# Chapter 1

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Chapter 1.

Literature Review

1.1. Introduction

Cancer is a major public health problem in the world. In India, according to ICMR National Cancer Registry Program data (2002), over 800,000 new cancer cases have been estimated per year. Epidemiological studies have shown that 70-90% of all cancers are environmentally-induced types. The major cause of cancer in India holds the use of tobacco for about 50% of all cancers in men. Also, dietary practices, reproductive and sexual practices, etc., accounts for 20 – 30% of cancers. In developed countries like The United States, currently, one in four deaths are due to cancer. In 2006, the estimated new cases are over 13 lakhs combining both sexes and of which highest number of 321,490 patients with cancers in genital systems (Jemal A, et al., 2007).

Several evidences are accumulated by the cancer research indicate that tumorigenesis is a multifactorial and multistep process and these steps reflect genetic alterations that drive the progressive transformation of normal human cells into highly malignant derivatives (Wild CP, et al., 1996.). In the incidence and the demand of toll of death, cancer being the second to cardiovascular diseases, even though its rate varies with geographical, racial, and ethничal and gender consideration.

1.2. Etiology of Cancer

More and more studies are revealing highly suggestive clues about the causes of cancer. The causes fall into two broad categories: environmental and genetical. In Table 1.1. known high-risk factors for cancer are depicted.

1.2.1. Environmental Factors

The role of environmental factors in carcinogenesis was demonstrated the English physician Percival Pott in 1775. He correlated
the effect of chimney soot in the development of scrotal cancer in men. Today, it is estimated that environmental factors may be involved in a significant percentage of cancer.

Table 1.1.

<table>
<thead>
<tr>
<th>Site</th>
<th>Predisposing Factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Age over 40; late first full-term pregnancy; early-age menarche; late menopause; familial history of breast cancer</td>
</tr>
<tr>
<td>Cervix</td>
<td>Women who begin sexual activity at an early age and have multiple partners; human papilloma virus exposure</td>
</tr>
<tr>
<td>Colon and rectum</td>
<td>Familial polyposis; ulcerative colitis over many years; diet high in fat, low in fiber</td>
</tr>
<tr>
<td>Lung</td>
<td>Cigarette smoking; exposure to asbestos, uranium, and nickel</td>
</tr>
<tr>
<td>Skin</td>
<td>Farmers and other outdoor workers; fair-skinned individuals who sunburn easily</td>
</tr>
<tr>
<td>Mouth</td>
<td>Alcohol plus smoking; smokeless tobacco</td>
</tr>
<tr>
<td>Thyroid</td>
<td>X-ray therapy of neck as infants</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Aniline-dye exposure; exposure to parasite Schistosoma haematobium; cigarette smoking</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Exposure to benzene; ionizing radiation</td>
</tr>
</tbody>
</table>

(Adapted from Weisburger JH, et al., 1995)

1.2.2. Industrial Environment

Different industrial wastes are attributed to the development of cancers, such as aniline dye industry for increased risk of bladder cancer; (Gan J, et al., 2004) nickel-refining plants predispose workers for cancer in sinuses. The inhalation of certain solvents like benzene causes leukemia (Natelson EA, 2007).
1.2.3. Ultraviolet and Irradiation

UV radiation from sun increases the risk for skin cancer (Meeran SM, et al., 2007). Myelogenous leukemia is also reported to be caused by irradiation (Rithidech KN, et al., 2007). Radiation enhances the possibility for the development of lung cancer in those who works in Plutonium, Uranium, Strontium, Nickel and Beryllium industries (Sunderman FW Jr., 2001; Brown SC, et al., 2004)

1.2.4. Cigarette Smoke

It is the major cause of lung cancer in males. Incidence of lung cancer in females is directly linked to cigarette smoke (Henschke CI, et al., 2006). Smoking related illness cause more than 4 lakhs premature deaths every year (Munteanu I & Didilescu C, 2007).

1.2.5. Diet

Increased number of colorectal cancer is due to nondigestable complex of carbohydrates in the Western type diet. The high fibre content food reduces the risk for colorectal cancer but increases the stomach cancer. Studies of Denis Burkitt and others have shown relationship between fibre content and intestinal bulk. The non-absorbable sugar in the bulk changes the intestinal milieu and help to transmit carcinogens formed in large bowel by the action of certain bacteria on bile salts in the colon is increased by low fat food. The high fat and low fibre food produce small stool that take longer time to pass through the intestine, allowing the bacteria to degrade bile salts to carcinogens and subsequent chance for carcinogenesis (Holmberg SB, 1991).

1.2.6. Drugs

Antimetabolites and glucocorticosteroids are associated with possible carcinogenesis in man. The major cancers produced are leukemias and lymphomas. Acute myelocytic leukemia is an unfortunate and rare complication of alkylating agents such as melphalan and
cyclophosphamide (Natori K, et al., 2007). Table 1.2. lists the different drugs associated with possible carcinogenesis in man.

Table 1.2.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Patient Population</th>
<th>Associated Cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cyclophosphamide</td>
<td>Cancer</td>
<td>Bladder cancer, acute myelogenous leukemia (AML)</td>
</tr>
<tr>
<td>Melphalan</td>
<td>Multiple myeloma</td>
<td>AML</td>
</tr>
<tr>
<td>Azathioprine and cyclosporine</td>
<td>Allotransplant recipients</td>
<td>Non-Hodgkin’s lymphomas, skin cancer</td>
</tr>
<tr>
<td>Nitrogen mustard, procarbazine, ionizing radiation</td>
<td>Cancer</td>
<td>AML</td>
</tr>
<tr>
<td>Synthetic (diethylstilbestrol)</td>
<td>Females</td>
<td>Vagina, cervix (adenocarcinoma)</td>
</tr>
<tr>
<td>Phenacetin-containing drugs</td>
<td>Over-the-counter pain treatment</td>
<td>Renal pelvis carcinoma</td>
</tr>
</tbody>
</table>

(Adapted from Weisburger JH, et al., 1995)

1.2.7. Proto-oncogenes, Oncogenes and Tumor Suppressor Genes

Most, if not all, cancer cells contain genetic damage that appears to be the responsible event leading to tumorigenesis. The genetic damage present in a parental tumorigenic cell is maintained (i.e. not correctable) and transmitted to subsequent generations. Genetic damage found in cancer cells is of two types:

1. Dominant and the genes have been termed proto-oncogenes. The distinction between the terms proto-oncogene and oncogene relates to the activity of the protein product of the gene. A proto-oncogene is a gene whose protein product has the capacity to induce cellular transformation
given it sustains some genetic insult. An oncogene is a gene that has sustained some genetic damage and, therefore, produces a protein capable of cellular transformation.

The process of activation of proto-oncogenes to oncogenes can include retroviral transduction or retroviral integration, point mutations, insertion mutations, gene amplification, chromosomal translocation and/or protein-protein interactions (Kiernan HP, 2007; Feng Q, et al., 2007).

2. recessive and the genes variously termed tumor suppressors, growth suppressors, recessive oncogenes or anti-oncogenes.

Given the complexity of inducing and regulating cellular growth, proliferation and differentiation, it was suspected for many years that genetic damage to genes encoding growth factors, growth factor receptors and/or the proteins of the various signal transduction cascades would lead to cellular transformation. This suspicion has proven true with the identification of numerous genes, whose products function in cellular signaling, that are involved in some way in the genesis of the tumorigenic state. The majority of these proto-oncogenes were identified by either of two means: as the transforming genes (oncogenes) of transforming retroviruses or through transfection of DNA from tumor cell lines into non-transformed cell lines and screening for resultant tumorigenesis. Table 1.3 lists some of the oncogenes and tumor suppressor genes associated with human cancers.

Table 1.3.

Examples of Oncogenes and Cancer Suppressor Genes Associated With Human Cancers

<table>
<thead>
<tr>
<th>Gene</th>
<th>Tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>MYC</td>
<td>Many solid tumors and hematologic malignancies</td>
</tr>
<tr>
<td>N-MYC</td>
<td>Neuroblastoma and lung cancers</td>
</tr>
<tr>
<td>Genes</td>
<td>Tumors Associated with</td>
</tr>
<tr>
<td>-------</td>
<td>------------------------</td>
</tr>
<tr>
<td><strong>L-MYC</strong></td>
<td>Small cell lung cancer</td>
</tr>
<tr>
<td><strong>RAS</strong></td>
<td>Many solid tumors</td>
</tr>
<tr>
<td><strong>p53</strong></td>
<td>Many sporadic malignancies (most commonly mutated cancer gene); when inherited, causes the Li-Fraumeni familial cancer syndrome</td>
</tr>
<tr>
<td><strong>BRCA1</strong></td>
<td>Breast cancer, ovarian cancer, prostate cancer</td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td>Breast and ovarian cancer</td>
</tr>
<tr>
<td><strong>APC</strong></td>
<td>Colon familial polyposis (a premalignant condition)</td>
</tr>
<tr>
<td><strong>MSH, MLH</strong></td>
<td>Mismatch repair genes associated with hereditary nonpolyposis colon cancer (HNPCC)</td>
</tr>
<tr>
<td><strong>DCC</strong></td>
<td>Colon cancer</td>
</tr>
<tr>
<td><strong>RB</strong></td>
<td>Retinoblastoma and some other tumors</td>
</tr>
<tr>
<td><strong>NF1, NF2</strong></td>
<td>Neurofibroma</td>
</tr>
<tr>
<td><strong>C-ERB-b2</strong></td>
<td>Breast cancer (also called HER-2/neu), other tumors</td>
</tr>
<tr>
<td><strong>ABL</strong></td>
<td>Chronic myeloid leukemia</td>
</tr>
<tr>
<td><strong>WT1</strong></td>
<td>Wilms’ tumor</td>
</tr>
<tr>
<td><strong>ATM</strong></td>
<td>Ataxia telangiectasia associated with multiple cancers</td>
</tr>
<tr>
<td><strong>MEN</strong></td>
<td>Multiple endocrine neoplasia syndrome</td>
</tr>
<tr>
<td><strong>HPC1</strong></td>
<td>Prostate cancer</td>
</tr>
<tr>
<td><strong>VHL</strong></td>
<td>Renal and some other cancers</td>
</tr>
<tr>
<td><strong>PTCH</strong></td>
<td>Basal cell cancers (Gorlin syndrome)</td>
</tr>
<tr>
<td><strong>p16</strong></td>
<td>Melanoma</td>
</tr>
<tr>
<td><strong>BCL-2</strong></td>
<td>Non-Hodgkin’s lymphoma</td>
</tr>
</tbody>
</table>
1.3. Characteristics of Cancer Cells

A cancer cell is characterized by six prominent alterations in its physiological processes that collectively dictate malignant growth (Figure. 1.1.); self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis (Hanahan D & Weinberg RA, 2000). Trosco et al., (2004) enlighten one more hallmark of carcinogenesis; stem cell and cell-cell communication.

1.3.1. Self-sufficiency in growth signals

Malignant signals pass a normal cell from its quiescent state into a proliferative state. Many of the oncogenes in a cancer cell act by mimicking normal growth signaling in order to maintain growth potential. Tumor cells invariably show a reduced dependence upon growth signals from exogenous source. The liberty from the dependence over exogenous signals disrupts important homeostatic mechanism that normally ensures the apt behavior of cells within a tissue. The autonomy brings about by adopting any of the three strategies; alteration of extracellular growth signals, or of transcellular transduction of those signals or of intracellular circuits that translate those signals into action. The cancer cells obviate dependence over growth signals from their microenvironment by manufacturing the growth factors themselves (Fedi P, et al., 1997). The production of PDGF and TGF-α by glioblastoma and sarcomas, respectively, are two examples (Brockmann MA, et al., 2003).

1.3.2. Insensitivity to growth-inhibitory (antigrowth) signals

Multiple antigrowth signals operate to maintain cellular quiescence and tissue homeostasis; these signals include both soluble growth inhibitors and immobilized inhibitors embedded in the extracellular matrix and on the surface of nearby cells.

Antiproliferative signals can block proliferation by forcing cells into quiescent (G₀) state or induced enter into post mitotic states, usually associated with acquisition of specific differentiation-associated traits. At
the molecular level, the antigrowth signals are funneled through retinoblastoma protein (pRb) and its two relatives, p107 and p130. pRB blocks E2F protein, an transcription factor, from inducing the genes essential for progression from G1 to S phase (Weinberg RA, 1995). pRB phosphorylation suppresses it and liberates the E2F. TGFβ causes synthesis of the p15INK4B and p21 proteins, which block the cyclin:CDK complexes responsible for pRB phosphorylation(Hannon GJ & Beach D, 1994; Datto MB, et al., 1997). The pRB signaling circuit can be disrupted in a variety of ways in different types of human tumors (Fynan TM & Reiss M, 1993). Some lose TGFβ receptors while others display mutated dysfunctional forms (Markowitz S, et al., 1995).

Cancer cells also avoid terminal differentiation step by expressing of c-myc in association with max, which is growth stimulatory in action. But, in normal development, max complexes with another transcription factor called mad, and this complex elicits differentiation-inducing signals (Foley KP & Eisenman RN, 1999). But over expression of c-myc can reverse this process, shifting the balance back to favor myc – max complex, thereby impairing differentiation and promotes growth.

1.3.3. Evading apoptosis

The rate of cell proliferation and rate of cell attrition determine the survival and expansion of tumor cell population. The acquired resistance towards apoptosis is a hallmark of most and perhaps all types of cancer.

In 1972, Kerr, Wyllie and Currie described the possibility that apoptosis serves as a barrier to cancer (Kerr JF, et al., 1972). The upregulation of bcl-2 oncogene in follicular lymphoma (Korsmeyer SJ, 1992) and its antiapoptotic activity (Vaux DL, et al., 1988) opened up the investigation of apoptosis in cancer at the molecular level. 50% of infrequent lymphomas arising in bcl-2 single transgenic mice had somatic translocation activating c-myc, confirming a selective pressure during lymphomagenesis to upregulate both bcl-2 and c-myc(McDonnel TJ & Korsemeyer SJ, 1991). The inactivation of p53 tumor suppressor protein, a
component of the apoptotic signaling circuitry, led to rapidly growing tumors containing low number of apoptotic cells (Symonds H, et al., 1994). The expression of mutated p53 gene resulting in functionality inactivation of its “translate,” the p53 protein, is seen in 50% of human cancers (Harris CC, 1996). Hypoxia and oncogene overexpression evoke the signals are also channeled to apoptosis by p53(Levine AJ, 1997).

1.3.4. Limitless replication potential

The acquired characteristic growth signal autonomy, insensitivity to antigrowth signals and evasion of apoptosis – uncouples a cells’ growth program from signals in its environment. In principle, the deregulated proliferation program should suffice to enable the generation of the vast cell population that constitutes macroscopic tumors. The disruption of cell-to-cell signaling not only suffice to bring about unmanaged replication potential but the intrinsic, cell- autonomous program that limits their multiplication is also disrupted.

The early works of Hayflick suggest that cells in cultures have a finite replicative potential (Hayflick L, 1997). After a certain number doubling, the cell populations stop growing a process called senescence. The senescence of cells is circumvented by disabling their pRB and p53 tumor suppressor protein in cultured human fibroblasts enabling the cells to continue multiplying for additional generations until they enter a state called crisis. This crisis state is characterized by massive cell death, end-to-end fusion of chromosomes and occasional emergence of variants, (1 in 107) cells that acquired this ability to multiply without limit, the trait termed immortalization (Wright WE, et al., 1989).

The loss of telomere at each cell cycle is the cause for chromosomal fusion. Telomere maintenance is evident in almost all types of malignant cell types (Shey JW & Bacchetti S, 1997; Ohali A, et al., 2006; Stewart SA & Weinberg RA, 2006). By upregulating telomerase, which adds 6bp repeats onto the ends of telomeric DNA (Bryan TM & Cech TR, 1999). By this or other mechanism, telomeres are maintained at a length above a
critical threshold, and this in turn permits unlimited multiplication of descendent cells.

Circumvention of cellular senescence is another factor for unlimited replication. The senescence of the cells in the cultures could be an artifact that does not reflect a phenotype of a cancer cell in the living tissue and doesn’t represent an impediment to tumor progression in vivo (Effros RB, et al., 2005).

1.3.5. Sustained Angiogenesis

The oxygen and nutrients supplied by the vasculature are crucial for cell function and survival. Once a tissue is formed, the growth of new blood vessels- the process of angiogenesis- is transitory and carefully regulated. The cells within aberrant proliferative lesions initially lack angiogenesis ability, curtailing their capability for expansion. In order to grow to a large size, incipient neoplasia must develop angiogenic ability (Cao Y, 2005).

Vascular endothelial growth factor (VEGF) and acidic and basic fibroblast growth factor transmit angiogenesis-initiating signals. Each binds to tyrosine kinase receptors on the endothelial cell (Fedi P, et al., 1997; Veikkola T & Alitalo K, 1999). There are currently more than a dozen angiogenic inducer factors and similar number of endogenous inhibitor proteins.

Tumors appear to activate the angiogenesis switch by changing the balance of angiogenesis inducers and countervailing inhibitors (Hanahan D & Folkman J, 1996). One common strategy for shifting balance involves altered gene expression. Many tumors are associated with elevated expression of VEGF and/or FGFs compared to their normal tissue counterpart. In others, the expression of endogenous inhibitors such as thrombospondin -1 level to fall, liberating endothelial cells from the inhibitory effects (Dameron KM, et al., 1994). The VEGF gene is also under complex transcriptional control. For example, activation of the ras oncogene or loss of the VHL tumor suppressor genes in certain cancer

1.3.6. Tissue invasion and Metastasis

During the causes of the development of the most types of human cancer, primary tumor masses spawn pioneer cells that move out, invade adjacent tissues, and thence travel to distant sites where they may succeed in founding new colonies. These distant settlements of tumor cells—metastases—are the cause of 90% of human cancer death (Sporn MB, 1996). Distant metastases represent the major cause of death after curative surgery of colorectal cancer (Losi L, et al., 2007).

Invasion and metastasis are exceedingly complex processes involving changes in the physical coupling of cells to their microenvironment and activation of extracellular proteases. E-cadherin is one of the cell-to-cell interaction molecules, which limit the cells from detaching from its neighborhood. Coupling between adjacent cells by E-cadherin bridges results in the transmission of antigrowth and other signals via cytoplasmic contact with β-catenin to intracellular signaling circuits (Christofori G & Semb H, 1999). E-cadherin expression is lost in majority of epithelial cancers, by mechanisms that include mutational inactivation of the E-cadherin or β-catenin genes, transcriptional repression, or proteolysis of intracellular cadherin domain.


1.3.7. Stem cell and cell-cell communication

In the analysis of carcinogenesis role of pluripotent stem cells and gap junctional intercellular communication (GJIC) must be brought into consideration (Trosko JE & Chang CC, 2003). In the initiation stage of carcinogenesis, a single cell is irreversibly blocked from terminal differentiation. The promotion phase is a potentially reversible or
interruptible clonal expansion of the initiated cell by a combination of
growth stimulation and inhibition of apoptosis. The progression phase is
achieved when the expanded initiated cells accrue sufficient mutations and
epigenetic alterations to become growth stimulus independent and
resistant to growth inhibitors and apoptosis. Stem cell and its early
progenitor cell are the target cells for the initiation event. These cells are
naturally immortal and become mortal only when they are induced to
terminally differentiate and lose their telomerase activity (Ju Z & Rudolph
KL, 2006). These two types of initiated cells are suppressed by either
secreted negative growth regulators (the stem cells) or GJIC (the early
initiated progenitor cells). Promoters inhibit either the secreted growth
inhibitor to initiated stem cells or GJIC between the initiated progenitor
cells and the normal progenitor cells (Trosko JE, et al., 1998). When a
stable resistance to the secreted negative growth regulator or permanent
downregulation of GJIC has occurred, the cell has entered the progression
phase. These two new concepts contradict the current paradigm that the
first phase of carcinogenesis is the immortalization of a normal cell

1.4. Different Genetical Routes To Cancer

1.4.1. Alterations in the cell signaling

The discovery of viral oncogenes was the first evidence for the host
genes could cause cancer. Normally the protooncogenes act as biochemical
switches to control and command the processes of a cell, specifically
transmitting signals from outside of cell to nucleus. The mutations in any
of the members of this relay process leads it constitutively activated,
resulting in the characteristic of a cancer cell- the uncontrolled growth. In
10% to 20% of human cancers are associated with mutation in the ras
oncogene (Mascaux C, et al., 2005). Ras is controlled by GTPase activating
proteins and transmits signals by activating raf, this in turn causes
expression of nuclear protein jun, fos, and myc(Staber PB, et al., 2004;
Duquette ML, et al., 2007). Myc is mutated and rearranged in lymphoid
malignancies and amplified in breast cancers. *jun, fos* and *myc* are the proteins that cause the expression of other genes. So the mutation in the relay nodes of these signal transduction pathways is potential for oncogenic conversion. Either *ras*-GTPase activating proteins or NF1 can regulate Ras; and stimulation of the ras pathway activates a number of MAP kinase for their constitutive expression and continuous transmission of signals to nucleus (Hiatt KK, *et al.*, 2001).

**1.4.2. Unwanted Expression of Proteins**

The expression of a normal protein that has no function in the biology of a specific tissue also leads to cancer. Several transcription factors come under this category: *myc, tal-1/SCL, lyl-1, Ttg-1* and *Ttg-2* are examples for this. *Myc* is expressed in all cells and has roles in cell division and differentiation (Littlewood TD & Evans GI, 1990). *Myc* expression could be seen deregulated in B-cell acute lymphocytic leukemia (Ryor Pokora B, *et al.*, 1991). *tal-1/SCL, lyl-1, Ttg-1* and *Ttg-2* are linked to T-cell acute lymphocytic leukemia. This type of oncogenic conversion is due to expression of protein in the inappropriate tissues. *Lyl-1* is usually expressed in myeloid and B-lymphoid cells; and *Ttg-2* expression is found in liver, spleen, and kidney; *lyl-1* in erythroid and myeloid precursors; all these are not expected to expressed in T-cells. The expression of these genes serves as a switch to induce malignancy.

**1.4.3. Relaxed Suppression**

Tumor suppressor genes by the loss of function contribute to malignant conversion (Turker MS, 2003). Tumor suppressor genes such as retinoblastoma gene, Rb-1 and p53 blocks cell proliferation at unwanted occasion by distinct pathways. Rb-1 binds with transcription factor E2F and loss of function of Rb-1 causes the release of suppression over E2F and induction of transcription. p53 enhances the expression of p21/CIP1, which is the suppressor of CDKs essential for the cell cycle to continue through different checkpoints (Wakasugi E, *et al.*, 1997; Lee WS, *et al.*, 2003). Loss of p53 and the attenuation of p21/CIP1 expression result in
unmanaged progression through the cell cycle. In congenital retinoblastoma, Rb-1 mutation and in Li-Fraumeni multicancer syndrome, p53 mutations are encountered (Petitjean A, *et al.*, 2007). As with oncogenes, presence of a single abnormal allele of tumor suppressor gene is not sufficient to develop cancer; other genetic lesions are also needed. The human papilloma virus (HPV) causes many cervical, anal and penile carcinomas in human beings, inhibits both Rb-1 and p53 through binding with and inactivation by viral protein E6 and E7 (Wu SY, *et al.*, 2006, 2007).

One category of tumor suppressor genes are CDK inhibitors such as p16, p27 and p57 and mutations in them are associated with many diverse cancers ranging lung, head and neck, breast and pancreatic cancers (Looi K, *et al.*, 2006; Pateras IS, *et al.*, 2006; Kalemi TG, *et al.*, 2007). p16 mutation is common in malignant melanoma (Conscience I, *et al.*, 2006). The disruption of the suppressor function leads to cancer.

### 1.4.4. Imbalance in Cell Death /Cell Proliferation Ratio

Current researches point that abrogation of programmed cell death (apoptosis) (PCD) is an important mechanism for neoplastic transformation (Jaattela M, 2004). The cells in response to many assaults bring about the PCD and it needs a concerted action of many components, of which caspases attain special attention. Some of the components of this system are involved in cancer and cancer treatment.

Bcl-2 is an oncogene that blocks apoptosis when overexpressed or abnormally expressed. The *bcl-2/bax* ratio is one of the determinant factors for whether the cell should (Cox AG, *et al.*, 2007) undergo apoptosis or not. *Bcl-x*, like *bcl-2*, is antiapoptotic (Zhou H, *et al.*, 2005), while *bax, bad, bak, bcl-Xs* are inducers of apoptosis. The ratio of this antiapoptotic and proapoptotic proteins in the cell is a responsible factor for responsiveness to chemotherapy or radiation (Scopa CD, *et al.*, 2001). Normally, when the cells exceed a critical level in their mutation burden, self-destruct processes are initiated. But, cancer may result when
genetically abnormal cells are not cleared but are allowed to proliferate, these accumulating mutations potentially important in cancer causes.

1.5. Signal Transduction Pathways in Cancer

A number of characteristic alterations gathered by the cell could be encountered during the course of tumor initiation and progression (Martin GS, 2003). Mainly, the transforming cell becomes independent of exogenous growth stimulatory or inhibitory signals for invading distant sites to metastasize and to elicit angiogenesis. They also acquire ability to evade cell proliferation limiting mechanisms like apoptosis and replicative senescence. These reflect the change in the signal transduction pathways in the cancer cell from normal counterparts.

The change in the component proteins of any of the pathway leads to the disruption or alteration in the interconnected signal transduction pathways. The basic problem still more to be unraveled is that how these complex pathways work in vitro and in vivo and how it is changed in cells when it is transformed.

The coordination of the different complex pathways by the network is achieved by the nodal action of component protein molecules of the network of which modular protein domains help to protein–protein interactions in order to receive and relay the signals to bring about effective output (Pawson T & Nash P, 2003). Different pathways involve in the characteristics like cell motility, invasion, cell growth, survival etc. of a cancer cell.

1.5.1. Src and FAK in Cell Motility and Invasion

v-src is the first protooncogene found in vertebrate genome. By interacting with FAK, (Focal Adhesion Kinase) src plays a crucial role in tumor cell invasion (Chang LC, et al., 2005). src and FAK are nonreceptor tyrosine kinases located to cell-matrix adhesions and mediate integrin signaling. Binding with integrins, FAK undergoes autophosphorylation at Tyr$^{397}$ and src was recruited to activated-FAK via an interaction between the SH2 domain of src and FAK pTyr$^{397}$ (Toutant M, et al., 2000). src
then phosphorylates FAK at different Tyr residues, making docking sites for different SH2 domain-containing signaling protein like Grb2 (Arola ST, et al., 2002). src and FAK are overexpressed in many epithelial tumors, especially in invasive cancers. Src and FAK are found in podosomes or invadopodia of cancer cells and which are involve in the degradation of extracellular matrix (Hauck CR, et al., 2002). These observations have provided circumstantial evidence for a role of src and FAK in tumor cell motility and invasion (Cox BD, et al., 2006).

Another mechanism works for the enhancement of tumor cell invasion by the interaction of src and FAK is through the activation of JNK (Cox BD et al., 2006). This involve a cascade of complex signaling network involving src, FAK, the docking protein Cas, the adapter Crk, and a guanine nucleotide exchange factor, DOCK180, which leads to the activation and secretion of matrix metalloproteinases such as MMP-2 & 9 (Hsia DA, et al., 2003). In contrast to in vitro, FAK promote the growth of src-transformed cells in vivo and is related to increased expression of VEGF and increased tumor angiogenesis (Laird AD, et al., 2003).

Src regulates cell motility and morphological transformation via different mechanism that involves both FAK dependent, which involves the local activation of Ras or Rac, and FAK independent in which FISH is phosphorylated and cofilin is dephosphorylated. It is obvious from the above description that Src and FAK are promising targets for inhibition of tumor cell invasion (Rishi AK, et al., 2006).

1.5.2. The Ras/MAP kinase pathways

Ras is mutated in significant fractions of human cancers. It has multiple effectors. One of which is Raf; through the Raf-MEK-ERK pathway. Many components of ERK/MAP kinase pathway are still to be identified and are under the intense investigation as possible target for anti-cancer drugs.

The MAP kinase pathway is controlled by a negative feedback loop. The EphA2, activated by MAP kinase in turn activates p120RasGAP (Miao
H, et al., 2001) that downregulates wild type Ras but not mutationally activated Ras. Activation of MAP kinases have antiapoptotic effect that are important for tumor cell survival. Recently, a novel target for MAP kinase has been described, the caspase 9. The ERK MAP kinases inhibit caspase-9 by phosphorylation. (Allan LA, et al., 2003). RKIP or Raf kinase inhibitor protein is one of the scaffold protein negatively regulates MAP kinase pathway by blocking interaction of Raf with MEK (Yeung K, et al., 1999). As an inhibitor Raf-MAPK pathway, RKIP may function as a tumor suppressor, and indeed RKIP expression is downregulated in metastatic breast and prostate cancers.

1.5.3. PI3 Kinases- Role in Cell Growth, Motility and Survival

PI-3 kinase is a lipid kinase phosphorylates its substrate to 3'-phosphoinositide. PI-3 kinase plays a role in cell growth, motility and survival (Michl P, et al., 2005; Kim D, et al., 2005). PTEN is a tumor suppressor dephosphorylates 3'-phosphoinositide and the gene encodes p110α, the catalytic subunit of a type 1A PI-3 kinase, is amplified in human cancers. The downstream targets of PI-3 kinase pathway includes a protein kinase Akt and a translation initiation factor eIF4E, are transforming in cell cultures. These observations show the importance of PI-3 kinase pathway in tumorigenesis (Xia C, et al., 2006)

1.5.4. Role of Akt/mTOR in Tumorigenesis

PI-3 dependent kinases PDK1 and Akt (PKB) initiate a kinase cascade that plays a key role in growth regulation. The disruption of the ubiquitously expressed member of the Akt family of genes, Akt1, in the mouse demonstrates a requirement for Akt1 in ErbB2-induced mammary tumorigenesis (Ju X, et al., 2007). PDK1 is the kinase phosphorylates Akt, which in turn phosphorylates mTOR. mTOR can regulate translation in two ways. First, activation of ribosomal S6 protein kinase, which is responsible for translation of TOPmRNAs that encode ribosomal proteins and other components of translational machinery and second, mTOR inactivates 4EBP-1, an inhibitor of eIF4E. In this way PI-3 kinase pathway
regulates the translational machinery and thus cell growth. Rapamycin can inactivate the mTOR, which prevents the transformation by avian sarcoma viruses encoding activated and membrane targeted forms of PI-3 kinases or Akt (Aoki M, 2001)

1.5.5. **Protein Kinase Cs in Cell Matrix Interaction and the Control of Cell Polarity**

Conventional isoforms of protein kinase C (cPKCs; α, β and γ) are of intense interest since they are targeted by tumor promoters, such as TPA (Hu LY, et al., 2006). The recently found novel PKCs and atypical PKCs play a critical role in the response to cell-cell and cell-matrix interaction and in the development of cell polarity. Novel PKCε integrates cytokine signaling (Ivaska J, et al., 2003). The major determinants of the cell polarity are a complex of GTPase, Cdc42, aPKC and two scaffolding proteins par3 and par6. In mammalian cells there is direct interaction between aPKC, par6 and mLgl, the mammalian homology of the *Drosophila* tumor suppressor lethal(2) giant larvae (Plant PJ, et al., 2003). Thus aPKC is directed to its substrate by specific protein–protein interaction that connect the par3–par6 cell polarity complex to other complexes that regulate cell polarity, vesicle trafficking, microtubule stability, cell junction formation and cell proliferation.

1.6. **Apoptotic Pathway – A Primary Target for Cancer Therapy**

Apoptosis, or programmed cell death is a major mechanism by which cells undergo death to control cell proliferation or in response to DNA damage if it is not repaired (Lowe SW, et al., 2000) and also that is essential for the development and maintenance of multicellular organisms. The malignant cells are either defiant or defective in the apoptosis regulatory pathways such as p53, NF-κB or PI3/Akt leading to cell immortalization or evasion of apoptosis (Kaufmann SH, et al., 2001). Some type of human cancer such as B-cell chronic lymphocytic leukemia
(CLL), follicular lymphoma (Tsujimoto Y, et al., 1985) and tumors infected by human T-cell leukemia/lymphoma virus-1 (Hengartner MO, 2000). Are characterized by the defect in apoptosis and immortalization of cells is resulted. Because the apoptotic pathways are altered in tumor tissue, there is a potential for differential effect for the therapy sparing the normal tissue.

1.6.1. Extrinsic & Intrinsic Pathways of Apoptosis

The pathways of apoptosis have been elucidated so far; of which the first, referred as extrinsic or cytoplasmic pathway, is triggered by Fas death receptor, a member of TNF receptor subfamily (Zapata JM, et al., 2001). The second, referred as intrinsic or mitochondrial pathway is initiated by the release of cytochrome c from inner membrane of mitochondria and activation of the death signal (Hockenberry D, et al., 1990). Both the pathways converge to caspase-3 to a common pathway of caspases to bring about the apoptosis(Figure 1.2).

1.6.1.1. Caspases

Caspases are cysteinyl aspartate proteinase. The first known member of this family was caspase-1, which was initially known as IL-1beta converting enzyme (ICE) (Dinarello CA, 2001) Not all the caspases have role in apoptosis (Thonberry NA, 1998).

By a proximity-induced activation (Fuentes-Prior P and Salvesen GS, 2004; Boatright KM and Salvesen GS, 2003) the procaspases are activated at the specific death-signaling complex. Actually the prodomains of the caspase mediate them to death signaling complex. The procaspases activated by attaching themselves to death signaling complex are initiator caspases and they in turn recruits further executioner caspases, in mammals caspase 8 and caspase 3 respectively in death receptor pathway. The main initiator caspases are caspase –2, -8, -9 and –10. Caspase 2 and 9 contain a caspase recruitment domain (CARD), while caspase 8 and 10 contain a pair of death effector domain. (DEDs). The prodomain in caspase play a role in their self-activation and those having not a
prodomain lacks the ability to self activate and need to be activated by
initiator caspases. Out of downstream caspases –3, -6 and –7, caspase-3 is
the main executioner caspase that cleaves the inhibitor of the caspase
activated deoxyribonuclease leads to its activation and nuclear apoptosis.
The downstream caspases induce cleavage of protein kinase, cytoskeleton
protein DNA repair protein, inhibitory subunits of endonucleases (CIDE
family) and finally destruction of “housekeeping” cellular function.
Caspases also affect cytoskeleton structure, cell cycle regulation and
signaling pathways ultimately to the manifestation of morphological
changes in apoptosis like DNA condensation and zeiosis (membrane

1.6.2. Particular Proteins Regulates Apoptotic Pathway

1.6.2.1. p53-Guardian of Genome

p53 is a transcriptional factor function in the normal cells response
to damage owing to cellular stresses such as DNA damage, oncogenic
stimulation, nutrient deprivation or hypoxia. P53 and its downstream
target genes like PCNA, p21, TIGAR, sco2 involved in the cell survival and
Puma, BAX, DR5 and PIG3 are annexed with apoptosis (Rozan LM, 2007)
p53 becomes activated upon the exposure DNA damaging agents like
gamma-radiation, UV or chemotherapeutic agents. The mechanism of
tumor suppression by p53 has not been identified completely. More
functions like involvement in the mitochondrial respiration and its impact
on cellular metabolism and tumorigenesis (Matoba S, 2006). Also, stromal
cell communication with tumor epithelia is p53 dependent manner (Hill R,
2005).

1.6.2.2. NF-κB

NF-κB is an important transcriptional factor with major roles in the
regulation of genes involved in the regulation of apoptosis, viral
replication, tumorigenesis, inflammation and many autoimmune diseases
(Maldonado V, 1997) in cells, NF-κB is sequestered in the cytosol in its
inactive form bound with its inhibitor protein of the IkB family. NF-κB
activation causes phosphorylation of IκB and its subsequent degradation. The translocated NF-κB in the nucleus binds with the consensus sequence of various genes and thus activates their transcription (Maldonaldo V, 1997). The proapoptotic and antiapoptotic properties of NF-κB depend on the origin of tissue. Normally under physiological condition upregulation of NF-κB activates complex proteins to induce resistance to death signals. The proteins activated are TNF receptor associated factor, IAP and X-linked IAP. In some instance the activation of NF-κB cause apoptosis (Kuhnel F, 2000). It may be explained as the activation of some proapoptotic protein like c-myc, interferon-regulated factor –1 and p53. Some of the virus-mediated apoptosis is also marked with NF-κB activation (Kuhnel F, 2000).

1.6.2.3. The Ubiquitin Proteosome System

It is a large proteinase complex that is responsible for the turnover of most intracellular proteins and consequently regulates cell growth and apoptosis (Myung J, 2001). The protein to be degraded selected by binding with ubiquitin molecules and subsequently 26S proteosome degrades them. Many cell cycle regulatory proteins like p53, cyclin and cyclin dependent kinase inhibitors and NF-κB are regulated by this pathway (Adams J, et al., 2000). Proteosomal inhibition leads to initial accumulation of p53, p27, proapoptotic bax & Bad or the activation of stress kinase, which results in the release of cytochrome c from mitochondria and triggers intrinsic apoptotic pathway (Adams J, et al., 1999).

1.6.2.4. PI3K

PI3 kinase plays a central role in signaling pathways important to cell survival, proliferation, motility and tissue neovascularization. PI3K is unregulated in many cancers (Hidalgo M, et al., 2000). The second messengers like phosphatidyl inositol 4, 5- bisphosphate 3 and phosphatidyl 3, 4, 5- triphosphate convey the messages from the
cytoplasm to cell surface. The former induce the activation of protein kinase 1, which in turn activate the kinase Akt. Akt activation promotes cell survival by releasing NF-κB (Cantley LC, 2002). Phosphorylation of Bax by Akt also leads to cell survival. The other phosphorylation events for the block of apoptosis are phosphorylation of caspase 9 and Fork-head related transcription factor 1.

mTOR, the downstream component of Akt pathway, is now being considered as target for novel therapies. The inhibition of mTOR blocks the signals to two pathways, the 40S ribosomal protein S6 kinase (p70S6K) and 4E-binding protein-1 (4E-BP1). These check the cells to transit from G1 to S phase and their inhibitor leads to growth arrest. Abnormalities in cyclin D, p53, pRB, and p16 can increase PI3K activity and make it more prone to mTOR inhibitor (Dancey JE, 2002).

1.6.2.5. The Bcl-2 Protein Family

The Bcl-2 protein family consists of proteins regulating apoptosis. Some of the members are proapoptotic and others are antiapoptotic. Induction of apoptosis by inhibiting the inhibitor of apoptosis is a strategy for the finding of novel drug molecules (Fesik S, 2005). The BH-3 only proteins can bind with antiapoptotic Bcl-2, Bcl-xL and MCL 1 through the interaction of BH3 domain with hydrophobic groove in the antiapoptotic protein. ABT-737 is a small molecule inhibiting these antiapoptotic proteins by mimicking the BH3 domain (Konopleva M, 2006; Van Delft M, 2006). ABT-737 inhibits Bcl-2 and induces apoptosis in multiple myeloma cells (Kline MP, et al., 2007).

1.7. Modalities of Cancer Therapy

1.7.1. Surgery

Being the oldest modality for cancer management, it remained as the only treatment that could cure patients with cancer until very recently. Surgery has the role in prevention of cancer, diagnosis and treatment of cancer.
1.7.2. Radiation

Ionizing radiation is energy that, during absorption, causes the ejection of an orbital electron. A large amount of energy is associated with ionization. Examples for particulate radiation are electrons, protons, α-particles, neutrons, negative pi mesons and atomic nuclei. Radiation may interact with cellular target molecules either directly or indirectly. The important target molecule is DNA while considering its importance in cell reproduction. Other important biological effects of radiation (eg. edema) are far more likely to be caused by its action on membranes.

Apoptosis is an important response to radiation in many cells (Dewey WC, et al., 1995). The relative proportion of cells undergoing apoptosis rather than pausing in cell cycle to repair radiation damage may be a very important determinant of the likelihood of the radiation curability of cancer. A number of genes associated with oncogenesis affect the likelihood of a cell demonstrating programmed cell death after DNA damage, including bcl-2, Bcl-Xs and p53 (Boise LH, et al., 1993; Thompson CB, 1995). A variety of factors can induce or stimulate apoptosis including those stimulated by radiation (Fuks Z, et al., 1994).

The most important modifier of biologic effects of radiation is molecular oxygen. For equivalent killing of cells at every level of survival, greater doses are needed under hypoxic condition than under oxic condition. Radiosensitizers augment the amount of injury induced by radiation to hypoxic cells that are relatively resistant, has increased the effectiveness of radiation therapy.

1.7.3. Chemotherapy

The introduction of chemotherapy has resulted in the development of curative therapeutic interventions for patients with several types of advanced solid tumors and hematologic neoplasm. The systemic treatment of cancer has its root in the work of Paul Ehrlich, who coined the term chemotherapy. Alkylating agents represent the first class of chemotherapeutic drugs to be used in the clinical setting. Meanwhile, the
development of alkylating agents as antitumor agents, Sidney Farber reported that folic acid has a significant proliferative effect on leukemic cell growth in children with lymphoblastic leukemia. This caused the development of folic acid analogues as drugs to inhibit folic acid metabolism in cancer cells.

Currently, chemotherapy has role in four different clinical settings (DeVita VT, 1988) 1) as induction treatment for advanced diseases. 2) as an adjunct to local methods of treatment 3) as primary treatment for some patients to whom local forms of therapy by themselves are inadequate 4) direct instillation into sanctuary sites of cancer.

1.8. Different Classes of Chemotherapeutics

Chemotherapeutic compounds are classified as either non-plant derived or plant derived (Figure.1.3).

1.8.1. Non-plant Derived.

They include,

1.) Antitumor antibiotics, 2.) Antimetabolites, 3.) Alkylating agents.

1.8.1.1. Antitumor Antibiotics- These are synthesized as a result of microbial fermentation. It comprises important compounds like bleomycin, anthracyclines, various unusual nucleotides, actinomycin D, mitomycin C and mitramycin. Anthracyclines comprise two important compounds, daunorubicin and doxorubicin.

1.8.1.2. Antimetabolites- Antimetabolites are chemical compounds itself as a proxy to the normal substrates for the metabolism to bring about the inhibition on vital metabolism essential for the maintenance of cell integrity. Aminopterin is the first studied antimetabolites proved to be active in clinic for the treatment of acute leukemia in children (Farber S, et al., 1948). Later, methotrexate (MTX) replaced aminopterin and currently MTX remain as the widely used antifolate in cancer chemotherapy. MTX is the tight – binding inhibitor of dihydrololate reductase (DHFR), a critical enzyme for folate metabolism. The other important antimetabolites are trimetrexate, tomudex, 5-fluopyrimidines, cytorabine, gemcitabine etc.
1.8.1.3. Alkylating Agents- A nitrogen mustard alkylating agent was the first non-hormonal chemical that demonstrated significant antitumor activity. The gas has vesicant effect on skin and mucos membrane and in addition to this, depression in hematopoeitic and lymphoid system was also observed in experimental animals. The important alkylating agents are nitrogen mustard, cyclophosphamide, ifosfamide, melphan, chlorambucil, thiota and busulfan.

The alkylating agents are potent electrophiles and react with many electron-rich molecules within the cell to be inactivated. One such principal molecule is glutathione (GSH), a tripeptide with a free cysteine sulfhydryl that is present at millimolar concentrations in cell. Cells can be sensitized to alkylating agents by exposure to inhibitors of GSH-S-transferases.

1.8.2. Plant-Derived Compounds As Antineoplastic Agents

Plants produce different complex structures with several chiral centres, which are important antineoplastic compounds such as the alkaloids, vinblastine, paclitaxel, camptothecin and the lignan molecule podophyllotoxin, the anthraquinone derivative, emodin etc (Wink M, et al., 2005)

From the early days of mankind, plants with secondary metabolites have been used by humans to treat infections, health disorders and illness (Mann J, 1992, Robert MF & Wink M, 1998, van Wyk BE & Wink M, 2004). The use of plant drugs for medical treatment is possible since plants have evolved bioactive secondary metabolites that have been selected during evolution as a means against microbes and herbivores (Wink M, 1998, 1993, 2000)

Many compounds, so far isolated as active and potent substances are morphine (pain killer) codeine (antitussive), papverine (phosphodiesterase inhibitor), ephedrine (stimulant) ajmalicine (antirrhythmic) etc. these active components of either chemotherapeutic
of chemopreventive activity are belong to classes like alkaloids, flavonoids and anthraquinone derivatives (Wink M, et al., 2005).

1.8.2.1. Alkaloids

Alkaloids, nitrogen containing heterocyclic basic compounds, form a large family of compounds with many important chemical leads for the drug development. Some of the important ones are reviewed here.

**Camptothecin**- This monoterpenoid isoquinoline indole alkaloid was originally isolated from *Camptotheca acuminata* by Wall ME & Wani MC in 1956. Camptothecin and its derivatives, topotecan and irinotecan, show activity against colorectal, stomach, small bowel and non-small cell lung cancers as well as melanoma (Rasheed ZA & Rubin EH, 2003). Camptothecin and its derivatives exert biological activity by prompting TOP1 enzyme to act as a cellular poison.

**Vinca Alkaloids**- Vinca alkaloids have been used in clinic for more than 30yrs(Leveque D & Jehl F, 2007). Today two natural compounds, vinblastine and vincristine and two semi-synthetic derivatives, vincristine and vinorelbine have been registered for cancer chemotherapy. Vinca alkaloids inhibit the tubulin assembly leading to mitotic arrest; assumed to be the cause for cytotoxic activity of these alkaloids. By combinatorial chemistry, a new derivative vinflunine, a diflurinated derivative, was selected for clinical testing (Hill BT, 2001). The pharmacological data of these derivatives couldn’t relate the *in vitro* and *in vivo* data, so rational design of new derivatives guided by structure activity relationship seems to be limited about this class of compounds. Also the lack of exact knowledge on the Vinca binding site(s) on tubulin and the mechanism of action of Vinca alkaloids remain unclear. Recent studies reveal that certain newly identified properties; such as antiangiogenic activities, could enlarge the therapeutic use of natural and semi-synthetic Vinca alkaloids (Campostrini N, et al., 2006; Kruczynski A, 2006). Thus, Vinca alkaloids remain as a drug family with continuing interest for future anticancer therapy (Duflos A, et al., 2002; Jean-Decoster C, et al., 1999).
**Sanguinarine and Ellipticine** - these are characterized by significant biological activities including a high antitumor potential; the following are the important targets of these agents (Faddeeva MD & Beliaeva TN, 1999).

- **DNA and other double helical polynucleotides** - Due to DNA–intercalating ability, sanguinarine and ellipticine can change the double helical structure and topological forms of polynucleotides. The presence of protonatable ring nitrogens distinguished ellipticine from other simple intercalators (Garbett NC & Graves DE, 2004).

- **ATP synthesis in mitochondria** - both sanguinarine and ellipticine belong to a group of penetrating (hydrophobic) cations, which are accumulated near the external side of inner mitochondrial membranes during the membrane energization. They neutralize the negative charges. This neutralization inhibits ATP synthesis and oxidative phosphorylation (Faddeeva MD & Beliaeva TN, 1999).

- **Cholinesterase system** - This was inhibited by sanguinarine and ellipticine due to their hydrophobicity and positive charges (Faddeeva MD & Beliaeva TN, 1999).

- **SH-dependent enzymes** - they also inhibit SH-dependent enzymes and proteins. Sanguinarine also inhibit SH-dependent ATPase (Faddeeva MD & Beliaeva TN, 1999)

### 1.8.2.2. Taxanes

The taxanes are an important new class of anticancer agents that exerts their cytotoxic effects on microtubules by a unique mechanism of action. Paclitaxel and docetaxel have significant activity in a broad rage of tumor types that are generally refractory to conventional therapy, including chemotherapy-resistant epithelial ovarian cancer, advanced breast cancer, small and non-small cell lung cancer, bladder cancer and head and neck cancer (Ojima I, *et al.*, 2002; Pienta KJ, 2001).
Paclitaxel was discovered as a part of a drug-screening program of NCI (Wani MC, *et al.*, 1971). Docetaxel is derived semi-synthetically from 10-deacetylbaccatin III. It is more soluble in water and is more potent antimicrotubule agent *in vitro* (Pronk LC, 1999).

### 1.8.2.3. Flavonoids

The flavonoids are polyphenolic compounds found an integral component of human diet. These compounds are present universally in flowering plants, particularly of food plants (Amic D, *et al.*, 2007). The flavonoids are phenyl substituted chromones (benzopyrane derivatives) consisting of a 15-carbon basic skeleton (C6-C3-C6, composed of a chroman(C6-C3) nucleus (the benzoring A and the heterocyclic ring C) also shared by tocopherols, with a phenyl (the aromatic ring B) substitution usually at the 2-position.

An impressive body of information exists on the antitumor action of flavonoids. *In vitro* work has concentrated on the direct and indirect action of flavonoids on tumor growth, kinase activity inhibition, apoptosis induction, suppression of the secretion of matrix metalloproteinases and of tumor invasive behavior (Kandaswami C, *et al.*, 2005). However, once absorbed, the bioactivity depends on the forms and polarity of the compounds circulating *in vivo* (Rice-Evans *et al.*, 2004).

The pharmacological effect of flavonoids is mainly due to their antioxidant activity and their inhibition of certain enzymes. The SARs and QSARs studies have provided useful tools for revealing the nature of flavonoids antioxidant action (Amic D, *et al.*, 2007). Katayama *et al.*, (2007) have demonstrated that 3’ 4’ 7’-trimethoxyflavone is potent to inhibit the activity of breast cancer resistance protein (BCRP) with RI(50) values of 0.012µM for SN-38 and 0.044µM for mitoxantrone.

*Silybin and silymarin* showed cytoprotective activity by its antioxidative and radical scavenging properties. The role of silybin in the modulation of signaling of NFkB, inhibition of EGFR-MAPK/ERK ½

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signaling, activity upon E2F and Rb protein, IGF-receptor signaling (Gazak et al., 2007).

**Genistein and genistin** are two flavonoids induce apoptosis in human ovarian cancer cell line, SK-OV-3. Genistein caused cell cycle arrest at G2/M phase in dose dependent manner. Genistin induced cell cycle arrest at both G2/M and G1 phase (Choi EJ, et al., 2007).

**Tageretine and nobelitin** induce inhibition on the proliferation and cancer cells growth *in vitro* and *in vivo*. The proliferation of human breast cancer cell lines MDA-MB-435 and MCF-7 and human colon cancer line HT-29 were inhibited by tangeretine and nobelitin (Morley KL, et al., 2007).

**Quercetin**, a flavonoid found in onion, grapes, green vegetables, etc., has been shown to possess potent antiproliferative effects against various malignant cells. It was found to downregulates matrix metalloproteinases 2 & 9 in prostate cancer cells (Vijayababu MR, et al., 2006). Quercetin induces apoptosis through the effector molecule insulin-like growth factor-binding protein-3 (IGFBP-3) in a p53 independent manner (Vijayababu MR, et al., 2006). Le Marchand et al., (2000) observed an inverse association between quercetin (Flavonol) intake and risk of lung cancer.

### 1.8.2.4. Anthraquinone Derivatives

Quinone moieties are present in many drugs such as anthracyclines, daunorubicin, doxorubicin, mitomycin, mitoxantrone and saintopin, which are used in the therapy for solid cancers. The cytotoxic action of these quinoidal drugs is due to the following two factors. 1). Inhibition of TOPII and 2). Formation of semiquinone radical that can transfer one electron to oxygen to produce superoxide, which is catalyzed by flavoenzyme *cytochrome P-450 reductase*. Both semiquinone and superoxide can generate OH’ radical, which is the cause for DNA strand breaks (Verma RP, 2006).

Because of the planar structure, anthraquinones are known to readily intercalate into DNA strands (Swanbeck G, 1966) and by virtue of
this platinum in complexed with anthraquinones, the Pt-1C3 complex may represent a system of Pt delivery to nuclear DNA (Alderden RA, et al., 2006). The amino substitution at C-9 and C-10 positions of anthraquinone skeleton convert it a promising group of anticancer agent (Zagotto G, et al., 2000). Anthraquinones with diamine side chains have a better intercalating property to DNA (Sadeghi-Aliabadi H, et al., 2004). The substitutions of hydroxyl group enhance the cytotoxicity of anthraquinones (Teng CH, et al., 2005).

Emodin is a trihydroxy anthraquinone with tyrosine kinase inhibitory activity. It blocks the tyrosine kinase activity of HER-2/neu and suppresses the tumor growth of human breast cancer cells in vitro and in nude mice (Hung MC, et al., 1999). It can also block protein kinase C, NF-kB, and mitogen-activated protein kinase (MAPK) signaling cascades. Aloe – emodin, a dihydroxy anthraquinone is known to induce apoptosis in T24 human bladder cancer cells mediated by activation of p53, p21, Fas/APO-1, Bax and caspase-3 (Lin JG, et al., 2006).

Recently two novel anthraquinones, lupinacidines A and B, have been isolated from culture broth of endophytic actinomycete belongs to the genus Micronosperma. Lupinacidines were found to show significant inhibitory effects on the invasion of murine colon 26-L5 carcinoma cells without inhibiting cell growth (Igarashi Y, et al., 2007).

1.9. The Camptothecin and Analogues

Camptothecin(CPT)(Figure.1.4.a) and its analogues are a very promising class of antitumor compounds with a very unique mode of action with its cellular target Topoisomerase I. the parent compound, camptothecin, belongs to the class of indole alkaloids, was originally isolated from the bark of Camptotheca acuminata(Wall ME and Wani MC, 1966), a tree native to China, during a drug discovery programe of NCI. The interest upon camptothecin is on a growing phase because of the unique mode of action which help to understand the topoisomerase I
activity and also as a therapeutic agent for various tumors (Pommier Y, 2006)

When we look back to the early days of CPT, after its discovery in the year of 1956, it entered into clinical trials only in 1970 and the phase II studies and because of the failure for a meaningful result over gastrointestinal cancer and malignant melanoma, the usage and experiment over camptothecin put aside into the dark side of drug discovery programme.

Later, a resurrection of CPT was made by the identification of the cellular target of CPT, the topoisomerase I (TOP 1) enzyme, which was never been a target of antineoplastic compounds known by that date. This finding accelerated for the synthesis of more soluble, more potent analogues of camptothecin like topotecan (Hycamtin) and irinotecan (CPT 11)(Figure.1.4.b.). Four CPT derivatives are now under clinical evaluation, including topotecan, irinotecan, 9-aminocamptothecin and GG221.

1.9.1. Chemistry Behind the Biological Activity- a Structure Activity Relationship

CPTs are indole alkaloids having rings with a chiral carbon at C-20 in the terminal lactone ring. The stereospecificity also confers to the activity of camptothecin. The (S) – isomer is more potent than (R)-isomer. Substitutions at positions 9 and 10 increases the activity while at 12, decreases the activity. The stabilization of lactone ring is another determining factor for the activity. This ring opens non-enzymatically to a carboxylate form at physiological pH in aqueous phase. This form is therapeutically inactive. This is why the severe cystitis observed while the patient is undergoing administration of water-soluble sodium salt of CPT.

The semi-synthetic analogue irinotecan is having a unique structure with a bis-piperidine side chain at C-10. Irinotecan gets metabolized by the catalysis of carboxylesterase to SN38 (7-ethyl 10-hydroxy camptothecin), the potent form, is 1000 fold inhibitorier to TOP1 compared to CPT.
1.9.2. Topoisomerases-The Relaxing Agents of DNA

Seven genes encode for topoisomerase in mammals: four of which encode for type I topoisomerase and the rest for type II Topoisomerases (TOP2α & TOP2β and SPO11).

The type I topoisomerase cleaves only one strand of duplex DNA whereas type II enzymes cleave both strands. This type I has been again classified into type IA and IB. The four type I topoisomerase genes includes nuclear topoisomerase (TOP1) and mitochondrial topoisomerase (TOP1MT)(Zhang H, et al., 2001; Zhang H, et al., 2004) gene and two genes for TOP3α and TOP3β (Champoux JJ, 2001; Wang JC, 2002). TOP3 enzymes and bacterial TOP1 belong to type 1A group, as they form 5’-phosphotyrosil adducts similar to type II isomerases. TOP1 and TOP1MT form 5’-phosphotyrosyl covalent bonds in eukaryotic cells. These forms are the targets of topoisomerase inhibitors (Pommier Y, 2006).

1.9.3. Poisoning The Top1 Enzyme- Mechanism of Action of CPT

CPTs are pharmacologically unique for several reasons. First, TOP1 is the only target of these drugs as shown in yeast in which TOP1 gene is genetically removed renders it resistant to camptothecin. (Eng WK, et al., 1988; Nitiss J and Wang JC, 1988)) CPT penetrates vertebrate cells readily and targets TOP1 within minutes of exposure. CPT then binds reversibly to the TOP1 cleavage complexes(TOP1cc). CPT neither binds to TOP1 alone nor binds to DNA alone and if, only very weekly (Leteurtre F, et al., 1993), but only to the complex formed by TOP1 with DNA for replication or transcription. It is also pointed that not all cleavage sites are blocked by the CPT to form TOP1cc but only at which the base pairs that flank the broken DNA are a thymine at the –1 position and a guanine at +1 position. The drug (CPT) binds simultaneously both to the DNA by hydrophobic stacking interaction and to TOP1 by a network of hydrogen bonds. (Figure 1.6). The polycyclic aromatic ring of CPT intercalate between –1 and +1 base pairs that flank the TOP1 cleavage site (Marchand C, et al., 2006)(figure 1.6) by stabilization of the TOP1cc by CPT causes the
collision of TOP1cc with the DNA tracking processes – replication and transcription complexes – leads to double strand breaks and subsequent downstream events leading to cell death via apoptosis (Figure 1.6).

1.9.4. Molecular Determinants of TOP1 Response to CPT and Non-CPTs

Because the removal of drug, the immediate religation of cleaved DNA could be seen by TOP1. Thereby TOP1 inhibitors do not directly damage DNA but it is TOP1 itself that damage DNA by DNA-helix tracking processes especially replication and transcription (Figure 1.6). Due to this inhibition over religation by TOP1 inhibitor, the replication and transcription complex were trapped and collide with DNA – TOP1ccs, generating irreversible TOP1 covalent complexes at the 5’ – end if the nicked DNA template becomes misaligned with substrate (Figure 1.6). Therefore it’s the transcription or replication that converts the TOP1cc that are trapped by drugs to irreversible TOP1 covalent complexes and DNA strand breaks. For the reason TOP1 inhibitors that trap TOP1cc are commonly referred to as TOP1 poisons (Staker BL, et al., 2005)

1.9.5. Rationale to the Use of CPT As a Therapeutic Drug and Development of Novel Lead Molecules

The CPTs are therapeutically effective, eventhough they are not curative as a single agent. Stress to given to find more efficient TOP1 inhibitor or otherwise enhance the potency of TOP1 inhibition of existing drugs. Development of the new inhibitors with low toxicity and improved pharmacokinetics are well appreciated. Moreover, the molecular determinants of drug sensitivity would have been evaluated in model systems or appropriate combinations of TOP1 inhibitors with other drugs or biological treatment on the basis of molecular network of the tumor are needed. Also, sensitive and non-invasive biomarkers are required to follow the early response or lack of response to the TOP1 inhibitors in combination with other treatments so that therapies can be rapidly and effectively adapted (Pommier Y, 2006).
1.10. Secondary Metabolite Production Through Biotechnology

It is economically feasible to produce high value secondary metabolites (SMs) through plant biotechnological approach. Screening, selection and medium optimization may lead to high 20- to 30- fold increase in the production of certain metabolites such as phytoalexins, but the chance and instants are rare for many compounds in cultures by elicitors. The culture of differentiated cell, such as root cultures or shoot cultures, is an alternative (Verpoorte R, et al., 2002).

Secondary metabolism in a plant plays a major role in the survival of the plant in its environment. Numerous plant secondary metabolites such as alkaloids, anthocyanins, flavonoids, quinines, lignans, steroids, and terpenoids have found commercial as drugs, dye, flavor, fragrance, and insecticide etc. A lot interest has been put into plant tissue cultures as a possible production method for plant secondary metabolite (SM) of commercial interest (Verpoorte R, et al., 1993; Alfermann AW & Petersen M, 1995). In some cases, success has been achieved for the development of feasible methods, like for pure compounds such as Shikonin, taxol and berberine. However, for many of the pharmaceuticals of interest the production is too low. This is usually due to the fact that production is controlled in a tissue specific manner, dedifferentiation resulting thus in loss of production capacity. The differentiated cells produce the same compound as the plant itself, but the large-scale production remains a bottleneck for the economy of such a production (Verpoorte R, et al., 2002).

1.10.1. Sustainable production of anticancer compounds from tissue culture

In an appropriate medium supplemented with phytohormones, it is possible to establish in vitro cultures of almost all plant species. Staring from callus culture, either cell suspension cultures or organized tissue culture can be followed by the appropriate manipulation of combination of
phytohormones. Taxus cell cultures have been established in reactors with ship impeller type stirrer for better mixing of cells (Phyton Biotch, Germany). Alternatively, shoot or root cultures can be grown in cultures. The growth of root cultures can be enhanced by transformation with Agrobacterium rhizogenes (Saito et al., 2001) Bioreactors have also been developed for shoot and root cultures that allow large scale production of plant metabolites (Ramakrishnan and Curtis, 2004).

Cell and organ cultures have been widely employed to study the formation of secondary metabolites. Only very few compounds are produced in unorganized callus tissue (eg. anthocyanins, betalins, phenolics, anthraquinones, berberine, nicotine), but most economically important products (vincristine, morphine, scopolamine, camptothecin) are produced in organized culture in a scalable quantity (van Hengel et al., 1992). Their capacity obviously depends on the sites of synthesis in the intact plant. A few SMs are produced in all tissues, but most of the SMs are produced in roots (eg. nicotine, tropane alkaloids, pyrrolizidine alkaloids) and are transported to aerial parts through either phloem or xylem. Some are produced in aerial part also (eg. Cardiac glycosides, quinolizidine alkaloids). It is likely that the SM-related genes are turned off in undifferentiated tissues, which would explain the failure of callus and suspension cultures to produce SM in significant quantities (review: Wink M, 1987a, b; Walton et al., 1999; De Luca V and St. Pierre B, 2000).

1.10.2. Improving Production of Secondary Metabolites

1.10.2.1. Screening and selection, medium optimization

In the most common approach is the screening and selection of the high producing cell lines and the optimization of culture condition. For plant cell cultures this approach has been effectively adopted by Sato F et al., (1982) for a 7g/l berberine production from Coptis japonica cell cultures. It has been reported the production of 3g/l shikonin from Coptis japonica cell cultures (Fuyita Y, 1988). The high production is often unstable and after few subcultures the production may decrease. An initial
production can be enhanced upto 20-30 fold and it is not possible to those cultures where initial cell cultures do not produce compounds of interest, as in the case of *C. roseus* (Verpoorte R, *et al.*, 2002).

**1.10.2.2. Differentiation to induce production**

Secondary metabolites are by definition a product of differentiation and so the undifferentiated cell suspension cultures of various plant species were unable to produce secondary metabolites. Unlike the suspension cultures, the differentiated cultures of root and shoot are similarly potential to produce secondary metabolites as *in vivo*. For example the tropane alkaloid hyoscyamine and scopolamine are produced quite well in root cultures (Caldentey O & Arroo R, 2000). A large scale set up of root and shoot culture *in vitro* is a major problem. The only success story is that of growth of ginseng roots (Hibino K & Ushiyama K, 1999).

**1.10.2.3.Elicitation**

Accumulation of certain metabolites have been found to be increased as a response to elicitors, such as addition of heavy metal ions, microbial attack and UV irradiation. In plants certain secondary metabolite pathways are induced by infection with microorganisms. The compounds formed are phytoalexins, low molecular weight compounds with antimicrobial activity (Smith CJ, 1996).

**1.10.3. Production of dimeric indole alkaloids**

Dimeric indole alkaloids, such as vincristine, vinblastine and ajmalicine are produced by *Catheranthus roseus*. Because of the chemotherapeutic value, substantial efforts have been made to produce these compounds by *in vitro* cultures. Despite the enormous efforts made in this direction, only very low traces or not at all amounts of these alkaloids were able to produce in *in vitro* cultures (Banthorpe DV, 1994). These cultures could biotransform stemmadenine to catharanthine, tabersonine and condylocarpine (El-Sayed M, *et al.*, 2004). Similarly ineffective were the trails with root and hairy root cultures (Tikhomiroff C & Jolicoeur M, 2002). However, shoot cultures had somewhat better
results (Satdivé RK, 2003) Since Catharanthus roseus is fast growing and easy to cultivate, in vitro cultures of do not provide a viable alternative.

1.10.4. Production of Paclitaxel

Since Taxus is slow growing tree, in vitro production would be a viable alternative. Several groups have shown that callus and suspension cultures of various Taxus species are able to produce Taxol or precursors. Methyl Jasmonate and other biotic and abiotic stress factors could increase the yield upto 295mg/l (Tabata H, 2004). The yields of paclitaxel and Taxanes, as published by several groups, were in the range of 25mg/l in a 20l bioreactor and 74mg/l in 500l bioreactor. Recently, a 70000l bioreactor has successfully installed by Biotech Pharma, Germany. Root and hairy root cultures of Taxus also reported to produce Taxanes and taxol. Part of the compounds are released into the medium (Roberts SC, et al., 2003) In consideration of the market value of taxol, the in vitro culture provides a sustainable and economically interesting alternative.

1.10.5. Production of Camptothecin

Plants from several unrelated families produce Camptothecin (CPT) (see Lorence and Nessler, 2004). In vitro cultures have been established from Camptotheca acuminata, Nothopodytes foetida and Ophiorrhiza pumila for the sustained production of CPT. Undifferentiated callus and suspension cultures usually fail to produce significant amounts of CPT. Production of CPT in callus and suspension culture were in the range of 0.002-0.004mg/g dry wt or even lower (Sakato et al., 1974; van Hengel et al., 1992) whereas intact plants of C. acuminata contain 0.2-2mg/g dry wt (Lopez-Mayer et al., 1994). Also in cell suspension cultures of N. foetida, CPT levels were 100-1000 fold lower than in the intact plant (Roja and Heble, 1994; Ciddi and Shuler, 2000; Fulzele et al., 2001; Thengane SR, et al., 2003). Obviously CPT biosynthesis and storage appears to be strictly under strict control of cellular differentiation and environmental factors, as in the case many other alkaloids and SMs(De Luca and St. Pierre, 2000).
A promising result was achieved by Saito et al., (2001) with hairy root cultures of *O. pumila* with a production of approximately 1mg/g dry wt of CPT. The authors have characterized these cultures in detail and have studied genes and enzymes of the pathway leading to this monoterpenoid indole alkaloid (Yamazaki et al., 2003a, b). Production of CPT has been scaled up to 3-litre bioreactors and a final concentration of 0.0085% CPT (fresh weight) was obtained; approximately 17% of CPT was released into the culture medium (Sudo et al., 2002).

Lorence et al., (2004) reported the establishment of hairy root culture of *C. acuminata* and the culture was shown to produce CPT and hydroxycamptothecin. The amount of CPT and HCPT were in the same range of intact plant, i.e. 1.0 and 0.15mg CPT and HCPT per g. respectively.

In *Ophiorrhiza mungos*, Michael Wink et al., (2005) has shown that differentiated root and hairy root cultures produce significant amount of CPT than the undifferentiated cell suspension and callus cultures. They also found that CPT is released into medium and biogenic elicitors can enhance this. HPLC-MS analysis revealed that CPT is the main product.

**1.10.6. Production of podophyllotoxin (PTOX)**

Kadkade PG (1982) was the first who reported production of podophyllotoxin by callus cultures of *Podophyllum peltatum*. In 2000, Sharma et al., included *P. hexandrum* in the list of PTOX producing cell cultures. Due to the experience that *Podophyllum* cell cultures do not grow well, interest has turned to *Linum* species (Mohagheghzadeh et al., 2003). *Linum* is an interesting group of species, as one can find lignans of various complexities in the different sections of this genus; for example *Linum album* and *L. persicum*, both contain aryltetraline lignan podophyllotoxin and 6-methoxypodophyllotoxin. Hairy root cultures from *L. album* and *L. persicum* produced derivative of podophyllotoxin, 6-methoxypodophyllotoxin. Rhizomes of 5-year-old *Podophyllum hexandrum* plants contain about 5%PTOX. These hairy root lines are now
under investigation for studies on lignan biosynthesis and lignan production on a biotechnological scale.

1.10.7. Production of Anthraquinones

Anthraquinones are a group of plant phenolic products encompassing several hundreds of compounds, differing in the nature and positions of the substituents. They were particularly wide spread in the families Rubiaceae, Gesneriaceae and Scrophulariaceae. Anthraquinones were not only common constituents of plants of the Rubiaceae, but also their tissue and cell cultures. The first paper dealing with this kind of secondary metabolites appears in 1981 when Amonkar and co-workers reported the isolation of 3-geranyloxy-6-methyl-1, 8-dihydroxyanthrone from root extracts of *Psorospermum febrifugum Spach var. ferrugineum* (Hook.fil) (Guttiferae) (Amonkar *et al.*, 1981). Further investigations on other parts of the latter plant led to the isolation of vismione D and a reinvestigation on root bark extracts in fact led to the isolation of two novel compounds, acetylvismione D and 3-geranyloxy-6-methyl-1, 8-dihydroxyanthraquinone. Vismione and acetylvismione D exhibited a valuable cytotoxic activity *in vitro* against Co-115 human colon carcinoma cell line with LD50 values of 0.15 mg/mL and 0.38 mg/mL, respectively. Four new emodin derivatives, namely 3-O- (2-hydroxy-3-methyl-but-3-enyl)-emodin, 3-O- (2-methoxy-3-methyl-but-3-enyl) emodin, 3-O- (3-hydroxymethyl-but-2-enyl) emodin and 3-O- (3-hydroxymethyl-4-hydroxy-2-enyl) emodin were isolated in 2000 by Morelli and co-workers from roots of the African shrub *Vismia guinensis* (L.) Choisy (Hypericaceae) (Bilia AR, *et al.*, 2000). Many compounds of this class have shown a wide variety of pharmacological activities such as antifungal, antimicrobial, anti-inflammatory, analgesic, antipyretic, antioxidant and antitumor activities (Kim YM, *et al.*, 2004; Chen RF, *et al.*, 2004; Huang Q, *et al.*, 2004; Cai Y., 2004; Alves DS, *et al.*, 2004; Sadeghi-Aliabadi H, *et al.*, 2004; Huang HS, *et al.*, 2004).
1.10.8. Anthraquinones from *Ophiorrhiza* species

Many *Ophiorrhiza* genus have been explored for anthraquinone production. Kitajima M, *et al.*, (1998), while studying for the production of camptothecin from tissue cultures of *O. pumila*, found that the cultures produce anthraquinones rather than camptothecin. They found three novel anthraquinones besides many known anthraquinones.

Recently, three new naturally occurring anthraquinones, ophiohayatone-A, -B, and -C, together with four known anthraquinones, were isolated from *O. hayatana* OHWI (Chan HH, 2005).

**Scope of the Present Study**

The present study we encompassed the *in vitro* production of camptothecin from shoot and root cultures of *Ophiorrhiza rugosa* var. *decumbens*. Various biotechnological strategies were adopted to study their impact on the production of camptothecin. We also investigated for the novel anticancer compounds from cultures of *O. rugosa* and isolated some anthraquinones and studied for their anticancer activity. We tested the anticancer efficacy in murine cell lines as well as *in vivo* model. Some of the compounds isolated from *O. rugosa* seems to be promising drug candidates, eventhough more studies are required.
Fig.1.1. Acquired Capabilities of Cancer Cell

- Self-sufficiency in growth signals
- Evading apoptosis
- Insensitivity to anti-growth signals
- Sustained angiogenesis
- Tissue invasion & metastasis
- Limitless replicative potential
Fig. 1.3. Different Classes of Plant-Derived Antitumor Compounds

**Alkaloids**

- Vincristine
- Vinblastine

**Taxane**

- Taxol

**Flavonoid**

- Silybin

**Anthraquinone**

- Rhein
Fig.1.3. Different Classes of Chemotherapeutics

Non-Plant derived

*Bleomycin* (Antitumor antibiotic)

*Methotrexate* (Antimetabolite)

*N-Mustard* (Alkylation agent)
Fig. 1.4.a. Structure of Camptothecin (lactone) composed of five ring system. The lactone ring is essential for the biological activity of CPT.

Fig. 1.4.b. Structure of CPT analogue irinotecan (CPT-11). Carboxylesterase enzyme metabolize CPT-11 to SN-38, which is the active form of prodrug CPT-11.

Fig. 1.5. Atomic Structure of CPT trap a TOP1

a) Overview of a ternary TOP1 cleavage complex (TOPcc) trapped by camptothecin. CPT (in green) in the centre of the boxed area. The TOP1 is shown in brown, the DNA double helix in blue.

b) Enlarged view of the cleavage site induced by TOP1 and trapped by CPT. The drug [spherical representation—C(green), O(red) and N(blue)] is staked between the base pairs that flank the TOP1 cleavage site. By convention, the base covalently linked to the TOP1 catalytic tyrosine (Y723; shown in magenta) is referred to as +1. The base pairs that flank the TOP1 cleavage site are shown in blue in stick representation. The nick is on the lower strand, and the religation of the 5’ end of the nicked DNA is precluded by the presence of the drug intercalated inside the cleavage site. The TOP1 polypeptide is shown in ribbon representation.

c) H bond network between CPT and TOP1 residues. The view is 90° vertical rotation of panel b.
Fig. 1.6. Mechanism of Action of Camptothecin

Fig. 1.6. a. Replication mediated DNA strand break by CPT
b. Transcription mediated DNA strand break by CPT