Chapter 1

Introduction and Review of Literature
INTRODUCTION

Cancer, or more correctly termed malignant neoplasm, involves the unrestricted growth of cells, invasion of local tissues and spread of metastasis to other parts of the body, especially bones and soft tissues causing secondary growth. Cancer is characterized by abnormal Cell proliferation in which several molecular changes involved to initiate normal cell to transformed cancerous cell. The transformation of a normal cell to a cancer cell begins with damage to DNA caused by chemical carcinogens, UV light, viruses or replication errors. (Colleen Smith et.al. 2005).

Cancer is a disease of multi cellular organisms whose basis is abnormal unregulated cell proliferation, often accompanied by abnormal differentiation. The formation of cancer is a multi step process in which multiple genetic alterations occur usually over the span to derail sufficiently the control of cell growth, division and differentiation. The recent application of transcriptional profiling to cancer had documental changes in the expression of thousands of genes, as normal cells undergo transformation into their neoplastic derivation (Golub 1999. Poron 2000).

The process of cancer development in humans generally takes many years through initiation, promotion and progression. Agents causing cancer fall into three broad groups; radiant energy, chemical compounds and viruses. In general these act by causing mutations or by introducing novel genes into cells. There are a number of familial conditions that cause cancer. These are due to mutations in specific genes (tumor suppressor genes) (Robert K Murray et. al. 2000).

Careful molecular analysis of cancerous tissue has shown that tumor development may result from mutations in several proto oncogenes. Cells have thus evolved with overlapping growth-control, when one is
compromised by mutation others take over. (Reginald H.Garret et.al.2005).

Cancer is the second most common cause of death after cardiovascular disease. It is truly a dreaded disease. In spite of all the advances in the diagnosis and treatment of cancer, only about 10% of cancers are curable at all stages. Some cancers like long and esophageal cancers are associated with 100 percent mortality. Also many patients suffer a miserable lingering death. Hence, from the humanitarian point of view prevention of cancer is highly desirable.

Natural remedies are responsible for maintaining the health of a person and for curing different metabolic diseases. It is mentioned in the Ayurvedic text that natural drugs enhance the bodies defense capacity against various causative factors and to improve the metabolism of different nutrients in the body (chakrapani Dutt 1978). The properties of natural drugs are similar to anti oxidants of modern biology.(Harman D. 1992.)

Antioxidants have a wide range of biochemical activities. These include inhibiting the generation of reactive oxygen species directly or indirectly scavenging free radicals, and altering the intracellular redox potential. (Migueł. J. et al., 1989). Antioxidants have been shown to trigger apoptosis in smooth muscle cells independent of oxidative reactions. (Tsai, J et. al., 1996). Antioxidant also can inhibit tumor initiation, tumor promotion and cell transformation. (Steele V.F. et. al., 1990, OBrien.P. 1994).

Plants remained a great source of therapeutic agents and have been looked at as a source of new therapeutic agents. Plants provide chemicals of unknown and unusual chemical structures. These compounds provide new pharmacological prospects and may serve as a starting material for more complex biologically active compounds.
Natural products compounds discovered from medicinal plants have provided numerous clinically useful drugs. In the areas of cancer and infectious disease, 60-75% of new drugs, originate from natural sources. A large number of plants have been screened in the last three decades for their chemical constituents as well as for pharmacologically active principles. Their efforts have resulted in a plethora of compounds for further evaluation.

The demand for natural products will increase in the coming two decades. This demand will exert tremendous pressure on already scant natural resources. Therefore biotechnological alternative methods hold great promise to meet the demand and the challenge.

A wide variety of compound classes were isolated and characterized. Clinically significant cancer chemotherapeutic agents that emerged included paclitaxel, hycamptamine, (topotecan),CPT-II and 9-amino camptothecin. The latter compounds are semi-synthetic derivatives of camptothecin (camptotheca acuminata, Nyssaceae.)

Plants offer enormous potentials for developing new drugs, chemical, ecology and traditional are can identify plants or plant parts of a plant that may be interesting source of bioactive compounds. Plant biotechnology offers the possibility for the production of interesting compounds and even the potential of generating novel compounds.

There has been much interest in using tissue culture to obtain adequate quantities of commercially valuable chemicals from plants. Plant tissue culture can be used to produce compounds for screening. Undifferentiated cell cultures produce secondary metabolites and the types of compounds produced can be varied by altering the culture conditions or by adding chemicals to elicit the expression of different metabolic pathways. Such manipulations can provide a wide range of chemical diversity from cell cultures that can be extracted directly from plant material (Stafford, A.M. et al., 1998). Similar approaches with different
elicitors can be applied to more highly differentiated plant cultures such as root and leaf cultures. (Gleba. D. et al 1999).

Jivanti or *Adakodien* is botanically called *Holostemma adakodien* and belongs to the family Asclepiadaceae. Jivanti is a handsome, extensive, laticiferous, twining shrub. Though its roots are medicinal, the leaves, flowers and fruits are eaten as vegetables. *Adakodien* root is the most accepted source of jivanti and reckoned as an important rasayana drug capable of maintaining youthful vigour and strength. Roots also possess various other uses. It has cooling, alternative, tonic and lactative properties and is also an astringent to the bowels and is sweet. The root made into a paste is applied to eyes in ophthalmic and also for scalding in gonorrhoea. In diabetes, the root rubbed into a paste is given in cold milk. In spermatorrhoea, the dried root with an equal quantity of the root of Ceiba pentandra powder is given in six doses with milk and sugar daily. It is employed in dicoction by the Santhals, as a remedy for cough and also for orchitis. It is a Munda stomach ache medicine. It also cures ulcers, biliousness and diseases of the blood, worms, itching and vesicular calculi. Its medicinal properties are attributed to the amino sugars present in the roots like alpha amyrin, lupeol and beta sitosterol. It also contains six amino acids like alanine, aspartic acid, valine, glycine, serine and threonine.
Fig. 1.1 *Holostemma adakodien*
REVIEW OF LITERATURE

Cancer is a unique disease, very different from all other diseases. It originates within the body itself from normal cells as an abnormal growth, due to defective growth regulatory and finally kills it, also destroying itself in this process. It is the only disease endowed with the twin abilities to invade and metastasize.

Cancer can be defined as any uncontrolled abnormal growth of cells in the body, which, if left untreated, will continue to grow and eventually kill the host. A single cell or a group of cells escape from the normal growth regulatory mechanisms, and start proliferating independently, giving rise to an abnormal mass of cells, known as a malignant tumor. A benign tumor remains localized and does not invade neighboring structures. It is usually confined within membrane or capsule formed by fibrous tissue.

A malignant tumor, on the other hand, is characterized by the twin properties of local invasion and distant metastasis. The malignant tumor consists of transformed cells, which proliferate continuously, in an uncontrolled manner, with absolute disregard for neighboring structures. They infiltrate into adjoining normal healthy tissues, destroy them and grow in that space also (local invasion).

A malignant tumor is also called “cancer”, from the Greek word, “Karakinos”, meaning a crab. Like a crab spreading out its claws, cancer also spreads out in the body, through infiltration and metastasis. Cancer is, essentially, an autonomous, parasitic growth in the body. It gets all its nutrients from the body, giving nothing in return. It depletes the body of its nutrients continuously, grow thing luxuriantly, even when the rest of the body is starving—truly malignant. Finally, it destroys the host, and in this process, kills itself too.
Molecular Basis of Cancer

Cancer is the term applied to a group of diseases in which cells no longer respond to normal restraints on growth. Normal cells in the body respond to signals, such as contact inhibition, that direct them to stop proliferating. Cancer cells do not require growth stimulatory signals and they are resistant to growth inhibitory signals. They are also resistant to apoptosis, the programmed cell death process whereby unwanted or irreparably damaged cells self-destruct. They have an infinite proliferate capacity and not become senescent (i.e., they are immortalized). Furthermore, they can grow independently of structural support, such as the extra cellular matrix (loss of anchorage dependence).

Damage To DNA Leading To Mutations

A. Chemical and Physical Alterations in DNA

An alteration in the chemical structure of DNA, or of the sequence of bases in a gene, is an absolute requirement for the development of cancer. The function of DNA depends on the presence of various polar chemical groups in DNA bases, capable of forming hydrogen bonds between DNA strands or other chemical reactions. The oxygen and nitrogen atoms in DNA bases are targets for a variety of electrophiles.

Many chemotherapeutic agents, who are designed to kill proliferating cells by interacting with DNA, also may act as carcinogens and cause new mutations and tumors while eradicating the old. Structural alterations in DNA also occur through radiation and through UV light, which causes the formation of pyrimidine dimers. More than 90% of skin cancers occur in sunlight-exposed areas. Thus, each chemical carcinogen or reactant creates a characteristic modification in a DNA base. The DNA damage, if not repaired, introduces a mutation into the next generation when the cell proliferates.
B. Mutations in Repair Enzymes

Repair enzymes are the first line of defense preventing conversion of chemical damage in DNA to a mutation. DNA repair enzymes are tumor suppressor genes in the sense that errors repaired before replication do not become mutagenic. DNA damage is constantly occurring from exposure to sunlight, background radiation, toxins, and replication error. If DNA repair enzymes are absent, mutations accumulate much more rapidly, and once a mutation develops in a growth regulatory gene, a cancer may arise. As an example, inherited mutations in the tumor suppressor genes Brca 1 and Brca 2 predispose women to the development of breast cancer.

**Oncogenes**

Proto-oncogenes control normal cell growth and division. These genes encode proteins that are growth factors, growth factor receptors, signal transduction proteins, transcription factors, cell cycle regulators, and regulators of apoptosis. The mutations in oncogenes giving rise to transformation are usually gain-of-function mutation; either a more active protein is produced or an increased amount of the normal protein is synthesized.

**A. Oncogenes and Signal Transduction Cascades**

All of the proteins in growth factor signal transduction cascades are proto-oncogenes.

1. Growth Factors And Growth Factor Receptors

The genes for both growth factors and growth factor receptors are oncogenes. Growth factors generally regulate growth by serving as ligands that bind to cellular receptors located on the plasma membrane (cell-surface receptors). Binding of ligands to these receptors stimulates a signal transduction pathway in the cell activating the transcription of certain genes. If too much of a growth factor or a growth factor receptor is produced, the target cells may respond by proliferating inappropriately,
growth factor, dimerization, kinase activity, or some other aspect of their signal transmission. In such cases, the receptor transmits a proliferative signal even though the growth factor normally required to activate the receptor is absent. In other words, the receptor is stuck in the “on” position.

2. Signal Transduction Proteins

The genes encoding proteins involved in growth factor signal transduction cascades may also be proto-oncogenes. Consider, for example, the monomeric G Protein Ras. Binding of growth factor leads to the activation of Ras. When Ras binds GTP, it is active, but Ras slowly inactivates itself by hydrolyzing its bound GTP to GDP and Pi. This controls the length of time that Ras is active. Ras is converted to an oncogenic form by point mutations that decrease the activity of the GTPase domain of Ras, thereby increasing the length of time it remains in the active form.

Ras, when active, activates the serine/threonine kinase Raf (a MAP kinase), which activates MEK (a MAP kinase), which activates MAP kinase. Activation of MAP kinase results in the phosphorylation of cytoplasmic and nuclear proteins, followed by increased transcription of the transcription factor proto-oncogenes myc and fos. Note that mutations in the genes for any of the proteins that regulate MAP kinase activity, as well as those proteins induced by MAP kinase activation, can lead to uncontrolled cell proliferation.

3. Transcription Factors

Many transcription factors, such as Myc and Fos, are proto-oncoproteins (the products of proto-oncogenes). MAP kinase, in addition to inducing myc and fos, also directly activates the AP-1 transcription factor through phosphorylation. AP-1 is a heterodimer formed by the protein products of the fos and jun families of proto-oncogenes. The targets of AP-1 activation are genes involved in cellular proliferation and progression through the cell cycle are the targets of the myc transcription
factor. The net result is increased production of the proteins that carry out the processes required for proliferation.

B. Oncongenes and the Cell Cycle

The growth of human cells, involving DNA replication and cell division in the cell cycle, is activated by growth factors, hormones, and other messengers. These activators work through cyclins and cyclin-dependent kinases (CDKs) that control progression from one phase of the cycle to another. For quiescent cells to proliferate, they must leave G0 and enter the G1 phase of the cell cycle. If the proper sequence of events occurs during G1, the cells enter the S phase and are committed to DNA replication and cell division. CDKs are made constantly throughout the cell cycle by repair binding of a specific cyclin to be active. Different cyclins made at different times in the cell cycle control each of the transitions (G1/S, S/G2, G2/M).

The activity of the cyclin-CDK complex is further regulated through phosphorylation and through inhibitory proteins called cyclin-dependent kinase inhibitors (CKIs). CKIs slow cell cycle progression by binding and inhibiting the CDK-cyclin complexes. CDKs are also controlled through activating phosphorylation by CAK (cyclin-activating kinases) and inhibitory hyper phosphorylation kinases.

4. Tumor Suppressor Genes

The tumor suppressor genes encode molecules involved in the regulation of cell proliferation provides several examples. The normal function of tumor suppressor proteins is generally to inhibit proliferation in response to certain signals such as DNA damage. The signal is removed when the cell is fully equipped to proliferate; the effect of their elimination of tumor suppressor genes is to remove the brakes on cell growth. The products of tumor suppressor genes frequently modulate pathways that are activated by the products of proto-oncogenes.
RB1  The retinoblastoma protein RB1 plays a critical role in cell cycle control by binding to and inhibiting the activity of E2F family transcription factors and components or the basal transcriptional machinery of RNA polymerases I and III. RB1 controls cell proliferation not only by arresting the cell cycle, but also limiting the rate of protein synthesis.

Tp53  The p53 protein is a transcription factor that regulates cell proliferation and responds to signals, including DNA damage, by arresting the cell cycle or inducing apoptosis. Cell-cycle arrest is facilitated by transcriptional activation of the genes encoding the p21/p27 family of cyclin-dependent kinase inhibitors, and repression of genes such as MYC whose activity leads to proliferation. The p53 protein induces apoptosis in certain cell types in response to oncogenic cell behavior, partly through regulation of the BAX and BCL2 genes.

NF1  NF1 is a RAS-GTPase activating protein (GAP), i.e. a protein whose function is to antagonize RAS signaling by accelerating the RAS intrinsic GTP but inactive when bound by GDP. Active RAS recruits RAF to the cell membrane, where it is phosphorylated and then able to initiate the MAP kinase signaling cascade.

WT1  The WT1 gene encodes at least four zinc finger proteins by alternative splicing. The main splice variant is a transcriptional repressor of several genes involved in growth regulation, including BCL2, IGF-II and MYC.

p21 and p16  p21 is a general cyclin-dependent kinase inhibitor and is activated by p53 in response to DNA damage. P16 is a specific inhibitor of CDK4 and CDK6-D cyclin complexes. Loss of functional mutations in the genes encoding both inhibitors prevents normal growth inhibitory signals blocking the cell cycle at G1.

BRCA1, BRCA2  BRCA1, BRCA2 mutations are responsible for most familial cases of combined breast and ovarian cancer, and about half of the cases where breast cancer appears alone. The proteins are
very similar. Each contains a zinc finger module and each is regulated by phosphorylation during the cell cycle.

Salient Features of Cancer

Immortalization

Neoplastic cell cultures can grow indefinitely, whereas, normal cell cultures die after some generations, e.g. human cell cultures die after 50 generations.

Loss of contact Inhibition

Cancer cells apparently lack proper recognition and communication. Contact inhibition is a process when the cells do not move and grow in a culture; this is because of the contact of plasma membranes of different cells and formation of gap junctions. Cancerous cells lose the property of contact inhibition. Such cells divide even after forming a monolayer. Loss of contact inhibition enables the cells to dissociate from neighboring cells and to infiltrate other organs. Such cells pass over or under one another, they grow on top of one another, and they infrequently form gap junctions.

Invasiveness

The transformed cells have the ability to invade other tissues. Normal cells lack this property. The transformed cells first invade extra cellular matrix and then enter blood circulation. These pass through the wall of vessel and form metastasis tumor. The invasion and metastasis are biologic.

Loss of Anchorage Dependence

Most normal cells must be attached to a rigid substratum (i.e. they must be anchored) in order to grow. Transformed cells can grow even when they are not attached to the substratum, as for example when they are suspended in a semisolid medium containing agar or methyl cellulose. The cells that have lost anchorage dependence generally form tumors with high efficiency when they are injected into animals that can not immunologically reject the cells.
Increased Sugar Transport

Tumor cell consumes much more glucose than normal cells, because they have to grow and multiply. There is a great increase in the rate of sugar transport across the surface cell membrane after transformation. This increases the intake of glucose by the transformed cells. More transporters (Permeases) are available on cell surfaces, perhaps due to the increased glycolytic activity of transformed cells, which give them a higher requirement for sugar transport.

Disorganization of the Cytoskeleton

Normal cells have a cytoskeleton of microtubules and microfilaments. These have a regular arrangement and bring about coordinate cell movement. In transformed cells the fibers are few in number, thinner and disorganized. These undergo depolymerization and disaggregate. The loss of cytoskeleton elements has been considered a possible cause of the increased mobility of cell surface proteins.

Protease Secretion

Cancerous cells secrete a protease called plasminogen activator, which cleaves a peptide bond of plasminogen (a serum protein), covering it to the protease plasmin. It results in loss of actin microfilaments, growth stimulation etc. Increased plasmin may help the cells penetrate the basal lamina, facilitating invasiveness of transformed cells.

Release of Transforming Growth Factors

Transforming growth factors (TGFs) are proteins secreted by transformed cells that can stimulate growth of normal cells.

Loss of Capacity for Growth Arrest

Normal cells suppress their own growth when the concentrations of any of the many critical nutrients or factors fall below a threshold value. When the concentration of isoleucine, phosphate, epidermal growth factors (EGF) or another substance that regulates growth falls below the necessary level, normal cells go into quiescence. Transformed cells are
deficient in their ability to respond by growth arrest to lowered nutrient or factor concentrations.

**Easier Agglutination by Lectins**

Transformed cells are agglutinated by lectins at a much lower concentration than required to agglutinate normal cells. Lectins are plant proteins such as concanavalin A and wheat germ agglutinin; these have multiple binding sites for specific sugars. Increased mobility of cell surface glycoprotein's in transformed cells allow low concentrations of lectins to make patches of receptor lectin complexes on the cell surface, patches of various cells can be cross-linked causing the cells to agglutinate.

**Cell Surface Alterations**

In transformed cells, their surfaces undergo changes; some of them may be as follows:

1. In transformed cells glycolipids and glycoproteins are modified. Protein lined N-acetyl neuraminic (sialic) acid is decreased.
2. Ganglioside content of all lipids decreases.
3. Cell surface proteins become more mobile, because of which antibodies can more easily agglutinate surface proteins.
4. Links between surface proteins and cytoskeletal elements are modified.

**Surface Fibronectin Alterations**

Fibronectin is a protein. Normal quiescent cells in monolayer culture become covered with a dense fibrillar network containing fibronectin as major protein content. This also covers the growing cells. Transformed cells either totally lack fibronectin or have greatly reduced amounts. They have difficulty in binding it to their surfaces.
Altered Gene Transcriptions

As compared to normal cells, in transformed cells some mRNAs have increased concentrations, some are decreased and about 3% of mRNA population is specific to transformed cells only. This small amount of RNA probably represents many different low-abundance mRNA molecules. The proteins encoded by these transformation-sensitive genes, although low in concentration, have profound effects on cell growth and morphology. Such specific mRNAs also appear in embryonic cells, and tumor cells have many proteins that are characteristic of embryonic cells; this would suggest that transformation may alter protein composition toward that characteristic of embryo.

Signal Transduction and Cancer

Modern man is confronted with an increasing incidence of cancer and it is the second leading cause of death after heart disease. Carcinogenesis is a multi-step process that proliferates in an unrestricted manner due to an imbalance between growth-promoting and growth-inhibiting mechanisms. An intricate network of signalling pathways is involved in these control mechanisms. The main focus of the current treatment regimes is to block potential points on key biochemical routes that result in the transformation of a normal cell into a cancerous cell. Some of the major signal transduction pathways in cancer that are commonly blocked by phytochemicals are briefly described below.

AP-1 and NF-KB activation pathway

The NF-KB (nuclear factor-κB) pathway plays an important role in the pathogenesis of several important human inflammatory diseases including cancer, diabetes, rheumatoid arthritis and atherosclerosis. Binding of NF-κB is sequestered in its inactive form in cytoplasm through interaction with 1kB binding sites in promoter sequences of several genes such as cyclin D1, apoptosis suppressor proteins such as Bcl-2 and Bcl-XL and those required for metastasis and angiogenesis. It is suggested
that NF-κB activation promotes cell survival and proliferation mechanisms. Dysregulation of NF-κB pathways is crucial for the development of various types of cancer (Shishodia & Agarwal, 2004). NF-κB works in concert with other transcription factors such as activator protein-1 (AP-1) (Shaulian E & Karin M, 2002) that regulates the expression of several genes associated with cell differentiation and proliferation. It is a complex of Jun and Fos and promotes the expression of genes involved in angiogenesis and the invasive growth of cancer cells.

**RTK related pathways of signal transduction**

Polypeptide growth factors, such as platelets derived (PDGF) and epidermal growth factors, can promote the tyrosine phosphorylation of cellular proteins. They interact with their specific receptors called receptor tyrosine kinases (RTKs) such as epidermal growth factor receptor (EGFR) HER2, HER3 and HER4 of subclass 1 (erb B) by sending a signal to the cells. Most of the human malignancies express high levels of growth factors and their receptors. RTK is essential for the activation of phospholipase C (PLC) – g (to activate a cascade of intracellular signaling) (Margolis et al., 1989) and signal transducers and activators of transcription molecules (STATS) (latent transcription factors) (Darnell, 1994) Constitutive over expression of RTKs is involved in the pathogenesis of a variety of tumors. The tyrosine protein kinases have now emerged as one of the most important groups of drug targets, accounting for 20% to 30% of the drug discoveries of many pharmaceutical companies.

**MAPK signaling pathway**

The signal transduction of the MAPK (mitogen-activated protein kinase) super family of protein kinases provides proliferative signals to the cells. Activation of various RTKs stimulates Ras, which in turn activates the protein kinases Raf-1. The latter further phosphorylated and activates MEK ½ (MAP kinase kinase). MEK ½ then phosphorylates the ERK ½
(extra cellular signal regulated kinase) pathway. In addition JNK 1/2/3 (C-Jun N-terminal kinase) and p38 pathways (belonging to the MAPK super family) also functions in parallel. Dysregulation of these MAPKs pathways are involved in a variety of tumors. Therefore targeting this pathway can be an effective strategy in cancer chemotherapy.

Cancer and Apoptosis

Apoptosis

Apoptosis or programmed cell death has been characterized as a fundamental cellular mechanism that occurs under a range of physiological and pathological conditions (Steller et al. 1995, Ellis R.E et al 1991, Martin S.J. 1995, Raff M.C 1992, White E. 1996). It plays an essential role as a protective mechanism against neoplastic development in the organism by eliminating genetically damaged or excess cells that have been induced improperly to divide by a mitotic stimulus (Hickman J.A 1992, Sen S. et al 1992). Growing evidence from both in vitro and in vivo studies demonstrates that suppression of apoptosis is involved in tumor promotion by chemical agents. Suppression of apoptosis may be a feature of tumor promotion by chemical carcinogens.

The term apoptosis frequently is used synonymously with programmed, active suicide cell death. However, this use does not take into account the existence of different types of programmed cell death, which are clearly distinct on the basis of morphological and biochemical criteria like condensation of cytoplasm, separation of cell from neighbouring cells, condensation of chromatin at the nuclear membrane, fragmentation of cell into apoptotic bodies, and phagocytosis by surrounding cells.

Apoptosis is a programmed cell death and a highly organized physiological phenomenon (Wyllie et al., 1990; Hickman, 1992; Sen.S and D'Incalci, 1992; Schulte- Herman et al., 2000). Induction of apoptosis thus is a highly desirable mode as a chemotherapeutic as well as a chemo preventive strategy for cancer control. Indeed, many chemo preventive
agents of natural origin act through the induction of apoptosis as a mechanism to suppress carcinogenesis (Bursch W et al., 1992; Kelloff G.J et al., 1996; Taraphdar A K et al., 2001).

A. Normal Pathways to Apoptosis

Caspase Activation by cell surface death receptors

One pathway that leads to caspase activation is initiated by the engagement of cell surface death receptors with their specific ligands. Cell surface death receptors are a family of transmembrane proteins that belong to the tumor necrosis factor (TNF)/nerve growth factor (NGF) receptor super family. Mammalian death receptors include Fas/APO-1/CD95, TNFR1, DR-3/Apo-3/WSL-1/TRAMP, and the TRAIL receptors DR4/TRAILR1 and DR5/TRAIL-R2/TRICK2/KILLER (Ashkenazi & Dixit 1998). These receptors share a conserved cysteine-rich repeat at their extra cellular domains. Although the regions of greatest sequence homology between super family members are extra cellular, Fas and TNFR1 share a region of homology at the cytoplasmic face (68 amino acids) termed the death domain. This domain, which is discussed below, is required for apoptotic signaling by both Fas and TNFR1. The activating ligands for these death receptors are structurally related molecules that belong to the TNF gene superfamily (Nagatu 1996). Fas CD95 ligand (FasL) binds to Fas, TNF and lymphotixin & bind to TNFR1, apo3 ligand (Apo3L) binds to DR3, and Apo2 ligand (Apo2L, or TRAIL) binds to DR4 and DR5 (reviewed in Ashkenazi & Dixit 1998).

When the Fas receptor binds its ligand, this recognition event is translated into intracellular signals that eventually lead to caspase activation. In particular, there are three distinct steps: ligand-induced receptor trimerization, the recruitment of intracellular receptor-associated proteins, and the initiation of caspase activation.

The binding of FasL to Fas receptor induces trimerization of Fas. The cytoplasmic region of Fas, which contains death domain (DD), recruits a DD-containing adaptor molecule designated FADD (Fas-associating
protein with death domain). FADD also contains death domains. A single point mutation in this domain abrogates the apoptotic signal, suggesting that the death domain is required for initiating the signal inside the cell (Boldin M.P et al 1995, Chinnaiyan A.M et al 1995). Several other novel proteins that contain homologous death domains have subsequently been identified, including TRADD (TNF-receptor associated death domain), RIP (receptor interacting protein), RAIDD, and MADD (reviewed in Cryns V & Yuan J 1998).

The death domain of FADD is necessary for physical association with the ligand bound death receptor complex (the death-inducing signaling complex, or DISC) the N terminus of FADD, which is termed the death effector domain (DED), is critical for recruiting the upstream procaspases such as procaspase-8 contains a caspase homology region. Immediately after recruitment, procaspase-8 is proteolytically processed to the active form that consists of large and small catalytic subunits (Boldin M et al 1996, Muzio M et al 1996).

Several lines of evidence suggest that procaspase-8 can be proteolytically activated by oligomerization following its recruitment to the DISC. First, chemically induced dimerization of membrane-targeted procaspase-8 resulted in its proteolytic auto activation and subsequent activation (Muzio M et al 1998). Likewise, transfecting cells with a chimeric caspase-8 construct in which it's prodomain had been replaced with an N-terminal CD8 dimerization domain resulted in caspase-8 auto activation and apoptosis (MartinD.A et al 1998). Recently, by using two inducible oligomerization systems, Yang et al showed that oligomerization activates auto proteolysis of procaspase-8, which in turn activates their cell death activity. This study further demonstrated that the prodomain of procaspase-8, which in turn activates their cell death activity. This study further demonstrated the prodomain of procaspase-8 is first separated from the protease domain, followed by the separation of the large and small protease subunits (Yang X et al 1998), suggesting that procaspases
may have weak proteolytic activity and cleave one another when they are brought into close proximity.

REGULATION OF CELL SURFACE DEATH RECEPTOR ACTIVATION

There are three distinct mechanisms involved in regulation of death receptor activity. The mechanism prevents pro-caspase recruitment and/or activation at the DISC. Recently, several endogenous inhibitors of death-receptor-induced caspase activation have been identified (reviewed by Cryns V & Yuan J 1998). One group of these inhibitors belongs to a family of viral proteins, FADD-like ICE inhibitory proteins (vFLIPs), which contain two DEDs (Hu S et al 1997, Thome M et al 1997). The presence of DEDs in these proteins prevents procaspases recruitment to the DISC by competing with the procaspases for binding to the DED of FADD.

A mammalian homologue of viral FLIP (cFLIP) subsequently identified by several laboratories is also known as Casper, I-FLICE, FLAME, CASH or MERIT (Srinivasula S.M et al 1997, Inohara N et al 1997), Hu S et al 1997, Golstein Y.V et al 1997, Han D.K.M et al 1997). There are two alternatively spliced forms of FLIP, FLIP-long and FLIP-short. Interestingly, in addition to the two N-terminal DEDs, FLIP-long possesses a C-terminal domain that resembles caspase-8, although it lacks protease activity due to the absence of several conserved residues at the caspase active sites. As expected, both isoforms of cellular FLIP bind to FADD, pro-caspase-8, and pro-caspase-10 via DED interactions (Irmler M et al 1997) and block the processing of procaspases at the DISC due to the completion for DED (IrmlerM et al 1997, Golstein Y.J et al 1997). Consistent with this mechanism, cells transfected with FLIP became resistant to death-receptor-inducing stimuli, but not to other apoptotic stimuli such as staurosporine or UV-irradiation (Irmler M et al 1997).

The second mechanism for inhibiting death-receptor-induced apoptosis is through the expression of decoy receptors for TRAIL. Decoy receptors are closely related to the TRAIL receptors DR4 and DR5.
(Golstein P 1997). However, this receptor lacks the cytoplasmic domain (DcR1) or contains a cytoplasmic region with a truncated death domain (DcR2), thereby specifically inhibiting TRAIL-induced apoptosis by sequestering the TRAIL ligand away from the death receptors DR4 and DR5 (Marsters S.A et al 1997). Interestingly, normal human tissues express these decoy receptors more abundantly than tumor tissues (Ashkenazi A & Dixit 1998), raising the possibility that the increased sensitivity to apoptosis in tumors is partly due to the decreased expression of decoy receptors.

Recently, the identification of a different type of decoy receptor has been reported. Unlike decoy receptors 3 (DcR3) can bind Fas ligand and block its binding to Fas receptor (Pitti R.M et al 1998). In addition, DcR3 is amplified in lung and colon cancer cells. Although its significance is not yet clear, it is intriguing to speculate that the over expression of DcR3 may provide a mechanism for tumor cells to evade the immune surveillance by cytotoxic lymphocytes.

Finally, the third mechanism for preventing death-receptor-inducting stimuli is by directly inhibiting the proteolytic activation of the initiator procaspases such as procaspase-8 or procaspase-10. An example of this class of inhibitor is the viral protein CrmA, a member of the serpin family that is a potent inhibitor of procaspase-8 (Ray C.A et al 1992, Komiyama T et al 1994). CrmA can inhibit both autoproteolytic activation of procaspase-8, as well as the ability of caspase-8 to cleave Bid (discussed below), which then leads to cytochrome c release and the activation of the downstream caspases (Luo X et al 1998, Li H et al 1998).

Most recently, a 60-kDa protein, silencer of death domains (SODD), has been identified (Jaing et al 1999). In the absence of TNF treatment, SODD is associated with the death domain of tumor necrosis factor receptor type 1 (TNF-R1), thereby preventing the spontaneous signalling by death domain-containing receptors.
Despite recent advances in understanding how ligand binding to cell surface death receptors initiates caspase-8 activation, one puzzle still remains. In some cell types, caspase-8 is activated within minutes of Fas activation. However, in other cell types, caspase-8 is activation proceeds much slower. The activation step often occurs within several hours and can be inhibited by over expression of Bcl-2 on the mitochondria. Interestingly, the levels of FADD and procaspase-8 are indistinguishable between these two cell types. Instead, the rate of the DISC formation is very different (Scaffidi C et al 1998). The explanation for this phenomenon is currently unknown. Unlike the Apaf-1 pathway (discussed below), the in vitro system for caspase-8 activation is not available; therefore, it is still unclear whether there are other factors involved in addition to FasL, Fas, FADD, and SODD.

**CASPASE ACTIVATION BY MITOCHONDRIA**

Another caspase-acting pathway was discovered by the observation that the addition of ATP, or preferably dATP, to cell extracts prepared from normally growing cells initiates an apoptotic program, as measured by caspase-3 activation and DNA fragmentation (Liu X al 1996). Biochemical fractionation and reconstitution experiments have led to the identification of three proteins that are necessary and sufficient to activate caspase-3 in vitro.

Absorbance spectrum, protein sequencing and immunoreactivity identified the first protein factor as human Cytochrome C. Cytochrome C isolated from other mammalian sources can substitute for human cytochrome C in this in vitro assay. In addition, the apoptosis-inducing activity of Cytochrome C seems to be independent of its redox status (Liu X et al 1996, Yang J et al 1997, Kluck R.M et al 1997, Bossy-Wetzel E et al 1998). Consistent with these observations, it has been shown that cytochrome C is indeed released from mitochondria in cells undergoing apoptosis induced by a variety of stimuli, including DNA damaging agents, kinase inhibitors, and activation of cell surface death receptors (Yang J et
al 1997, Scaffidi C et al 1998). Once released from the mitochondria, Cytochrome C works together with the other two cytosolic protein factors, Apaf-1 and procaspase-9, to activate caspase-3.

Apaf-1 is a 130-kDa protein consisting of three distinctive domains. The N-terminal 85 amino acids shows homology with the prodomain of several caspases such as caspase-1, caspase-2, and caspase-9. This domain is proposed to function as the caspase recruitment domain (CARD) that binds caspases with a similar CARD (Hofmann K et al 1997). Of all the CARD-carrying caspases, only procaspase-9 is activated by Apaf. Following the CARD, Apaf-1 contains a stretch of 310 amino acids that shows 50% similarity in primary amino acid sequence to the C. elegans death-promoting protein CED-4. The most noticeably conserved regions of this domain are the Walker’s A and B boxes believed to be required for nucleotide binding (Zou H et al 1997). Mutations in this nucleotide binding site abolish both Apaf-1 and CED-4 function (Zou H et al 1999). The C-terminal half of Apaf-1 is composed of 12-13 WD-40 repeats (from different spliced forms), a motif that mediates protein-protein interactions. Deletion of the WD-40 repeats renders Apaf-1 constitutively active in vitro, independent of ATP/dATP and cytochrome c (Srinivasula A.M et al 1998). However, the activated caspase-9 cannot be released from Apaf-1 when the WD-40 repeats are truncated, indicating that this domain normally has dual functions that inhibit Apaf-1 activity and to help release the activated caspase-9 (Srinivasula A.M et al 1998, zou H et al 1999).

Procaspsase-3 activation by Apaf-1 and caspase-9 has been characterized using highly purified recombinant Apaf-1 and procaspase-9. Biochemical analysis reveals a multi step reaction leading to caspase-3 activation. First, Apaf-1 binds ATP/ATP and hydrolyzes it to ADP and dADP, respectively. This hydrolysis, however, does not have any functional consequence if Cytochrome C is absent. Likewise, Cytochrome C will bind Apaf-1 in the absence of ATP. This complex, however, is unstable and inactive. In contrast, in the presence of Cytochrome C, the
binding and hydrolysis of ATP/dATP promote the formation of a multimeric Apaf-1/cytochrome c complex. This multimeric complex is fully functional in recruiting and activating procaspase-9 (Zou H et al 1999). Therefore, the formation of this multimeric complex of Apaf-1/cytochrome c represents the commitment step in caspase activation. Once this complex is formed, procaspase-9 is recruited to the complex at approximately 1:1 ratio to Apaf-1 and becomes activated through proteolysis. The active site mutant of procaspase-9 cannot be activated even though it can be recruited to the complex. This finding suggests that Apaf-1-mediated procaspase-9 activation is through auto catalysis (Zou H et al 1999).

Finally, activated caspase-9 is subsequently released from this complex to cleave and activate downstream caspases such as caspase-3, -6, and -7. The formation of this multimeric Apaf-1/Cytochrome C complex may serve two purposes: First, to increase the local concentration of procaspase for intermolecular cleavage and, second, to set the threshold of caspase activation relatively high so that occasional leakage of Cytochrome C will not cause cells to commit to apoptosis. The linear caspase activation pathway that begins with mitochondrial damage followed by Cytochrome C release and Apaf-1 activation has been confirmed in vivo, as shown by the recent results from the gene knockout experiments. First caspase-3, caspase-9, and Apaf-1 knockout mice show remarkably similar phenotypes. All these knockout mice display excessive neuronal cells, both progenitors and mature neurons, in their brains. These mice die within one or two days postnatal. Furthermore, in Apaf-1 knockout mice, caspase-9 and caspase-3 cannot be activated in response to various apoptotic stimuli, even though cytochrome c release still occurs. Likewise, caspase-3 activation is abolished in caspase-9 knockout mice (Hakem R et al 1998, Kuida K et al 1998, Youshida H et al 1998, Cecconi F et al 1998).
REGULATION OF MITOCHONDRIAL-INITIATED CASPASE ACTIVATION

The primary regulatory step for mitochondria-mediated caspase activation might be at the level of Cytochrome C release. Cytochrome C normally resides exclusively in the inter membrane space of mitochondria, whereas its cofactors, Apaf-1 and procaspase-9, are both cytosolic proteins. Micorinjection or electroporation of cytochrome C induces apoptosis in certain cell types (Garland J.M & Rudin C 1998), indicating that in these cells cytochrome C release might be the key regulatory step. The known regulators of Cytochrome C release are Bcl-2 family proteins. Overexpression of Bcl-2 or Bcl-xL blocks Cytochrome C release in response to a variety of apoptotic stimuli (Yang H et al 1997, Kluck et al 1997, Vander Heiden M.G et al 1997, Scaffidi C et al 1998). On the contrary, the proapoptotic members of Bcl-2 family proteins such as Bax (Rosse T et al 1998, Juergensmeier J.M et al 1998) and Bid (Luo X et al 1998, Li H et al 1998, Kuwana T et al 1998, Gross A et al 1999) promote Cytochrome C release from the mitochondria. The precise biochemical mechanisms of Cytochrome C release and its regulation by Bcl-2 family proteins remain elusive. Currently, three theories have been proposed: the permeability transition pore theory of the Kroemer group (Kroemer G et al 1997), the ion flow model of the Thompson group (Vander Heiden et al 1997), and the BH3-containing protein model (Cosulich S.C et al 1997).

Cancer cells Bypass Apoptosis

Apoptosis should be triggered by a number of stimuli, such as withdrawal of growth factors, elevation of p53 in response to DNA damage, monitoring of DNA damage by repair enzymes, or by release of TNF or other immune factors. However, mutations in oncongenes can create apoptosis-resistant cells.

One of the ways this occurs is through activation of growth factor-dependent signalling pathways that inhibit apoptosis, such as the
Fig. 1.2  **Apoptosis**  
(cell shrinks, chromatin condenses)  

Viable Cell  

Apoptotic Bodies are phagocytosed; no inflammation

“Budding”

Necrosis  
(cell swells)  

Cell becomes leaky, blebbing  

Cellular and nuclear lysis causes inflammation

Fig. 1.3

A. Mitochondrial pathway of caspase activation

Bid, Bax, Bak  →  Mitochondria  →  Cyto c release  →  Formation of the Apoptosome  →  Activation of Caspase-3

B. Apoptosome Formation and Activation

Apaf-1  →  Cyto C, dATP  →  $7 \times$  →  Apoptosome  →  Active Caspase-9 dimers
Fig. 1.4

Chemoprevention to chemotherapy (targets and goals)

- Demethylation of transforming growth factor 2 expression
- Suppression of multi-drug resistance expression
- Suppression of JAK/STAT activation pathways
- Inhibition of growth factor activation pathways
- Inhibition of expression of oncogenes and tumor suppressor genes
- Inhibition of activation protein-1 activation
- Suppression of the NF-κB activation pathway

Chemopreventive agents as inhibitors of glutathione S-transferase-P1-1
PDGF/Akt/BAD pathway. Nonphosphorylated BAD acts like Bid in promoting apoptosis. Binding of the platelet-derived growth factor to its receptor activates PI-3 kinase, which phosphorylates and activates the serine/threonine kinase Akt (protein kinase B). Activation of Akt results in the phosphorylation of the pro-apoptotic BH3-only protein BAD, which inactivates it. The PDGF/Akt/BAD pathway illustrates the requirement of normal cells for growth factor stimulation to prevent cell death. One of the features of neoplastic transformation is the loss of growth factor dependence for survival.

**Prevention of cancer**

Two strategies can be employed to prevent cancer. The first is to avoid exposure to carcinogens and tumor promoters. In day-to-day life, it will imply refraining from smoking and chewing tobacco; avoiding pollution; discarding overheated, over fried and charred foods; minimizing salt and fat intake; avoiding all possible exposure to carcinogens at the work place, factories, etc.

The second strategy to adopt is “chemo prevention” of cancer. In effect, it amounts to regularly consuming vegetables and fruits, which are rich in the biological antioxidants, vitamins A, C and E; and also carotene. Consumption of “protective foods”, particularly yellow-green vegetables and fruits, has been found to decrease the incidence of some cancers in the West, particularly breast cancer; and has been vigorously advocated in the west and in Japan, for the past two decades.

**Modern Trends in Chemo protection and Chemo prevention**

Cancer is ultimately the end stage of a prolonged, pathological process characterized by abnormal cell and tissue differentiation. This process, which currently can lead to the malignancy and metastasis, is called carcinogenesis. Both time and causative mechanisms are important determinants in carcinogenesis, during the 20 years or more latent period before invasion and metastasis occur. Currently, DNA damage and mutations are clearly recognized as a trigger of carcinogenesis process
particularly those relating to the action of autocrine, paracrine, and endocrine regulatory molecules including inflammatory cytokines, prostaglandins and others.

Epidemiological studies report high differences in the cancer incidence, such as 20 fold differences in colon cancer, 40 fold differences in prostate cancer and 5-8-fold differences in breast cancer. However reasons for these large racial/ethnic differences cancer incidence are unclear. According to the recent epidemiological studies on teens in Scandinavia, over 70% of cancer cases are triggered by non hereditary factors i.e. by environmental factors (Lichtenstein P. et al 2000, Hemminiki K. 2001). Genetic polymorphism, however, can be an important factor determining the high risk of cancer in human populations. Genetic polymorphism of enzymes including DNA repair system, cellular detoxication, and antioxidant status of cells may influence the process of carcinogenesis. For example, a high pulmonary induction of the detoxication phase I cytochrome p450,involved in bioactivation of polyaromatic hydrocarbons and tetrachlorodibenzo-p-dioxins,is considered as primary toxicological and carcinogenic relevance(Wei C, et al 2002).

Chemo prevention is recognized as a scientifically justified pharmacological approach in order to prevent, suppress, or reverse the process of carcinogenesis. The proof of principle of chemo prevention has been clearly demonstrated in both animal and clinical studies (Lippman S.M et al 1998, Sporn M.B 2000). The concept of developing new chemo preventive agents is based on the understanding of their mechanism of action. The general area of promise is molecular and cellular study of carcinogenesis and subsequent interaction of chemo prevention agents. Several new agents show activity and promise in both preinvasive and invasive carcinogenesis .The appropriate use of a chemo preventive agent depends on the understanding of its mechanisms of action at all levels, molecular, cellular and tissue/organ level. Therefore, mechanistic and animal data are prerequisite for the selection of a new agent for a major
clinical chemo prevention trial. Chemo prevention based on mechanism is the overall strategy for design and/or discovery of new chemo preventive drugs. Currently, several categories of new agents are considered, which hold a promise for eventual clinical use.

Prostaglandins in inflammation and cancer progression

Prostaglandins are small lipophilic molecules produced from arachidonic acid in response to inflammatory stimuli. In contrast to the role of cyclooxygenase-1 (COX-1) present in most tissues and maintaining homeostatic processes in the body, the inducible enzyme cyclooxygenase 2 (COX-2) is believed to be primarily involved in the regulation of inflammation (Haris S.G et al 2002). For example, proliferation of T-cells is inhibited by PGE, one of the best-known prostaglandins, playing an integral role in infections and diseases. PGE, inhibits tumor-cell apoptosis, promotes tumor cell survival, proliferation and has been found at higher concentrations in tumor tissues than in normal tissues. Recent studies of prostaglandin E receptor-knockout mice indicate that PGE2 contributes to colon carcinogenesis through its action mediated through EP1 and EP2 receptors. Antagonists for these two receptors are considered candidates as chemo preventive agents against colon cancer (Mutch M et al 2002). However, the role of prostaglandins in the inflammation and cancer are diverse and complex. For example, the PGE2 and the 15-deoxy-delta[PGJ2, which are considered as key regulators of inflammation, have profound but opposing effects on tumorigenesis. Unlike the prostaglandin PGE2, which is clearly involved in the promotion and persistence of carcinogenesis (Haris S.G et al 2002). For example, the 15-d-PGJ2 inhibits the production of inducible nitric oxide synthetases (iNOS), tumor necrosis factor-alpha (TNF-alpha) and interleukin 1-beta (IL-1-beta). There are reports of the inhibition of tumor-cell growth both in vitro and in vivo by 15-d-PGJ2 in a variety of tissues, including breast, prostate, colon, lung, bladder and esophagus (Haris S.G et al 2002).
Over-expression of the COX-2 enzyme has been noted in many cancers, including, but not limited to, cancers of the breast, colon, and prostate. More precisely, COX-2 enzyme is undetectable in most normal tissues, but when it's induced by cytokines, growth factors, or oncogenes, this enzyme contributes to the synthesis of prostaglandins in inflamed and neoplastic tissues. Although the concept linking the inflammation and carcinogenesis as related processes is by no means new, selective inhibitors of COX-2 are recently considered promising chemoprotective as well as adjuvant therapy for the treatment of established cancer. Studies are under way to evaluate COX-2 inhibitors combined with cytotoxic drugs in Patients with recurrent colorectal cancer, non-small-cell lung cancer, and cervical cancer (Chau I and Cunningham D 2002).

Regression of established adenomatous polyps in patients with genotypically proven familial adenomatous polyposis (FAP) that received sulindac, a nonsteroidal anti-inflammatory drug (NSAID) was described in case reports, however, not confirmed in a recent randomized clinical trial (Editorials. New Engl. J.Med. 2002). Of 32 epidemiologic studies, 30 have shown a protective effect of NSAIDs against adenomatous polyps, invasive cancer, or both, although no dose-response relation could be established. In animal models, NSAIDs reduce the occurrence of colorectal neoplasia. This effect is most likely due to the inhibition of COX-2. However, other mechanisms of chemopreventive potential of NSAIDs in colorectal cancer can be possible. A small group of NSAIDs (e.g. sulindac) do not inhibit COX enzymes significantly but can reduce the synthesis of prostanoids by alternate mechanisms, such as inhibition arachidonic acid from phospholipids (Raz A. 2002). Recent studies have shown that NSAIDs stimulate the production of sphingomyelinase, resulting in hydrolysis of sphingomyelin to ceramide, which promotes apoptosis of tumor cells. It was also demonstrated a potential involvement of peroxisome proliferators-activated receptor (PPAR) delta as an adenomatous polyposis coli-regulated target of NSAIDs in colon cancer. Moreover, NSAIDs can up-regulate the (proapoptotic) prostate apoptosis
response for gene in human colon carcinoma cells (Mutoh M et al 2002). Overall, selective COX-2 inhibitors are considered promising chemo preventive agents. There was a significant reduction in the number of colorectal polyps in patients receiving celecoxib a selective cox 2 inhibitor, which led the Food and Drug administration (FDA) to approve celecoxib as an adjunct to endoscopic surveillance and surgery in patients with familial adenomatous polyps (Chau I and Cunningham D 2002). Studies in Europe and in the United States are under way to assess the efficacy of rofecoxib and celecoxib in preventing the recurrence of sporadic adenomatous polyps after polypectomy.

**Selective estrogen receptor modulators (SERMs)**

The term selective estrogen receptor modulator (SERM) is used to describe compounds that interact with the estrogen receptor but have different tissue-specific activities. A variety of compounds classified as antiestrogens are known to have estrogen-agonistic or estrogen-antagonistic properties. It was also known for decades that environmentally derived phytoestrogens could profoundly affect the reproductive function. These substances, which most often influence estrogen receptor (ER)-regulated pathways, stimulate cellular differentiation and proliferation of mammary gland and uterine growth. Two subtypes of estrogen receptor ER-alpha and ER-beta have distinct cellular distribution, regulate separate sets of genes and oppose each other’s action on some genes (Palmieri C et al 2002, Gruber C.J et al 2002). The first subtype ER-alpha (encoded by chromosome 6) was first cloned in 1986 and the second ER-beta (located on chromosome 14) was recently cloned in 1996. These two receptor subtypes vary in structure; their ligand-binding domains share only 55% of amino acid sequence. These receptors are hormone-dependent transcriptional regulators which, in the presence of appropriate ligands, bind to estrogen-response elements (EREs) on DNA. Today we know that It is known that only 5-10% of breast cancers are due to inherited genetic mutations. Women with mutations of BRCA-1
and BRCA-2 genes have approximately 50-80% lifetime risk of developing breast cancer and these mutated genes together are estimated to be involved in 60-70% of all hereditary breast cancers (Arun B and Hortibagy 2002, Schwab M et al 2002). However, diet, lifestyle, and environmental factors are believed to be involved in the majority of breast cancer cases. The triphenylethylene derivatives raloxifene and tamoxifen affect transcriptional regulation by the estrogen receptors ER-alpha and ER-beta. It is estimated that about two-thirds of ER-alpha-positive patients respond to tamoxifen (Palmieri C et al 2002). In 1998, tamoxifen achieved positive results in the Breast Cancer Prevention Trial, leading to the Food and Drug Administration (FDA) approval for tamoxifen, for the risk reduction in women at high risk of breast cancer. Tamoxifen is the only approved agent for the prevention of breast cancer in high-risk women. Novel third-generation SERM agents are under clinical trials (Munster P.N et al, 2001). In addition, combination of SERMs with hormone replacement therapy using dihydroepiandrosteron (DHEA) is proposed to be beneficial for women at menopause (Labrie F et al 2001).

The mortality rate is approximately 30%, making breast cancer the highest cause for death among women 50-55 years of age (Schwab M et al 2002). Since the benefit of tamoxifen in preventing breast cancer was only seen in ER-positive cancers (although some patients classified as ER-alpha-negative, do benefit from tamoxifen therapy), there is an urgent need for chemo preventive agents for the ER-negative breast cancers. Selective COX-2 inhibitors seem to be suitable candidates.

Aromatase inhibitors

Aromatization is the last step in estrogen formation, in which estrone and estradiol are formed of their obligatory precursor's androstenedione and testosterone, respectively. More precisely, the aromatase enzyme is particularly good target for inhibition because it mediates the last series of steps in steroid biosynthesis and is rate-limiting for estrogen synthesis. Therefore, inhibition of aromatase will not interfere
with downstream steroid synthesis. The aromatase inhibitors represent another prospective class of hormonal agents for the management of breast cancer. New aromatase inhibitors replacing megestrol acetate, such as anastrozole (arimidex), exemestane (Aromasin), letrozole (femara), were selected for randomized clinical trails addressing the value of these agents in sequence with, instead of, and in combination with tamoxifen (Ingle J.N 2001, Brodle A 2002). Combining letrozole or arimidex with tamoxifen or faslodex was not more effective than the aromatase inhibitors alone, but was more effective than tamoxifen alone. Steroidal substrate analogs, such as formestane and exemestane, inactivate aromatase by binding irreversibly to it. Generally, letrozole, anastrozole and exemestane, the three aromatase inhibitors currently approved in the USA, are highly effective in inhibiting peripheral aromatization (98.9%, 96.0%, and 97.9% inhibition, respectively) and reducing estrogen levels in patients (Brodle A 2002).

5-alpha reductase inhibitors

Prostate cancer risk is much lower in the peoples of Southeast Asia compared with Americans. In Japanese men, this difference rapidly disappears on immigration to the United States (Barnes S 2002). Epidemiological studies, however inconclusive, have hinted at the association between soy phytoestrogens and reduction in prostate cancer. For example, in Japanese men living in Hawaii, consumption of tofu led to threefold reduction in prostate cancer risk. A few glasses of soy milk daily was associated with 70% reduction in risk of prostate cancer in an Adventist population in the United States.

Androgens, male sex hormones, are formed in the testes and adrenal glands, as well as in peripheral tissues such as the prostate and skin. In men, the prostate is a major site of non-testicular production of dihydrotestosterone. Testosterone is converted irreversibly to dihydrotestosterone by the prostate 5-alpha-reductase type 2. In addition, the 5-alpha reductive type 2 is involved in a two-step conversion of
androstenedione to dihydrotestosterone. It is estimated that 65-75% of dihydrotosterone, considered to be 10 -times more potent androgen than testosterone, arises through the action of 5-alpha reductive in peripheral tissues such as prostate and skin. Inactivation of dihydrotosterone in the prostate is considered a potential modulator of androgenic activity in the prostate (Hsing A.W 2001). Abnormal metabolism of endogenous androgenic hormones is believed to be involved in many cases of human prostate cancer, however, it was also suggested the role of the prostate epithelial cell, capable to metabolize chemicals of dietary origin to mutagens (Lawson D and Kolar C 2002). It is not known, how much dihydrotosterone is necessary to saturate the androgen receptor within the prostate and induce the cellular proliferation, however, the hormonal hypothesis remains one of the most important hypotheses in prostate cancer etiology. Subsequently, the dihydrotosterone-supplying enzyme 5-alpha-reductase type 2 appears to be a molecular target in prostate cancer prevention, Androgenic blockers, such as flutamide, bicaludamide, niludamide, are considered to cause serious side effects (Brawley O.W 2002). Finasteride was the first 5-alpha-reductase inhibitor to enter human trials, approved in 1992 for the treatment of benign prostatic hyperplasia. Recently, a combined androgen blockade in the treatment of prostate cancer was proposed, where the testicles were blocked by an LHRH agonist while the androgens made locally in the prostate from DHEA were blocked by an antiandrogen (Labrie F et al 2001).

**Inductors of programmed cell death (PCD)/apoptosis**

Induction of apoptosis is considered as a protective mechanism against neoplastic development in which genetically damaged or improperly divided cells are eliminated. Both *in vitro* and *in vivo* studies have demonstrated that suppression of apoptosis is involved in tumor promotion caused by chemical agents. Isothiocyanates (ITCs), occurring as glucosinolates in cruciferous vegetables, have been shown to induce
apoptosis. Isothiocyanates are known chemo preventive substances, affecting phase I (inhibition of p450 cytochrome enzymes) and phase II (activation of detoxifying enzymes). Recent studies have shown that ITCs induce apoptosis via activation of mitogen-activated (MAP) kinases and p53 gene pathways. Animal studies confirmed that administration of ITC conjugates with N-acetylcysteine in the diet inhibited the post-initiation stages of B(a) P-induced lung tumorigenesis (Yang Y.M et al 2002). Overall, isothiocyanates are candidates for chemo prevention, thus far identified from animal bioassays, considered to potentially reduce the risk of lung cancers when administered after exposure to tobacco carcinogens.

**Farnesyl transferase inhibitors (FTI)**

An important goal of cancer therapeutics is to target specifically the genetic lesions responsible for the malignancy. Deregulation of growth-promoting oncogenes such as Myc and Ras, and subsequent loss of G1 restriction point control, induce aberrant cell proliferation. One of the directions in oncogene-targeted therapy is the development of farnesyltransferase inhibitors (FTIs) that prevent the function of Ras proteins by blocking post-translational attachment of phenyl moieties to the -COOH terminus of the Ras, there by inhibiting membrane localization and function (Zhang B et al 2002). It has been shown that Ras transformation can induce down-regulation of fas gene expression, thus rendering tumor cells resistant to Fas-induced apoptosis. Although it appears that many chemotherapeutic agents induce cancer death through apoptosis, in most cases this seems to occur through the mitochondrial pathway with the generation of the Apaf-1/caspase9 / cytochrome C complex. However in some cases, it appears that there may be a critical role for the Fas/FasL system. Therefore, it was proposed that treating Ras-transformed cells with FTI could result in reversal of the inhibition of fas gene expression and could render FTI-treated cancer cells susceptible to Fas-induced apoptosis. The farnesyl transferase inhibitors LB42722 (Taejon, Korea) and GGTL 286 (Calbiochem, La Jolla CA) were shown to
inhibit in vitro the H-Ras protein processing at micro molar concentrations. At least five different FTIs have recently entered clinical testing.

**Selective PPAR-gamma modulators (SPARMs)**

Peroxisome proliferators-activated receptor gamma (PPARgamma) is a nuclear receptor and transcription factor that regulates the expression of many genes relevant to carcinogenesis. Deficient expression of PPARgamma can be a significant risk factor for carcinogenesis, although in some cases over expression enhances carcinogenesis (Sporn M.B 2001). By analogy to the SERM concept, the SPARMs are considered clinically important for chemo prevention and for chemo therapy of cancer.

**Retinoid X receptor (RXR) ligands**

Natural and synthetic vitamin A metabolites and analogs (retinoids) were found to suppress head and neck and lung carcinogenesis in individuals with premalignant lesions and a high risk to develop cancers of the aerodigestive tract. These effects are thought to result from changes in the expression of genes that regulate cell growth and differentiation. The retinoid selective for retinoid X receptors (RXRs) represent a new category of chemo preventive agents, now called “rexinoids”. Rexinoids were found to be highly effective as preventive agents in animal models of mammary carcinogenesis. By the ability to modulate several receptors of the nuclear receptor super family, rexinoids play a central, integrative role in cellular physiology. Interestingly, new rexinoids with no affinity to the retinoid acid receptor (RARs), do not have classical toxicological profile of typical retinoid.

**Antioxidant/electrophile response element (ARE/EpRE)**

The activity of detoxifying enzymes of phase I is critical for carcinogenic activation of xenobiotics, whereas the enzymatic activity of phase II is critical for xenobiotic neutralization. Natural flavanoids from fruits and vegetables are potential activators of the phase II enzymes. The synthetic dithiolthione oltipraz activates a battery of phase II enzymes and
inhibits chemically induced tumors in a variety of target organs (Wilkinson J & clapper M.L 1997). The antioxidant or electrophile response element ARE/EpRE) found at 5'-flanking region of these phase II genes may play important role in mediating their induction by xenobiotics including chemo preventive agents. For example, phenolic antioxidant butylated hydroxyanizol and isothiocyanate sulporaphane were show to be involved in the transcription activation of ARE-mediated reporter gene (Kong A.N et al 2001). Similarly, a dietary flavanol fisetin, widely distributed in fruits and vegetables, was found to induce the phase II enzyme quinine oxidoreductase (QR) activity by transcriptional activation of the QR antioxidant/electrophile-response element ARE/EpRE(Hou D.X et al 2001).

Frequent setbacks in conventional chemotherapy had led to developing alternative strategies to fight cancer. In 1979, Sporn and Newton defined chemo prevention as prevention of cancer by the use of pharmacological agents that inhibits or reverse the process of carcinogenesis". The simplest answer, what distinguishes chemotherapy from chemo prevention, is that chemotherapy involves agents to treat advanced cancer, whereas chemo prevention involves agents to prevent cancer at the stage of premalignant lesion. As trends shift in cancer pharmacology, the last century's clinical trial designs for drugs with broad cytotoxic properties may fade. In the relatively brief history of cancer chemo prevention, hundreds of chemo preventive trials have been reported. Most research has focused on relatively non-toxic and selective drugs potentially applicable for cancer chemo prevention, and understanding of their mechanisms of action. Four classes of new agents, namely selective inhibitors of cycloxygenase-2 (COX-2), selective estrogen receptor modulators (SERMs), retinoids that bind selectively to retinoid X receptors (rexinoids,RXRs) and selective ligands for peroxisome proliferators-activated receptor gamma (SPARMs) appear to be most promising for future research. Theoretically, chemo prevention involves treatment of healthy persons and may require administration of agents
over long periods of time. Therefore, chemo preventive agents must have low toxicity in order to be clinically useful. Furthermore, because their purpose to keep something from occurring, definitive clinical studies to demonstrate their efficacy are necessarily randomized, blinded, long term, and large in size. Dietary intervention is not classically considered chemo prevention, but could be very important in the prevention of many cancers. Epidemiological studies point out the role of phytochemicals in lowering the risk of several common cancers, including cancers of colon, breast and prostate.

Treatment of Cancer

The three main modalities of cancer treatment are surgery, radiotherapy, and chemotherapy. If the tumor is localized and confined to a specific site, surgery is the preferred mode of treatment. Wherever applicable, surgery is still the best form of treatment. Surgery is not possible, if the tumor is adhering to vital structures like the aorta; or may be very difficult if the tumor is not in an accessible area. In both these cases, radiotherapy is used, if the tumor is radiosensitive and will respond. But, if the tumor is spread all over the body, as in leukemia, the only form of treatment available is chemotherapy, that is, systematic therapy with drugs.

Since cancer cells have originated from normal cells, and resemble them closely, with very little biochemical differences, drugs which kill cancer cells will also seriously affect and damage the normal cells from which they originated. Therefore, all drugs used in the treatment of cancer are toxic to some extend to normal tissues also.

Plant products

The vinca alkaloids, vincristine ("Oncovin"), and vinblastine, derived from the periwinkle plant, Vinca rosea, are the most important plant products in the chemotherapy of cancer. They act by inhibiting the
formation of the structural protein, tubulin, inside the cell, and arresting mitosis. Vincristine is used in the treatment of lymphomas and childhood leukemia and sarcomas. Vinblastine is used for treating Hodgkin’s disease. Another plant product, “Etoposide” acts by inhibiting the enzyme, DNA topoisomerase II. It active against a wide range of tumors, lymphomas and leukemia’s and is particularly valuable in the treatment of small cell lung cancer.

**Catharanthus Alkaloids**

Medicinal properties of *catharanthus roseus* have been described in traditional and folk medicine of several countries. The extracts yielded four active alkaloids vinblastine, vincristine, vinleuosine and vinsodine. Vinblastine and vincristine, two indole- dihydroindole alkaloids, are typical representation of catharanthus on alkaloids and have been developed as commercial drugs.

The catharanthus alkaloids are all cycle specific agents and similar to colchicins and podophyllotoxin, block mitosis by dissolutions of cell mitotic spindles and cause metaphase arrest. These alkaloids bind with tubulin protein and prevent tubulin formation from the protein. Though vincristin and vinblastine have antiproliferative properties, the two have different patterns of cytotoxic effects and are use in combination (K.G. Ramawat).

**Vinblastin**

Vinblastin is a potent antitumor drug that is widely used in cancer chemotherapy. It was discovered in extracts of leaves of the cathar anthus roseus. For many years the drug was thought to act primarily as a microtubule depolymerizer (Bensch K.G., et al 1969). However, recent advances have demonstrated its plant stabilizing effects on microtubule at low concentrations in the absence of microtubule depolymerization. These studies revealed that vinblastine acts, quite surprisingly, by stabilizing spindle microtubule dynamics at low concentrations (Wilson.L et al., 1995, Jordan.M.A. et al., 1992).
Taxol and taxanes from Taxus

Taxol, a diterpene, is the latest among antineoplastic drugs of natural origin. Thus obtained from the bark of *Taxus brevifolia*, *T. baccata* and other taxus species. It binds with tubulin and promotes the assembly of microtubules. The mode of action of the taxol is unique. It promotes tubulin polymerization and stabilizes microtubules against dipolymerization. It shifts polymer equilibrium of tubulin towards a polymeric state. Taxanes acetylated at C-13 were effective in inhibiting DNA and protein synthesis. (Ramawat K G 2007).

**Taxol**

Taxol was isolated in 1971 from *Taxus brevifolia* (Wani.M.C. et al., 1971) and is effective in the treatment of breast, ovarian and lung carcinomas. Taxol appears to arrest cells in mitosis by stabilizing spindle microtubules (Schiff.P.B et al., 1980, Derry.W. et al., 1995).

A wide variety of cytotoxic agents are used in the treatment of various cancers. They include alkylating agents, antimetabolites, antitumour antibiotics, plant products, steroids and miscellaneous others. An alkylating agent, as its name implies, donates an alkyl group to a DNA molecule, forming an adduct with it.

**Flavanoids**

The term ‘flavanoids’ covers a large group of naturally occurring phenolic compounds in which two benzene rings are linked by a propane bridge. (Geissman,1962). Since the biologic and pharmacologic functions of flavanoids are many and varied. The biological functions of flavanoids in man and animal was first suggested by Szent Gyorgi, who reported that the flavanoids present in citurs peels are more effective in preventing capillary bleeding and fragility associated with Scurvy. Cytotoxic flavanoids and flavones have been isolated from Eupatorium semiserratum and Baccharis sps (Kupchan et al., 1969). Structure and activity relationships among the flavanoids suggested that strong bacterial
mutagenicity required a double bond between positions 2 and 3 and a hydroxyl group at position 3 (Nagao M et al., 1981). Oshima et al., 1998 reported that a number of flavanoids are considered as antioxidants. A number of flavones and flavanoids have been identified as topo I and II poisons. (Austin C.A et al., 1992; Kashiwada et al., 1993; Finlay G.V et al., 1994). Flavanoids are the only group of polyphenols to have detailed structure activity relationships published for topo II inhibition, other polyphenols such as ellagic acid can also interact with topo I or topo II enzymes (Constantinou et al., 1995). Those polyphenols that effect topo II enzymes may be of particular concern. Topo II enzymes play an essential role in chromosome condensation, and disruption of their function prevents accurate chromosome segregation, as well as increasing recombination (Holm C et al., 1989).

Biotechnological approach for the production of plant secondary metabolites

The nature is a potential important source of useful drugs has been recognized since ancient times. This has resulted in the use of a large number of medicinal plants to treat various diseases and some drugs in western medicine are based on the traditional use of such drugs. Some are used as pure compounds from the traditional medicinal plant such as atropine, morphine, quinine and digitoxin and others as modifications of such compounds, such as aspirin and local anesthetics.

Our dependence on plants for natural products is expected to continue because some compounds are difficult to synthesize due to their structural complexity. With increasing demand, most of the plants have been indiscriminately exploited from their natural habitat for the isolation of various valuable therapeutic agents. The biotechnological application such as plant tissue culture seems to be a viable option for the production of the high value therapeutic compounds without destroying the natural flora.
Cell culture provides means for the production of high value therapeutic compounds unaffected by climatic and political instability. It also avoids the risk of crop failures due to natural hazards and the danger of extinction of natural flora.

Various important strategies are used to optimize product yield from plant tissue cultures, namely improvement of culture condition, selection of high yielding cell lines, elicitation, immobilization, hairy root cultures, bio transformation etc.

**Improvement of culture conditions:**

The environment of selected plant cells of organ cultures should provide optimum conditions for the cells to express their genetic information concerning secondary metabolite formation. Conditions which have been reported to influence the productivity of the culture, are composition of culture medium, light, temperature and aeration.

**Selection of high yielding cell lines:**

A plant is selected and cultures are initiated from various types of tissues like leaf, anther, root, stem etc. From these several types of cultures like callus, cell suspension, shoot, root etc. can be regenerated. The cultured cell lines are heterogenous in their ability to produce useful compounds selection is an active process, which deliberately favors only the survival of the wanted variant while the wild type cell does not survive (Berline and sasse, 1985)

**Immobilization**

Fixation of plant cell in a matrix, for example, polyurethane foam or entrapment of the cells in calcium alginate beads provides an artificial surrounding for the cells which protects them from hydrodynamic stress. The advantages of immobilization are the extend viability of cells in the stationary stage, enabling maintenance of biomass over a prolonged time period, simplified down stream processing, the promotion of differentiation linked with enhanced secondary metabolism etc.
Large scale immobilized alkaloid production system have been developed for Catheranthus roseus using glass fiber mats (Di Cosmo, 1990). Polyurethane foam was also used for bio transformation of codeinone of codeine. (Corchete P and Yeoman M.M, 1989) immobilization of coffea cell in calcium alginate resulted in a 13 fold increase of purine alkaloid production. (Haldimann P and brodelius P, 1987)

Hary root cultures

The use of Agrobacterium rhizogens has been recurring attention recently in secondary metabolism research. It inserts the Ri-plasmid into wounded tissue, causing the growth of very fine adventitious roots, so called hairy roots.

For most alkolid producing plants, having root cultures, have been initiated. Hyoscyamus, Datura, Atropa, Nicotiana, catheranthus, Cinchona and peganum. The alkaloid contents found in the hairy roots are similar to those founding normal plant roots. According to (Deyes xie et al., 2000) 0.54% artemisin was obtained from hairy root culture of Artemisia annua.

Permeabilization

The technique used to facilitate the forced release of metabolites is known as permeabilization. Various strategies have been used chemical permeabilization and electric permeabilization chemical permeabilization comprises the use of organic solvents such as DMSO, Chloroform, Tween 20 and surface active chemicals. Results with the various techniques used are always achieved at the cost of cell viability and this might hamper further applications. A short treatment with tween 20, combined with L-phenyl alanine feeding amplified the level of hyoscyamine released into the medium in the case of tropane alkaloid production from Datura innoxia transformed roots. (Boitel-conti et al., 2000 ).
Elicitors

Elicitors are compounds of biological origin involved in plant microbe interaction. In the context of product accumulation by plant cell cultures elicitors are mediator compounds of microbial stress (biotic elicitors) or are stress agents, e.g., UV light, alkalinity, osmotic pressure or heavy metal ions (abiotic elicitors). Molecules which stimulate secondary metabolism leading to the induction of stress metabolites are called elicitors and those derived from fungi may be referred to as fungal elicitors. It has been demonstrated that fungal – induced stress of normal, intact plant tissues leads to the induction and accumulation of phytoalexins. The ability of culture plant cells to produce certain metabolites in response to molecules isolated from fungi appears to be a general phenomenon, and must be correlated with physiological stress imposed on the cells.

Chemically defined elicitors (actinomycin-D, arachidonic acid sodium salt, chitosan, nigeran, poly-L-lysine and ribonuclease –A) or fungal preparations (whole extract or cell wall fraction) from common pathogenic fungi (Pythium, Alternaria, Helminthosporium, Fusarium, Colletotrichum, Sclerotinia, etc.) are used. Fungus is grown on fresh culture medium from stored stock cultures, homogenized and autoclaved at 121°C for 20 minutes and suitably diluted fungal preparations or chemicals are used to evaluate the elicitation effect.

It has been confirmed that elicitor-treated Catharanthus roseus cells released phenolics in the culture medium which were independent of alkaloid accumulation (Seitz H U et al., 1989). Exposure of plant cells to the elicitors requires a minimal period of contact to induce the physiological response. It was further concluded that elicitors rapidly discharged the plasmalemma potential of the cells resulting in differential uptake.