesis in rats (Regi et al, 2006). *P. amarus* inhibited the sarcoma development in mice induced by 20-methylcholanthrene and increased the life span of animals bearing sarcoma (Rajeshkumar et al, 2002). *P. amarus* also acts as a hepatoprotective agent against CCl$_4$ induced liver dysfunction (Sane et al, 1995). *P. amarus* has significant antiviral activity against HBV. In HBV carriers the administration of *P. amarus* reduced the levels of HBsAg. Extracts of *P. amarus* inhibited the DNA polymerase of HBV and related viruses (Blumberg et al, 1989). Treatment with *P. amarus* in human hepatoma cells reduced the hepatitis B surface antigen gene expression (Yeh et al, 1993). *P. amarus* down-regulates HBV mRNA transcription by a specific mechanism involving interactions between HBV enhancer I and C/EBP transcription factors (Ott et al, 1997). *P. amarus* also found to inhibit wild type human immunodeficiency virus-1 and several types of reverse transcriptase inhibitor resistant variants of HIV-1. *P. amarus* mainly blocks HIV-1 attachment. Isolated ellagitannins like geraniin and corilagin were shown to be most potent mediators of these antiviral activities (Notka et al, 2003; 2004). An aqueous extract of *Phyllanthus niruri* inhibited human immunodeficiency virus type-1 reverse transcriptase (HIV-1-RT) and the inhibitory property is because of the presence of repandusinic acid A monosodium salt (RA) (Ogata et al, 1992). A variety of hydrolysable tannins purified from *P. amarus* were potent inhibitors of rat liver cyclic
AMP-dependent protein kinase (Polya et al, 1995). *P.amarus* showed antimutagenic activity against several direct acting mutagens as well as mutagens needing activation (Regi et al, 2003; Sripanidkulchai et al, 2002). *P.amarus* reported to have antiulcer and anti-inflammatory activity (Regi and Kuttan, 2003). In murine macrophages the induction of iNOS, COX-2 and TNF is inhibited by *P.amarus* mainly via inhibiting the activation of NF-kB (Kiemer et al, 2003). The lignans obtained from *P.amarus* also showed anti-inflammatory activity (Kassuya et al, 2005) *P.amarus* also possessed anti-diabetic effects in alloxan induced diabetic models in rats (Regi et al, 2002). The antigenotoxic property of a crude extract of *P.amarus* was evaluated using the root meristem of *Vicia faba* L. as the in vivo test system. It was observed that the root meristem post-treated with *P.amarus* showed a significant reduction in the frequency of chromosomal alterations (Gowrishanker and Vivekanandan, 1994). *P.amarus* also reported to have diuretic and hypotensive activities (Srividya and Periwal, 1995). *P.amarus* has potential anti-diarrhoel activity against croton oil induced diarrhea (Odetola and Akojenu, 2000).

2. Curcumin:

Curcumin (1, 7-bis (4-hydroxy-3-methoxypentyl)-1, 6-hepadiene-3,5-dione) also known as diferuloyl methane belongs to the class of polyphenols (5-10%) present in the rhizomes of Turmeric (*Curcuma longa* L.). Structure of Curcumin is given Figure 1.8a. Turmeric is extensively used in the Indian subcontinent. Turmeric is used as an indigenous medicine for the treatment of various ailments for many centuries. Curcumin is demonstrated to have a wide spectrum of pharmacological properties (Srimal and Dhawan, 1973; Surh, 2003; Aggarwal et al. 2003; Maheshwari et al. 2006). Curcumin possess significant antioxidant activity. It inhibited the lipid peroxidation in different animal models. Curcumin protected the oxidative cell injury of kidney cells by inhibiting lipid degradation, lipid peroxidation and cytolysis (Cohly et al, 1998). Several *in vitro* as well as *in vivo* studies demonstrated the anti tumor and anticarcinogenic activity of curcumin. Curcumin was found to have antiproliferative and differentiation inducing properties in different types of cell lines in vitro. The mode of action of curcumin varies considerably with the cell type, concentration of
Curcumin and the time of the treatment. Curcumin inhibited DLA induced solid tumor as seen from the reduction in tumor volume and also inhibited ascites tumor in mice. Administration of curcumin enhanced the percentage of survival of animals bearing ascites (Kuttan et al. 1985; Soudamini and Kuttan, 1988). Curcumin has been shown to inhibit benzo[a]pyrene (B[a]P) induced forestomach papilloma in mice (Nagabhushan and Bhide, 1992). Curcumin was found to inhibit N-ethyl-N'-nitro-N-nitrosoguanidine induced duodenal tumorigenesis in C57BL/6 mice, and azoxymethane induced colon tumorigenesis in CF-1 mice. Histopathological analysis revealed that Curcumin inhibited the number of adenomas and adenocarcinomas of the duodenum and colon (Huang et al. 1994). Curcumin also inhibited the N-nitrosomethylbenzylamine (NMBA)-induced esophageal carcinogenesis in male F344 rats and administration of curcumin significantly decreased the expression of cell proliferation biomarkers (Ushida et al. 2000). Curcumin was found to be highly antitumor and inhibited the pulmonary melanoma induced by B16F10 cells in C57BL/6 mice (Menon et al. 1995). Curcumin inhibited the HIV-1 replication by down regulating the long terminal directed p24 expression (Li et al., 1993).

3. Picroliv:

Picrorhiza kurroa Royle ex Benth (Family- Scrophulariaceae) is a well-known herb in the Ayurvedic system of medicine and has traditionally been used to treat disorders of the liver and upper respiratory tract, reduce fevers, and to treat dyspepsia, chronic diarrhea, and scorpion sting. Picroliv was isolated from the roots and rhizomes of the plant Picrorhiza kurroa and is a mixture of two iridoid glucosides namely kutkoside and picroside-1 in a ratio of 1:1.5 w/w. (Dhawan, 1995) and the structure is given in Figure 1.8b. Several pharmacological activities of Picroliv have been reported. Picroliv has been shown to possess protective effect against myocardial ischemia (Tandon et al, 1995). Picroliv regulates the genes associated with hypoxia such as HIF-1 and VEGF (Gaddipati et al, 1999a; Gaddipati et al, 1999b). Picroliv also modulated the antioxidant status and down regulated the expression of AP-1 transcription factor after hemorrhage shock (Seth et al, 2003). Picroliv was also found to have antioxidant and
hepatoprotective activity (Dwivedi et al, 1991; Chander et al, 1992). Dimethyl Hydrazine-induced hepatic carcinogenesis in rats was inhibited by oral administration of Picroliv (Rajeshkumar and Kuttan, 2003). Picroliv was an effective inhibitor of hepatocarcinogenesis induced by NDEA in rats (Rajeshkumar and Kuttan, 2000). Picroliv was also found to inhibit the sarcoma induced by 20-methylcholanthrene (20-MC) and 7, 12-dimethylbenz[a]anthracene (DMBA)-initiated papilloma formation in BALB/c mice (Rajeshkumar and Kuttan, 2001).

4. Berberine:

Berberine is an isoquinoline alkaloid isolated from the roots of the plant Berberis asiatica Roxb. ex. DC non Griff (Family-Berberidaceae). The plant is common on the dry outer Himalaya, Assam Mount Abu etc. The structure of Berberine is given in Figure 1.8c. Berberine has been shown to have antidiabetic properties (Lee et al, 2006). Berberine was found to be cytotoxic to different types of cell lines of various origins (Anis et al, 1999; Lin et al, 2006). Berberine induced cell cycle arrest, not only at the G0/G1-phase, but also at the G2/M-phase in a dose-dependent manner. The berberine-induced G2/M-phase arrest accompanied by increased levels of Wee1 and 14-3-3sigma, but decreased levels of Cdc25c, CDK1 and cyclin B1 (Lin et al, 2006). Berberine also induces G1-phase cell cycle arrest and caspase-3-dependent apoptosis in human prostate carcinoma cells (Mantena et al, 2006). Berberine was shown to have antitumor activity in mice against transplanted tumors and also acts as an adjuvant along with cancer treatment modalities like radiotherapy and chemotherapy (Anis et al, 1999). Berberine also possesses significant anti-inflammatory activity (Kuo et al, 2004). Berberine showed significant anticarcinogenic activity. Berberine was found to inhibit the sarcoma induced by 20-MC and increased the life span of animals harboring sarcoma. The hepatocellular carcinoma induced by NDEA was also inhibited by the treatment with Berberine (Anis et al, 2001).

1.10. **Scope of the present study:**

In the present study we have evaluated the role of P.amarus, Curcumin, Picroliv and Berberine in the treatment and prevention of cancer. Initially we have evaluated the antiviral activity of P.amarus,
Curcumin, Picroliv and Berberine against Poliovirus I, Newcastle disease virus and EDS76 virus. The inhibitory effect of *P. amarus*, Curcumin, Picroliv and Berberine on Friend Virus induced erythroleukemia in mice was studied. Induction of apoptosis by *P. amarus* and its possible mechanism of action were studied in murine and human cancer cell lines. The adjuvant role of *P. amarus* during radiotherapy and chemotherapy was studied in mice. The effect of *P. amarus* on drug metabolizing enzymes (Cytochrome P450) in the liver induced by cyclophosphamide and phenobarbitone were evaluated. The results reported in this thesis gave ample evidence on the use of non-toxic natural compounds for their use as cancer therapeutic agents.
Figure 1.1: Acquired capabilities of cancer

Adapted from the landmark paper of Hanahan and Weinberg, 2000
Figure 1.2: Metabolic activation of B(a)P

![Chemical structure of benzo[a]pyrene (BP) and its metabolites](image)

*Adapted from Perantoni, 1998*

Figure 1.3: Metabolic activation of DMBA

![Chemical structure of DMBA and its metabolites](image)

*Modified from Sugiyama et al, 2002*
Figure 1.4: Schematic representation of events taking place during apoptosis

Adapted from Korsmeyer and Zinkel, 2001
Figure 1.5: Schematic representation of events mediated by viral onco-proteins involved during viral carcinogenesis

Key
◆ Stimulation by
◆ Inhibition by
◆ Disruption by

Cell stress (viral infection, anti-growth signals, DNA damage, carcinogens, etc.)

p53 proteasomal degradation

◆ HPV E6
◆ KSHV LANA-1
◆ KSHV LANA-2

Transcriptional control of p53-regulated genes

◆ KSHV LANA-1 (via Sp1)
◆ HPV E6, EBV (via c-myc)
◆ EBV LMP-1
◆ KSHV v-FLIP

Telomere shortening after mitosis

◆ EBV LMP-1
◆ Anti-apoptotic proteins (e.g., bax, caspases)

Normal cell senescence

Telomere stabilization

◆ KSHV LANA-1 (via Sp1)
◆ HPV E6, EBV (via c-myc)

Cell crisis

Apoptosis

DNA repair

DNA replication

◆ EBV LMP-1 (via NF-κB)
◆ HPV E7
◆ KSHV LANA-1

pRb-bound E2F

pRb\(^{\text{P}}\) + free E2F

Mitotic spindle machinery

◆ HPV E7
◆ HBV X

Cell proliferation

Adapted from de Oliveira DE
Figure 1.7: *Phyllanthus amarus* Schumach and Thonn
Figure 1.8: Structure of compounds

(a)

(b)
Cinnamoyl

Picroside-I

Vanilloyl

Kutkoside

(c)
### Table 1.1: Tumor inducing viruses

<table>
<thead>
<tr>
<th>Virus</th>
<th>Virus family</th>
<th>Genome</th>
<th>Associated cancers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human papilloma virus (HPV)</td>
<td>Papoviridae</td>
<td>Circular DNA</td>
<td>Anogenital cancers, skin cancers, oral cancers</td>
</tr>
<tr>
<td>Hepatitis B Virus (HBV)</td>
<td>Hepadnaviridae</td>
<td>Circular DNA</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Epstein-Barr Virus (EBV)</td>
<td>Herpesviridae</td>
<td>Linear DNA</td>
<td>Nasopharyngeal carcinoma, Burkitt’s lymphoma, B and T-cell lymphoma, Hodkin’s lymphoma, stomach cancer</td>
</tr>
<tr>
<td>Human herpesvirus 8 (HHV8)</td>
<td>Herpesviridae</td>
<td>Linear DNA</td>
<td>Kaposi’s sarcoma, body cavity lymphoma, multiple myeloma</td>
</tr>
<tr>
<td>Hepatitis C Virus (HCV)</td>
<td>Flaviridae</td>
<td>Linear RNA</td>
<td>Hepatocellular carcinoma</td>
</tr>
<tr>
<td>Human T-cell lymphotropic Virus-I &amp; II (HTLV)</td>
<td>Retroviridae</td>
<td>Linear RNA</td>
<td>Adult T-cell leukemia, HTLV-associated myelopathy and tropical spastic paraparesis</td>
</tr>
</tbody>
</table>