REVIEW OF LITERATURE

CHAPTER 1

REVIEW OF LITERATURE
Cancer is a term used to represent a group of more than 100 types of diseases involving any of the organs in the body. It is recognized by the behavior of a population of abnormal cells within a normal tissue exhibiting a diverse set of phenotypic abnormality including loss of differentiation, increased motility, invasiveness and decreased drug sensitivity (Bishop, 1991). These phenotypic abnormalities arise from a stepwise accumulation of genetic changes that liberates neoplastic cells from homeostatic mechanism that govern normal cell proliferation (Bishop, 1991). The presence of multiple genetic alterations in human cancers strongly indicated that alterations accumulate during tumor progression. Comparative analysis of these genetic alterations in early and late stage tumors led to the hypothesis of a multistage carcinogenesis in colorectal cancer progression which now is a widely accepted genetic tumor progression model (Kinzler and Vogelstein, 1996)

Transformation of normal cell into cancerous cell is referred to as oncogenesis. It involves several steps. An initiator that generally transforms a normal cell to cancer cell whereas promoter triggers changes in gene expression. Some genetic oncogenic changes contribute to uncontrolled growth or loss of senescence. Some oncogenes cause uncontrolled growth by activating persistent growth stimulatory signal transduction pathways and also by altering critical nodes in the cell cycle. Uncontrolled growth can be caused by deregulation at the level of DNA transcription factors. After the initial neoplastic transformation the tumour cell undergoes progressive proliferation which is accompanied by further genetic changes resulting in generation of heterogeneous tumour cell population with varying degree of metastatic potential (Shereen Keleg et al., 2003)
I CONVENTIONAL TREATMENT MODALITIES

Surgery, chemotherapy and radiotherapy are the most established strategies used to eradicate cancer cell in patient’s body. Physical removal of cancer mass is the foundation of surgery. Radiotherapy and chemotherapy use exposure to toxic ionizing radiations and cytotoxic chemicals respectively to destroy cancer cells without finding and remove them.

1.1 Chemotherapy

Cancer chemotherapy had its roots in the work of Paul Ehrlich who coined the term chemotherapy. Modern chemotherapy might have started on 1948 with the introduction of nitrogen mustard (Goodman et al., 1946). Most chemotherapeutic agents currently in use appear to exert their effect primarily on the cell multiplication and tumour growth. Because cell multiplication is characteristic of normal cells as well as cancer cells, most of the chemotherapeutic agents also have toxic effect on normal cells (DeVita, 1991). Inhibition of cell multiplication and tumour growth can take at several levels within the cells such as 1) macromolecular synthesis and function 2) cytoplasmic organization and 3) cell membrane synthesis and function.

Currently chemotherapy has a role in four different clinical setting (DeVita, 1991) as an induction treatment for advanced disease, as an adjuvant to local methods of treatment, as the primary treatment for some patients who present with localized disease in whom local forms of therapy by themselves are inadequate and by direct instillation into sanctuary sites or by site-directed perfusion of specific regions of the body directly affected by the cancer.
The term induction chemotherapy has been used to describe drug therapy given as the primary treatment for patients who present with advanced cancer for which no alternative treatment exists (Holland, 1986).

Adjuvant chemotherapy denotes the use of systemic treatment after the primary tumour has been controlled by an alternative modality such as surgery and radiation therapy (Cokgor et al., 1999). The selection of an adjuvant treatment program for a particular patient usually based on response rates in separate groups of patients with advanced cancers of the same histologic type. Determination of a population of patients as suitable for adjuvant treatment is based on available data about their average risk of recurrence after local treatment alone. Currently adjuvant chemotherapy is considered standard treatment for early stage breast and colorectal cancer (Fuchs and Mayer, 1995).

Use of chemotherapy as the initial treatment for patients who present with localized cancer denotes neo-adjuvant chemotherapy. For chemotherapy to be used as the initial treatment of a cancer partially curable by either surgery or radiation therapy, there must be considerable evidence for the effectiveness of drug program in question against advanced disease of same type. At this time neo-adjuvant therapy has been effectively used in the treatment of anal cancer, bladder cancer, breast cancer etc. (Goldie, 1987; Bonadonna et al., 1990; Jacobs, 1991).

Combination chemotherapy using conventional cytotoxic agents accomplishes several important objectives which not possible with single-agent treatment. Standard single drugs at clinically tolerable doses have been unable to cure cancer (Potter, 1951; Nathanson et al., 1969). In the early years of cancer chemotherapy drug combinations were developed based on known biochemical actions of available anticancer drug rather

Combination chemotherapy using conventional cytotoxic agents has some advantages over the single agent treatment. It provides maximal cell kill within the range of toxicity tolerated by the host for each drug as long as dosing is not compromised. The combination chemotherapy provides a broader range of interaction between drug and tumour cells with different genetic abnormality in heterogeneous tumour populations (Norton and Day, 1991).

Chemotherapeutical drugs cause many side effects like bone marrow suppression, mucositis and hair loss (Smith et al., 1996). Some of them cause cumulative dose-dependent toxicities to slowly- proliferating or non-proliferating normal tissues like kidney, liver, nervous system and heart. Cytotoxicity results when exposed cells attempt cell division before repairing the critical damage. The goal of cytoreductive therapy is often stated as reducing the surviving number of malignant cells to less than one. Since chemotherapy often produces severe toxic side effects, the drug dose that can be administered is determined by the patient tolerance rather than by the therapeutic advantage. Moreover, high cost of chemotherapy reduces the accessibility of this treatment to poor patients. Chemotherapy in fact usually fails in its goal of totally eradicating tumor cells and sparing normal cells.

1.2 Surgery

A surgical procedure used to physically remove malignant tissue is the most effective modality for treating localized disease. There are reports of higher probability of
surgical cure in early stages. Adjacent healthy tissue is also usually removed to provide a surgical ‘margin’ between the diseased and healthy tissues. This area is used by the pathologists to assess whether the tumour is invading the adjacent tissue or not. Local lymph nodes may also be removed to estimate a probability of systemic disease.

Cancer surgery carries the risk of surgery in general, dangers of anesthesia, loss of hemostasis and infection (Dripps et al., 1988). Resurrection of brain stem tumours of the same size is a higher risk procedure in a tissue that cannot grow and morbidity can be much higher. Besides, experience of surgical team with the procedure can also be an important factor.

1.3 Radiotherapy

Radiotherapy is a very important tool in the fight against cancer. It is used in the treatment of as many as 50% of all cancer patients. More than half a million-cancer patients receive radiation therapy each year either alone or in conjugation with surgery chemotherapy or other forms of cancer therapy.

Radiation therapy may be used to treat almost all types of tumour (Kligerman, 1977). It is useful in cases where surgical removal of cancer is not possible or when surgery might debilitate the patients. Together with image guided treatment planning, radiation therapy is a powerful tool in the treatment of cancer, particularly when the cancer is detectable at early stage (Harris., et al 1984). Radiation therapy can be used following surgery to destroy any cancer cells that were not removed by surgery or prior to surgery to shrink a previously incorporable tumour to a manageable size to enable surgical excisions. Radiation can also be used to destroy any remaining cancer cells after
surgery (Hellman 1980; Harris., et al 1984). Chemotherapy and radiation therapy may also be used together to effectively treat the cancer.

Radiation therapy uses high energy X-rays (ionizing radiations) to stop cancer cells from dividing. During this therapy high-energy ionizing radiation deposit energy in the area being treated, damaging the genetic materials of cells and making it impossible for these cells to divide. Although, radiation damages both cancer cells and normal cells, the normal cells are usually able to repair them and function properly (Thompson and Suit, 1969). Like surgery radiation therapy is a local treatment as it affects only the cells in the treated area.

Radio-sensitizers and radio-protectors are chemicals that modify a cell response to radiation. Radio-sensitizers are drugs that make cancer cells more sensitive to the effect of radiation therapy. But radio protectors are those protect normal cells from the damage caused by radiation therapy. These agents promote the repair of normal cells that are exposed to radiation (Sweeney, 1979). Many of the radiosensitizers and protectors are limited in application because of toxic nature (Sweeney, 1979). The most common side effects are hair loss, tiredness, skin reactions, loss of appetite and nausea (Bloomer and Hellman, 1975). Radiotherapy may also cause a decrease in the number of white blood cells and inducing severe immunosuppression (Praveen Kumar et al., 1996; Jagetia et al., 2002).
II. CANCER INVASION AND METASTASIS

Invasion and metastasis are the most insidious and life threatening aspect of cancer and distinguishing feature of malignant cell (Hart, 1982; Fidler, 1973). Once a neoplasm become invasive it acquires the capacity to disseminate through lymphatic and vascular channels (Hart, 1982).

Metastasis is the spread of cancer cells from a primary tumour to vital organs and distant sites in the cancer patient’s body. The distribution of metastasis varies widely depending on the histologic types and anatomic location of primary tumour. The most frequent organ location of distant metastasis in many types of cancer appears to be first capillary buds, encountered by the circulating cells (Terranova et al., 1986). On the other hand there are many metastatic sites that cannot be predicted on the basis of anatomic consideration alone but can be considered as an example of organ tropism (McCarthy et al., 1984; Terranova et al., 1986; Muller et al., 2001).

It is now well recognized that most neoplasms consists of several tumour cell populations, which vary, widely in several important biologic characteristics. These characteristics include growth rates, karyotype, production of growth factors and stimulators of angiogenesis, hormone production, susceptibility to cytotoxic agents, immune response and hypoxia (Fidler, 1973; Hart, 1982; Hanahan and Weinberg, 2000). It was logical to assume that the size of the aggressive subpopulation in primary tumour would reflect the propensity of that tumour to metastasis (Borsi et al., 2002). This assumption would be of prognostic significance if a clinical assay of primary tumour were able to detect and determine the presence of the highly aggressive subpopulation.
II.1 Metastatic cascade

The formation of a metastatic lesion is the result of a complicated, multistep process that is collectively referred to as the metastatic cascade (Poste and Fidler 1980). Formation of metastatic foci is a continuous process commencing early in the growth of primary tumours and it increase with time. The process of metastasis is a cascade of linked sequential steps involving multiple host tumour interactions (Fidler, 1973; Hanahan and Weinberg, 2000). By this scheme, the nutritional requirements of a growing tumor likely exceed the available blood supply, leading to establishment of a localized hypoxic state that in turn triggers an angiogenic switch (Hanahan and Folkman, 1996) (as described in Fig 1.1). The result is angiogenesis, a host response that facilitates neovascularization. As a consequence, and with continued tumor growth, some cells will lose expression of cell–cell adhesion molecules, such as E-cadherin, (Bussemakers and Schalken, 1996) and express matrix metalloproteinases (MMPs), such as MMP2, (Fang et al., 2000) which will allow them to detach from the primary tumor site and invade through the basement membrane. Subsequently, the invasive cells may enter the lymphatic or hematogenous circulation where they can be carried to distant sites. In order to avoid detection by the immune system, it is believed that metastatic cells form aggregates with other cells as well as with fibrin deposits and platelets (Albelda, 1993). Ultimately, the metastatic cells extravasate across the blood vessel endothelium and invade the extracellular matrix at the distant site. It is generally accepted that the metastatic cascade can only be complete if the microenvironment at the distant site is suitable for the establishment of a viable macroscopic metastatic lesion. (Fig 1.1)
Fig 1.1 Metastatic cascade

Primary Malignant Neoplasam → Vascularization → Invasion

Extravasation → Adherence → Embolism

Establishment of microenvironment → Tumour Cell Proliferation → Metastasis
Experimental evidences show that these interactions are highly complicated. Large foundations of experimental work suggest that during each stage of the process, only the fittest tumour cells survive. A very small percentage of circulating tumour cells ultimately initiates successful metastatic colonies. Thus metastasis is a highly selective competition, favoring the survival of a minor subpopulation of metastatic tumour cells that present within the primary tumour (Glinsky et al., 2000).

II.1a Adhesion

The first step of basement membrane invasion requirement is tumour cell attachment. Intravital microscopy shows that initial arrest of cancer cells occurs primarily by size restriction in the capillaries (Chambers et al., 1992). Adhesion among endothelial cells is mediated by several major cell-cell adhesion molecules (CAMs) like members of the immunoglobulin and calcium-dependent cadherin families and integrins (Hanahan et al., 2000). Notably, all of these "adherence" interactions convey regulatory signals to the cell (Aplin et al., 1998). Changes in integrin and immunoglobulin superfamily expression are also evident in invasive and metastatic cancer cells (Skubitz, 2002; Maurer et al., 1998). The importance of integrins in tumor cell dissemination is their role in cellular adaptation to changing tissue microenvironments as found in metastatic organs. This is achieved through various permutations in the spectrum of the more than 22 integrin subtypes. Experimental changes in expression of integrin subunits in cultured cells induces or inhibits invasive and metastatic growth, which confers their role as central determinants of these processes.
II 1b Invasion

Tumor invasion encompasses the process of tumor cell penetration or infiltration into adjacent tissue. This event is also central and related to the development of metastasis. The process of invasion is not only a passive one due to pressure from excessive cellular proliferation but it is an active dynamic process that requires protein synthesis and degradation (Kleiner and Stetler-Stevenson, 1999). Tumour cell must traverse the extra cellular matrix in the process of invasion and must be able to either secrete or activate enzymes that can degrade the major compound of the matrix such as collagen, fibronectin and proteoglycan.

Matrix proteolysis has been recognized as a key part of the mechanism of tumour cell invasion. A variety of proteases has been implicated in this process including matrix metalloproteinase, serine proteinase and cystein proteinase etc. The most important family of proteases are matrixin or matrix metallo proteinase. (Kleiner and Stetler-Stevenson, 1999)

II .1c Cell migration

Transendothelial migration is a dynamic process that involves the constant breaking and remaking of intercellular contacts and is accompanied by drastic changes in cell shape and cytoskeletal reorganization in both the tumor cell and its neighboring endothelial cells (Voura et al., 1998; Brandt et al., 1999). Many motility factors for cancer cells and non malignant cells were described first as growth factors. Motility factors converts a cell from static to a motility status, a transition that is characterized by the appearance of membrane ruffling, lamella, and pseudopodia. Several motility factors have been described for cancer cells including autocrine motility factor. Autotoxin and
scatter factors may also act as positive and negative growth factors. Insulin like growth factors stimulates chemokinesis and chemotaxis. Cell adhesion molecule also play a role in the formation of heterotypic contacts between cancer cells and endothelial cells (Voura et al., 2001). Antibody and peptide inhibition studies suggest the major involvement of multiple CAMs in cell migration. N-cadherin considered to be a potential candidate because transendothelial migration can be retarded by antibodies against N-cadherin (Sandig et al., 1997).

**II.1c. Angiogenesis**

As the tumour grows and central tumour cells become hypoxic, the tumour initiates its own blood supply. This process is called angiogenic switch. Oxygen and nutrients supplied by the vasculature are crucial for cell function and survival. Angiogenesis is permissive for local and systemic expansion of the tumor mass and can be induced by multiple molecules that are released by both cancer cells and stromal cells (Bergers et al., 1999). Angiogenesis itself encompasses a cascade of sequential processes emanating from microvascular endothelial cells, which are stimulated to proliferate and degrade the endothelial basement membrane of parental vessels, migrate, and penetrate into host stroma and initiate a capillary sprout (Holmgren et al., 1995). The recruitment of endothelial cells during angiogenesis and the formation of vascular tumors depends on the breakdown of basement membranes, which occurs under the control of numerous activating factors that were shown to be overexpressed in pancreatic cancer, including vascular endothelial growth factor (VEGF), bFGF, angiogenin, members of the TGF and FGF gene families, and interleukin-8 (IL-8) (Bergers et al., 1999)
II 2. Matrix metalloproteinases and their role in cancer metastasis

Since collagen represents the major structural protein of all tissues and the chief obstacle of migration of tumour cells, it has long been postulated that collagenolytic enzymes play a pivotal role in facilitating the dissemination of cancer (Kleiner and Stetler-Stevenson 1999). Matrix metalloproteinase are a family of closely related metal-dependent endopeptidase which once activated, degrade a variety of extra cellular matrix components. Secreted MMPs with specificity for which interstitial collegen (types I-III) basement membrane (type IV) or type V collagen has been purified from several different types of tumour cells (Kleiner and Stetler-Stevenson, 1999).

The expression and activity of MMPs are increased in almost every type of human cancers and this correlates with advanced tumour stage increased invasion and metaslasis and shortened survival. Considerable experimental data from many laboratires indicated that interaction between host cells and tumour cell have a dynamic symbiotic effect in controlling the breakdown of connective tissue stroma during cancer invasion by different types of cancer. Recent studies have implicated gelatinase A, gelatinase B and matrilysin in playing major roles in cancer invasion and metastasis (Kleiner and Stetler-Stevenon, 1999).

II.2a Regulation of Metallo proteinase

The MMP are secreted as inactive zymogens (pro MMPs). They are kept inactive by an interaction between a cysteine-sulphydryl group in propeptide domain and the zinc ion bound to the calatytic domain. Activation requires the poteolytic removal of the propeptide domain.
Extracellular MMP regulation is carried out mainly by an endogenous inhibitor Tissue Inhibitor of Metalloprotenase (TIMPS). Cytokines and growth factors also appear to play an important role in the modulation of MMP secretions in different tissue especially during inflammation and wound healing. Over expression of TIMP inhibitors and MMP 2, 3, 13 and 14 promotes invasion of cell lines through either collagen type I optic nerve explants or matrigel (Matsuyama et al., 2002). Levels of TIMP 1 or TIMP 2 production by tumour cell has been inversely correlated with invasive potential of various experimental tumours (Matsuyama et al., 2002).

11.3 The plasminogen activator /plasmin system

In addition to the MMP family, the plasminogen activator/plasmin system has been implicated in tumor invasion and metastasis. Plasmin participates in tissue degradation and is activated from the inactive precursor plasminogen by two types of plasminogen activators – uPA (urokinase plasminogen activator) and tPA (tissue plasminogen activator) (Wang, 2001). The proteolytic activity of uPA plays a dominant role in cell migration, angiogenesis, and tumor metastases and is tightly regulated by proteolytic cleavage. It is released from various cell types as an inactive proenzyme (pro-uPA) which upon cleavage by proteinases becomes enzymatically active (Schmitt et al., 1992). uPA binds to a specific cell surface receptor the Urokinase Plasminogen Activator Receptor (uPAR). Upon binding, uPA converts the zymogen plasminogen to plasmin, an enzyme which degrades fibrin and numerous other components of the extracellular matrix, such as type IV collagen, fibronectin and laminin (Dano et al., 1985). This likely enables tumor cells to migrate through tissue barriers (Dano et al., 1985; Friess et al., 1997). Several studies provided evidence that the expression of active
uPA by malignant cells correlates with their invasive potential (Bramhall et al., 1997). Elevated levels of uPA/uPAR have been reported in numerous tumors, including pancreatic cancer (Cantero et al., 1997)

II.4 Metastasis as a Therapeutic Target

The recognition that invasion and even metastasis are early events that leads to the logical application of these disciplines to clinical translation. Thus regulation of adhesion, proteolysis, migration and targeted signaling may be direction for translational application.

Many research studies have documented an important role for cell adhesion in tumour progression, invasion, and metastasis (Hanahan et al., 2000). Recent studies in the area therefore has been given rise to attempt to interfere with adhesiveness in attempt to control tumour invasion and metastatic spread. Many studies have investigated various anti galactoside binding lectins (Platt and Rraz, 1992) and agglutinin binding sugar chains (Platt and Rraz, 1992) and their role in modulation of metastatic spread. Additional recent studies of adhesion molecules and cancer metastasis have emphasized the role of CD44. Certain studies have indicated that anti CD44 monoclonal antibody inhibited the formation of metastatic tumours and prolonged survival in animals bearing human melanoma xenograft metastasis (Guo et al., 1994)

Many growth factors can stimulate both tumour and endothelial cell behaviors ranging from proliferation to attachment, motility and proteolysis. For that reason they are logical targets for therapeutic intervention. Vascular endothelial growth factors have been targeted through the genus drug development approaches. Small molecular receptor antagonists and monoclonal antibodies are the two class of molecule developed against
growth factor receptor. These molecules bind the growth factor binding site of its receptors. An antibody directed against VEGF receptor is in clinical trails (Harris, 2000).

Regulation of TIMP/MMP balance is critical to the localization inhibition of matrix breakdown for both physiologic invasion of angiogenesis and the malignant invasion of metastasis. The tissue inhibitors of metalloproteinases (TIMPs) represent a family of ubiquitous proteins which are natural inhibitors of the matrix metalloproteinases (MMPs). Each MMPs each have different substrate specificities within the ECM and are important in its degradation. The analysis of MMPs in human mammary pathology showed that several MMPs were involved in degradation of the ECM: collagenase (MMP1), which degrades fibrillar interstitial collagens, gelatinase (MMP2), which mainly degrades type IV collagen, and stromelysin (MMP3) which has a wider range of action (Bramhall et al., 1996; Bramhall et al., 1997). There are four members of the TIMP family. TIMP-1 and TIMP-2 are capable of inhibiting tumor growth, invasion, and metastasis which has been related to MMP inhibitory activity. Furthermore, both TIMP-1 and TIMP-2 are involved with the inhibition of angiogenesis. Unlike other members of the TIMP family, TIMP-3 is found only in the ECM and may function as a marker for terminal differentiation. Finally, TIMP-4, is thought to function in a tissue-specific fashion in extracellular matrix hemostasis (Gomez et al., 1997).

Tissue inhibitor of metalloproteinase-2 (TIMP-2) is a 24kD protein that is also known as metalloproteinase inhibitor 2. The gene encoding TIMP-2 has been described by Stetler-Stevenson et al. (Stetler-Stevenson et al., 1990). Metalloproteinase-2 (MMP2) which plays a critical role in tumor invasion is complexed and inhibited by TIMP-2.
Thus, TIMP-2 could be useful to inhibit cancer metastasis (Musso et al., 1997). When B16F-10 murine melanoma cells (a highly invasive and metastatic cell line) were transfected with a plasmid coding for human TIMP-2 and injected subcutaneously in mice, TIMP-2 over-expression limited tumor growth and neoangiogenesis in vivo (Valente et al., 1998).

Administration of MMP regulators that block the synthesis of MMPs prevent MMPs from interacting with molecules that direct their activities to the cell surface is one of the newly developed cancer therapy. (Matsuyama et al., 2002; Gress et al., 1995) Bastimastat and marimastat hydrazamate molecule targeted to interact with MMPs at the activation site by blocking chelation of the metal ion thus mimicking the physiologic action of TIMPs.

The loss of balance in the cellular communication process may allow for deregulation leading to tumourigenecity, invasion and metastasis. Therapeutic efforts in cancer prevention and treatment are being focused at the level of signaling pathways or selective modulatory proteins. Investigations into the signaling pathways underlying metastasis have suggested that protein kinase activity, calcium homeostasis and ras activation are important signals and therefore many be key regulatory sites for therapeutic intervention. Several natural products have been found to inhibit protein tyrosine kinase activity and many possess anti-proliferate or anti-invasive property (John Mann, 2002). Another recently developed therapeutic target in metastasis is ras oncprotein signaling cascade (van't Veer et al., 2002) and several agents have been taken for clinical trial. Investigators have further demonstrated its utility as a therapeutic target.
though studies that tie ras to the action of cytoskeleton and its function (van't Veer et al., 2002).

III. IMMUNE SYSTEM AND CANCER

Cancers arise from the uncontrolled proliferation and spread of clones of transformed cells. From an immunologic perspective cancer can be viewed as altered self cells that have escaped normal growth regulated mechanism. The concept of immune surveillance states that a physiologic function of immune system is to recognize and destroy clones of transformed cells before they grow into tumour and to kill tumours after they are formed. In experimental animals tumour antigens can be shown to induce both humoral and cell mediated immune responses resulting in the destruction of tumour cells (Rosenberg et al., 2004).

III. 1 Immune surveillance

The immune surveillance hypothesis suggest that a major function of immune system is to control the development of cancer (Shevach, 2004). The host provides number of effector mechanism against tumour cells. The important effectors include tumour specific antibodies, mononuclear phagocytes, natural killer cells; cytotoxic T lymphocytes and lymphokine activated killer cells.

III. 1a Natural killer cells (NK cells)

NK cells are distinct subpopulation of lymphocytes that without prior sensitization or requirement of MHC restriction can kill some cancer cell as well as nonmalignant non self cells. In some cases Fc receptors on NK cells can bind to antibody coated tumour cells leading to antibody depending cellular cytotoxicity (ADCC)
III 1b Macrophages

Macrophages and neutrophils from normal donor are generally slightly cytotoxic to tumour cells in *in vitro*. However macrophages and neutrophils can be activated by bacterial products *in vitro* to cover selective cytolysis or cytostasis of malignant cells (Srivastava et al., 1998; Suto and Srivastava, 1995). TNF produced by activated macrophages can account for all of the classical tumoricidal effects against some tumours *in vitro*. Activated macrophages also synthesize nitrogen oxides from L arginine and reactive nitrogen intermediates that also appear to be important mediators of killing of tumour cells. Macrophages also express Fc receptors enabling them to mediate ADCC.

III 1c Complement

The complement system is composed of a group of serum proteins most of which are β globulins with protease activity. Binding of complement components to the appropriate immunoglobulin subclass initiates a cascade of complement activation and macromolecular aggregation that results in the release of anaphylotoxin which cause neutrophil chemotaxis, neutrophil activation, increased vascular permeability, release of histamine from host cell and smooth muscle contraction. (Szebeni et al., 1998)

III 1d Antibodies and B cells

The role of B cells in regulating tumour immunity is poorly understood. In certain tumour model in which CD4+ helper T cells are required for tumour rejection, B cells appear to be necessary for T-cell priming and tumour resistance (DeFranco, 1999).

Human antisera and monoclonal antibodies with autologous tumors have been isolated. Normal or malignant cells of hematopoietic origin are generally lysed quite effectively by antibody and heterologous components *in vitro*, however certain normal or
malignant cells derived from solid tissue may be much less affected even when expressing higher levels of antigen. The reason for this striking difference depending on the source of complement, the antigen distribution or repair of complement mediated lesions may be involved. Some tumour cells are killed by a process involving coating with antibody (opsonization) and subsequent phagocytosis by macrophages. This process may be enhanced by the presence of complement. Alternatively antibody coated tumour cells may be killed in the absence of phagocytosis by ADCC when cultured with macrophages, NK cells or neutrophils (Sliwkowski et al., 1999).

III.1e Lymphocyte.

It has been demonstrated convincingly that T cell mediated immunity is of critical importance for the rejection of virally and chemically induced tumours by immunized mice or for the rejection of allogenic and UV light induced tumours by normal mice (Jardetzky et al., 1994). The relative importance of various T cell subsets in tumour rejection has been the subject of repeated and probably necessary controversies. T cells may use their NKG2 receptor to counteract cutaneous carcinogenesis and to kill skin carcinoma cells in vitro (Mingari et al., 1997)

III.2 Tumour escape mechanism

Human tumors, like viruses, have evolved an elaborate assembly of tricks designed to escape from the immune system, that are are “borrowed” from viruses (Lybarger et al., 2005). In general, tumors employ two strategies to avoid recognition: they either “hide” from immune cells thus avoiding recognition or they proceed to disable or eliminate immune cells. It has been recognized for a long time that tumors are adept at shedding surface antigens or down-regulating expression of key molecules necessary for
interactions with immune cells (Whiteside et al., 2006). In this way, tumors can evade the host's immune response by being either a poor stimulators of T cells or a poor targets for tumor-specific T cells (CTL). Expression of molecules such as TAA, HLA class I molecules or antigen processing machinery components (APM) is often down-regulated or altered in tumor cells (Ferris et al., 2006). As a result of abnormalities in the APM components, which might include their down-regulation, absence or mutation (Meissner et al., 2005), peptides are not generated from tumour associated antigens (TAA) or are generated in a form not allowing for the formation of HLA class I-peptide complexes recognized by T cells (Meissner et al., 2005). Tumors are not effective antigen-presenting cells, and they frequently mis-process and mis-represent processed TAA, so that immunogenic peptides cannot be made or are defective and thus do not fit into the HLA class I groove.

A wide variety of soluble immunosuppressive factors such as TGF, IL-10, ROS, enzymes, and inhibitory ligands such as FasL or TRAIL, that are released by tumor cells or other cells in the tumor microenvironment, suppressor cell populations, i.e., regulatory T cells (CD4+CD25) or myeloid-derived suppressor cells have been shown to play a key role in down-regulation of anti-tumor host immunity (Shevach, 2004; Gabrilovich, 2004). Generally, immunosuppressive effects of tumors are best seen locally, at the tumor site. Functional aberrations of TIL freshly isolated from human tumors are well documented in the literature (Whiteside et al., 1993)

Certain tumor specific antigens have been disappearing from the surface of tumour cells. Such antigen loss variants are common in rapidly growing tumours and can be readily induced in tumour cell lines by culture with tumour-specific antibodies or
cytotoxic T lymphocytes (CTLs). Malignant transformation of cell is often associated with a reduction (or even complete loss) of class I MHC molecule and a number of tumours have been shown to express decreased level of class I MHC molecule. Since CD8+ CTL recognize only antigen associated with class I MHC molecule, any attraction in the expression of class I MHC molecule on tumour cells may exert a profound effect on CTL mediated immune response.

Among less known but clearly important immunosuppressive effects tumors mediate is the ability to induce T-cell apoptosis (Whiteside, 2002). Different mechanisms may account for the high frequency of T-cell apoptosis observed in patients with cancer. Binding of Fas ligand (FasL) to the Fas receptor has been known for some time to induce apoptosis of T cells responding to autologous antigens and maintain tolerance to normal tissue antigens. Furthermore, chronically stimulated T cells are likely to undergo activation-induced cell death (AICD) mediated by the Fas/FasL pathway, or they may die because appropriate cytokines are not secreted (Van Parijs et al., 1996).

The mechanisms of escape evolved by human tumors are varied and ingenious. They appear to target components of the innate as well as adaptive immune system; they operate at the local as well systemic levels, and interfere with molecular pathways responsible for the key cellular functions of immune cells. Furthermore, progressing tumors co-opt tissue cells to participate in creating a microenvironment especially unfavorable for immune interventions in situ. As a result of these mechanisms, tumors have become adept at avoiding immune surveillance, and it might be predicted that their escape from the host’s immune system is likely to be difficult to overcome by immune therapies.
IV. CANCER IMMUNOTHERAPY.

As a result of the limited efficacy of conventional radiotherapy and chemotherapy regimens for treating advanced cancers and the significant morbidities associated with surgical treatment of localized disease, other approaches for treating different cancers are actively under investigation. Among these, immunotherapeutics represent a spectrum of alternative modalities that have proven to be effective in many malignancies such as renal cell carcinoma and malignant melanoma.(Parmiani et al., 2002; Glaspy, 2002)

In general, immunotherapies are designed to augment or manipulate the host immuneresponse to eradicate neoplastic cells. One major hurdle for this approach involves the high similarity between the genome and proteome of a normal cell and it’s corresponding malignant counterpart. Not surprisingly, very few cancer-specific genes or proteins have been identified. This is in sharp contrast to immunotherapies targeted toward microbial pathogens in which many proteins are unique to the infectious agent. The practical ramifications of the high genotypic and phenotypic identity exhibited between normal and neoplastic cells involves the ability of an immune based therapy to break immunological tolerance and to avoid toxicity directed toward normal host cells. Nonetheless, the immune system does exert significant anticancer effects as demonstrated by the presence of cytotoxic lymphocytes infiltrating tumors,(Rosenberg, 2001b) the increased incidence of malignancies such as cervical carcinoma in immunocompromised individuals, (Rosenberg, 2001b) and the graft-versus-leukemia effect observed in allogeneic bone marrow transplantation.(Weiden et al., 1981)
However, the very existence of neoplastic diseases and the almost certain progression without intervention underscores the failure of the natural surveillance systems to effectively police every tumorigenic event. The result of allowing even one critical transforming incident to progress unimpeded can be catastrophic for the human host. Thus, the primary objective of oncological immunotherapeutic approaches is to enhance the potency and effectiveness of the immune response toward the recognition and eradication of neoplastic disease.

Cancer immunotherapies can be broadly categorized into those utilizing active or passive mechanisms. Active immunotherapy entails vaccinating patients with antigens and adjuvants that activate tumor-specific T cells, the major cellular immune effector component. T cells identify target cells through a membrane-bound protein known as the T-cell receptor (TCR). The TCR recognizes short peptide antigens in association with major histocompatibility complex (MHC) molecules displayed by antigen-presenting cells (APCs). Thus, generating tumor-specific immunity is consequently dependent on an appropriate target antigen and the effective presentation of that antigen to the patient’s immune system. The critical roles for APCs in this process have recently been appreciated as APCs such as dendritic cells are responsible for the uptake, processing, and presentation of antigens to cytolytic-T (CD8) and helper-T (CD4) cells in the context of MHC class I and class II molecules (Guermonprez. et al. 2002). To generate an immune response, initial efforts to produce cancer vaccines utilized irradiated neoplastic cells derived from the patient (autologous) or from other individuals (allogeneic) to inoculate cancer patients. (Sedlacek, 1994)
However, this approach could be expected to have greater side effects due to cross-reactivity with normal host antigens, and the immune response may not be optimized due to the potential low expression level of any particular target. Subsequently, for some malignancies, focused efforts to identify and characterize cancer-specific antigens have distinguished well-defined targets for T cell recognition (Scanlan et al. 2002; Mullins et al. 2001).

IV.1 Immuno conjugates in Immunotherapy.

Immuno conjugates are cell targeting molecules such as mAbs, cytokines or soluble receptors that have been genetically or biochemically coupled to cytotoxic moieties (Niculescu-Duvaz and Springer, 1997). Thus cell-targeting portion of an immuno conjugates is used as a delivery system for toxins, radio isotopes, drug enzymes that can activate pro-drugs, liposomes or effector cell recruiting strictures.

IV.2 Cellular strategies

The cellular arm of the immune system plays a key role in maintaining antitumour immunity. In cellular therapy immune cell with antitumour activity are transferred to a tumour-bearing host. Cellular immunotherapeutic strategies can be aimed directly or indirectly at the tumour cells. Successful cellular therapy depends on the types of cells transferred and their effector functions, the ability of the cell to reach tumour site and their ability to overcome tolerance or immune suppression in the host.

IV.2a. Tumour Infiltrating Lymphocytes (TIL)

TILs are lymphocytes that infiltrate growing tumours. They are lymphocytes that have been obtained from tumour tissue and are considered to be a component of an
inflammatory host response to the tumor. The resulting single cell suspension is cultured for several weeks after the TILs can be harvested.

**IV 2b. Lymphokines activated killer cells.**

Lymphocytes from a tumor bearing patient are cultured in IL2 to expand and activate cytotoxic LAK cells primarily NK cells. They are then infused in to the patients with or without more IL2. Although some tumour regression occurs with this approach, significant toxicity is evident when high doses of IL2 are used (Lanfrenier and Rosenberg, 1985).

**IV 2c. Macrophage activated killer cells.**

Monocytes isolated from peripheral blood of tumour bearing patients are cultured in vitro with cytokine, which activate these cells for enhanced cytotoxicity before re-injection into patients.

**IV 3. Administration of immunomodulators.**

Agents that enhance the immune response of the host against cancer, infections disease or immunologic disorders are referred to as immunomodulators (Tzianabos, 2000). Immunomodulators belongs to a highly heterogeneous group of molecule with different mechanism of action. Immunomodulators can be administrated with antigen to increase their local retention and these by facilitate their slow release onto the body. In cancer treatment these immune response modifiers are widely used (William, 1997).

With recent advances in the understanding of how cells communicate with each other to signal effector functions, it has become possible to conceive of strategies to manipulate these signaling pathways in order to influence host responses. Compounds
that are capable of interacting with the immune system to upregulate or down regulate specific aspects of the host response can be classified as immunomodulators or biologic response modifiers. Several classes of these compounds, such as proteins, peptides, lipopolysaccharides, glycoproteins, and lipid derivatives, have all been characterized as molecules that have potent effects on the host immune system. Peptides such as cytokines and chemokines are well-known examples of such molecules. Whether certain compounds enhance or suppress immune responses can depend on a number of factors, including dose, route of administration, and timing of administration of the compound in question. The type of activity these compounds exhibit can also depend on their mechanism of action or the site of activity.

IV. 4 Cytokines

Cytokines are soluble mediators secreted by virtually all nucleated cells in the body and particularly by the cells of immune system (William 1997). They are relatively small proteins that influence the behaviour of other cells expressing appropriate receptors. Cytokines have both autocrine and paracrine activities and virtually all act on multiple cellular targets, Cytokines are involved in both the growth and death of malignant cells. Antiapoptote signals generated by cytokines can promote cell survival and signal transduction pathways involved in the pathogenesis of neoplasia. Conversely cytokines are crucial for the activation and development of immune response against tumour cells. Therefore these two effects must be manipulated in a manner that will be therapeutically useful. (William, 1997)
IV. 4a Interleukins (IL)

Interleukins is a generic name for a hormone like substance produced by the body. It stimulates the growth of blood cells important to body’s immune system. They are particularly important as they regulates inflammatory and immune response.

IL was among the earliest cytokines identified as it has so many potent activities. IL-1 is a powerful inducer of inflammatory process. The most profile IL-1 producing cells are macrophages following stimulation with variety of microbial product or other agents including cytokines (Rosenburg, 1993). Blocking IL-1 activity via receptor antagonist soluble receptors or newly screened drugs shows promise in controlling inflammatory disease such as rheumatoid arthritics and septic shock probably most effectively if combined with blockade of other inflammatory cytokines such as TNF, and IL-6 (Rosenburg, 1993).

IL-2 was the first cytokines to be molecularly characterized. It is a molecule whose functions are highly pleotropic. It is involved in the activation of antigen-specific T and B cells and it also triggers innate immunity by stimulating several functions of NK cells and macrophages. IL-2 can circumvent defective or suboptimal antigen mediated activation and thus overcomes tolerance. This findings suggests that IL-2 may be useful in tumour immunotherapy by enhancing the activity of NK cell or by activating tolerant or poorly responsive antitumor T cells (Kawakami and Rosenburg, 1997).

IV 4b Interferons.

Interferons are family proteins that are produced by cells in response to viral infections or stimulation with double stranded RNA antigens or mitogens (Kurzrock et al., 1991). Interferons have antitumour activity against a variety of tumour types
including hairy cell leukemia chronic myelogenous leukemia, cutaneous T cell lymphoma and Kaposi’s sarcoma (Devita et al., 1991). Three types of interferons are available. Interferon alpha has the greatest efficacy in the treatment of hematologic malignment diseases such as hairy cell leukemia and lymphoma (Pegram et al., 1998). IFN-α inhibits cell growth by inducing G1 arrest. The success of IFN-α therapy is greater when it is combined with other anticancer agents (Quesada et al., 1998).

IFN-γ is best known for its ability to augment the cytotoxic activity of CTLs and NK cells and to increase the expression of MHC molecules on various cells. It also activates monocytes and macrophages. IFN-γ has been used in patients with metastatic renal cell cancer and although the response rate was only 15% some response are durable (Motzer et al., 1928).

IV 4c. Tumour necrosis factor (TNF)

TNF-α was originally identified as a cytokine responsible for endotoxin induced necrosis (Bazzoni, and Beutler, 1996). Several independent groups reported that therapy with recombinant TNF-α was effective against several types of murine models of hepatic (Nishiyama et al, 1989; Scheringa et al., 1989) and pulmonary metastasis (Schultz, and Altom, 1990). TNF-α and TNF-β have been shown to exhibit direct antitumour activity, killing some tumour cells and reducing the rate of proliferation of others while sparing normal cells. In the presence of TNF-α, or TNF-β, a tumour undergoes visible hemorrhagic necrosis and tumour regression. TNF-α has also been shown to inhibit tumour-induced vascularization (angiogenesis) by damaging the vascular endothelial cells in the vicinity of a tumour, thereby decreasing the flow of blood and oxygen that is necessary for progressive tumour growth. TNF has potent antitumour activity against
large tumour burdens in some murine models (Haranaka et al., 1984; Creasey et al.; 1986). However, humans can only tolerate 2% of the systemic TNF dose (by weight) required in mice, due to dose limiting hypotension (Feinberg et al, 1988; Mortiz, et al, 1989). High doses of TNF, administered locally via direct tumour injection (Bartsch, 1989) or isolated limb perfusion (Lienard, 1992) can result in dramatic tumour regression in some cancer patients.

IV 5. Plant derived immunomodulators

Administration of artificially prepared immunomodulatory agents including various cytokines produce certain toxic side effects and it affect their therapeutical efficacy. Immunomodulatory agents that are free from side effects and which can be administrated for long duration to obtain a continuous immune activation are highly desirable for the prevention of diseases. Use of plant products as immunostimulants getting more and more importance in the field of cancer research because of their nontoxic nature. Some of the plants with known immunomodulatory activities are *Viscum album* (Kuttan and Kuttan, 1992), *Panax ginseng* (Singh et al.,1984), *Tinospora cordifolia* (Mathew and Kuttan, 1997), etc; components such as polysaccharides, lectins (Tzinabose, 2000) present in plants have been shown to stimulate the immune system. Curcumin, an active ingredient present in *Curcuma longa*, proved to be a immunopotentiator (Antony et al., 1999). Administration of an extract from the powdered root of the plant *Withania somnifera* was found to stimulate Immunological activity in Balb/c mice (Davis and Kuttan 2000). It also reported to be increase cytokines production in normal as well as tumour bearing animals (Davis and Kuttan, 1999) and proved as potent activator for CTL production both in vivo as well as in vitro .(Davis and Kuttan}
Intraperitoneal administration of *Withania extract* was found to enhance the proliferation of lymphocytes, bone marrow cells and thymocytes in responses to mitogens. Both PHA and Con A mitogens along with Withania treated splenocytes, bone marrow cells and thymocytes could stimulate proliferation twice greater than the normal (Davis and Kuttan 2002b). After treatment with five doses (20 mg/dose) of naturally occurring sulphur compounds such as diallyl sulphide (DAS), diallyl disulphide (DADS) and allyl methyl sulphide (AMS) the total white blood cell (WBC) count was found to enhanced significantly in Balb/C mice (Kuttan, 2000; Manesh and Kuttan, 2003). Administration of alcoholic extract of *Piper longum* (10 mg/dose/animal) as well as piperine (1.14 mg/dose/animal) were reported to be a immunostmulant and increased the total WBC count, the number of plaque forming cells in spleen, bone marrow cellularity and alpha-esterase positive cell number in Balb/c mice (Sunila and Kuttan, 2004).

V PLANT PRODUCTS IN CANCER THERAPY

Cancer chemoprevention is defined as pharmacological intervention with synthetic or naturally occurring compounds that may prevent, inhibit or reverse carcinogenesis, or prevent the development of invasive cancer (Wattenburg, 1993). Reports from the World Health Organization (World Health Organization. 1990) and the UK Department of Health (Department of Health. 1994, Department of Health. 1998) also provide evidence of beneficial effects of fruits and vegetables. Higher consumption of vegetables and fruit significantly reduces the risk of many chronic diseases including cancers of the mouth pharynx, oesophagus, lung, stomach, and also of colon and rectum (World Cancer Research Fund 1997). The cancer inhibitory potential of human nutrients
derived from plants, as well as of non-nutrient constituents (phytochemicals) has been confirmed in various animal models (Crowell, 1999). Dietary consumption of foods and herbal medicines is a convenient method of administrating potentially beneficial phytochemicals in a cost-effective manner.

Medical benefits from plant and plant products forms have been recognized for centuries. Herbs have been used in traditional medicine for thousands of years to cure diseases and heal wounds, even though their biogenesis and pleiotropic actions has not impacted on the practice of western medicines (Nanjoo et al., 1998). Several medicinal plants are being screened for their antitumour properties in India, China, Korea, Brazil and some other countries. *Selaginella tamariscina* a traditional medicinal plant for therapy of advanced cancer patients in the ‘Orient’ which has been shown to modify gene expression and cytokine production (Kuo et al 1998). There are several evidences that water extract of this plant efficiently increased p53 gene expression and induced G1 arrest and suggested that this might contribute to cytotoxic effects by causing apoptotic DNA fragmentation in human leukaemia cell line, U-937 and human ovarian cancer cell line A-2780 (Lee et al., 1999). Hot water extract of the bark of *Nikko maple* (*Acer nikoensa*), a traditional crude drug, has been known for its cytotoxicity (mediated through apoptosis) in susceptible and resistant mouse leukaemia P-388 cell lines, and this extract also increased the expression of sialylated glycoconjugates in apoptotic cells (Nitta et al., 1999). *Uncaria tomentosa* (Willd.) DC, also known as ‘Cat’s claw’, has been used in South American traditional medicine. The native Indians particularly of the Amazon region use tea made of the bark or roots for the treatment of a variety of health disorders including cancer, arthritis and infectious diseases. Organic solvent extracts of this plant
were shown to have cytostatic, contraceptive and anti-inflammatory activity (Keplinger, 1982). Sheng et al. reported that water extract of this plant induced cytotoxicity in human leukaemic cell lines HL-60, K-562 and human EBV-transformed B-lymphoma cell line Raji (Sheng et al., 1998). Extracts of \textit{Solanum muricatum} (Pepino) have been shown to induce apoptotic cell death in prostate (PC-3, DU1-45), stomach (MKN-45), liver (QSY-7721, SKHep-1), breast (MDA-MB-435), ovarian (OVCAR), colon (HT-29) and lung (NCI-H-209) cancer cells and some normal (NHP, HUVEC, WI-38) cells in culture. \textit{Alpinia oxyphylla Miquel} (Zingiberaceae) used in traditional Oriental herbal medicine has been shown to induce apoptosis-mediated cytotoxicity in HL-60 cells in culture (Lee et al., 1998). \textit{Salvia miltiorrhiza} is a traditional Chinese herbal medicine commonly used to treat liver diseases in China for centuries. This plant extract exerted clear cytotoxic effects and strongly inhibited the proliferation of human hepatoma cell line, HepG (2) cells (Liu, et al 2000). Water-soluble macromolecular components of \textit{Artemisia capillaris Thunberg} exhibited inhibition of cell proliferation and apoptosis when studied on human hepatoma cell line (SMMC-7721) (Hu, et al., 2000). \textit{Phyllanthus orbicularis}, a plant of genus \textit{Phyllantus}, is used in Indian traditional medicine for its antiviral activity against Hepatitis B virus and A and B flu virus. The aqueous extract of this plant induced apoptosis in Chinese hamster ovary (CHO) cells (Sanchez-Lamar et al., 1999). Seeds from \textit{Acalypha wilkesiana} (Euphorbiaceae) are essential components of a complex plant mixture used empirically by traditional healers in south-west Nigeria to treat breast tumours and inflammation (Bussing et al, 1999). Bussing et al. observed an induction of apoptosis and generation of reactive oxygen intermediates in granulocytes by an aqueous extract of the seeds (Bussing et al, 1999). This extract induced the release
of the pro-inflammatory cytokines TNF-α and interleukin-6 (IL-6) and also T-cell associated cytokines interleukin-5 (IL-5) and interferon-gamma (IFN-γ).

Natural products in modern medicine have been the mainstay of cancer chemotherapy for the past 30 years. Vinblastine and vincristine (Johnson 1968; Gidding et al., 1999) were first introduced plant products as cancer therapeutics and have contributed to long-term remissions and cures with childhood leukaemia, testicular teratoma, Hodgkin’s disease and many other cancers. Several structural analogues are also in clinical use, and most notable of these are vinorelbine and vindesine (Fahy, 2001). Taxol, a natural product obtained from the bark of the Pacific yew Taxus brevifolia which shows efficacy against refractory breast and ovarian cancers. Camptothecin, from the Chinese ornamental tree Camptotheca acuminate, was blighted by severe bladder toxicity, but chemical manipulation of its structure subsequently produced analogues, including topotecan (Hycamptin) and irinotecan (Camptosar), that have been approved for clinical use (Jonsson et al., 2000). These days camptothecin is valued as a biological tool to understand the functions of the enzyme topoisomerase I, for which it is a specific inhibitor (Chen and Liu, 1994). The human diet contains a complex mixture of plant polyphenols and it is believed that human individuals may consume as much as one gram of plant phenols per day in their diet (Tanaka1994; Chung et al.,1993; Meyer et al., 1993). Studies have shown that cytotoxic effect of these phenols against different tumours (Inone et al., 1994). Curcumin, a phenolic compound that has been identified as the major pigment in turmeric, induces apoptosis in transformed rodent and human cells in culture, ( Wall et al.,1993; Samaha et al., 1997; Kuo et al., 1996; Jiang et al., 1996). In past etiological studies, intake of certain kinds of polyhydroxyphenols such
as flavonoids or lignans in the diet has been correlated with low incidence of colon cancer and breast cancer (Setchell et al., 1981; Adlercreutz et al., 1982). The past decade has seen a dramatic resurgence in research on carbohydrates involved in diseases and their potential use as therapeutics (Persidis, 1997). Several plant polysaccharides have been described with \textit{in vitro} and \textit{in vivo} immunostimulating activity (Tomoda et al., 1994). Their major effect seems to be the activation of macrophage cytotoxicity against tumour cells. Likewise, other branched plant heteropolymers have been reported to enhance cytotoxicity of NK cells by inducing the production and/or release of cytokines (Mueller et al., 1990a; Mueller et al., 1990b; Hauer et al., 1993). Some polysaccharides have shown potent activity against various tumours when tested in implanted animals (Yamada et al., 1990). The mechanism proposed has been the blockage of metastasis by covering galactose-specific binding sites (Hagmar et al., 1991). These activities may have possible therapeutic implications in cancer treatment, from the approach of modulating the immunological functions or by blocking metastasis. Roots of \textit{Ashwagandha (Withania somnifera)} a common ingredient of many Ayurvedic preparations have shown very promising cancer therapeutic effects in clinical study (Davis and Kuttan, 2000). \textit{Withania somnifera} has shown to be immunomodulator and metastatic inhibitor (Leyon and Kuttan, 2004a). \textit{Boerhaavia diffusa} a medicinal plant has proved its antimetastatic activity (Leyon et al., 2004). Similarly Piperine a natural product from \textit{Piper longum Linn} has shown to be a potent inhibitor of metastasis (Pradeep and Kuttan, 2002) nuclear factor-kappaB (NF-kappaB), c-Fos,CREB, ATF-2 and proinflammatory cytokine gene expression in B16F-10 melanoma cells (Pradeep and Kuttan, 2004). It also proved to inhibit the matrix metalloproteinase production in highly metastatic B16F-10 melanoma.
Intraperitoneal administration of the synthetically prepared natural compounds tetrahydrocurcumin (THC), salicyl curcumin (SC) and curcumin III (C-III) proved to reduce the number of tumour directed capillaries induced by B16F-10 melanoma cells in C57BL/6 mice (Leyon and Kuttan 2003). Administration of Indian medicinal plant *Tinospora cordifolia* proved to inhibit angiogenesis and also inhibit the production of pro inflammatory cytokines and the direct endothelial cell proliferating agent vascular endothelial cell growth factor (VEGF) during angiogenesis (Leyon and Kuttan, 2004b). Administration of the polysaccharide fraction from *Tinospora cordifolia* was found to be very effective in reducing the metastatic potential of B16F-10 melanoma cells and also inhibit the production of the matrix metalloproteinase (Leyon and Kuttan, 2004c). The natural products from *cruciferase* family allyl and phenyl isothiocyanates proved their anti metastatic activity in highly metastatic B16F-10 melanoma cells and it also proved to inhibit the production of pro inflammatory cytokines and matrix metalloproteinase (Manesh and Kuttan, 2003). Beta-carotene a naturally occurring polyterpene proved to inhibit the lung metastasis induced by B16F-10 melanoma cells in C57BL/6 mice (Pradeep and Kuttan, 2003) Curcumin, an active ingredient present in *Curcuma longa* shown to inhibit lung metastasis induced by B16F-10 melanoma cells (Menon et al., 1999).

The production of monoclonal antibodies led to a new approach to targeting cancer cells in the 1980s opened an approach that combined this new means of tumour targeting with natural products: antibody-directed enzyme-prodrug therapy (ADEPT). This technique uses an antibody specific for a tumour cell that is conjugated to an enzyme ((Niculescu-Duvaz and Springer, 1997). The conjugate is administered to the
patient and, in principle, it accumulates at the tumour site as the antibodies bind to antigens on the tumour cell surface. After a short period, during which excess conjugate clears from the system, a non-cytotoxic prodrug is given and this is activated by the enzyme in the antibody–enzyme conjugate to yield a cytotoxic anticancer drug. In principle, the cytotoxic agent is only revealed close to the tumour cells, so nonspecific cytotoxicity is reduced. The other advantage is that one molecule of enzyme catalyses the production of numerous molecules of drug. Many naturally occurring cytotoxic agents have been converted into prodrugs, and for much of the past decade they have been used with the ADEPT technology more as experimental tools than as clinically viable drugs.

VI TERPENOIDS

Terpenoids perhaps are the most structurally varied class of plant natural products. The name terpenoid, or terpene, derives from the fact that the first members of the class were isolated from turpentine. All terpenoids are derived by repetitive fusion of branched five-carbon units based on isopentane skeleton. These monomers generally are referred to as isoprene units because thermal decomposition of many terpenoid substances yields the alkene gas isoprene as a product and also because suitable chemical conditions can induce isoprene to polymerize in multiples of five carbons, generating
Fig 1.2a Terpenoids

Carvone

Limonene

Perillic acid

Ursolic acid

Oleanolic acid
Fig 1.2b Terpenoids

Glycyrrhizic acid

Nomilin
numerous terpenoid skeletons. The enzyme isoprene synthase is present in the leaf plastids of numerous plant species, but the metabolic rationale for the light-dependent production of isoprene is unknown. C10 terpenoids, although they consist of two isoprene units, are called monoterpenes. The triterpenes, which contain 30 carbon atoms, are generated by the head-to-head joining of two C15 chains, each of which constitutes three isoprene units joined head-to-tail. This large class of molecules includes the brassinosteroids, the phytosterol membrane components, certain phytoalexins, various toxins and feeding deterrents, and components of surface waxes, such as oleanolic acid of grapes. These compounds are important intermediates in the biosynthesis of steroids. Most of the terpenes are colourless fragrant liquids having boiling points between 150°C and 200°C. They are lighter than water and are ready steam volatile. They dissolve in organic solvent but usually not in water. Chemically they are considered hydrocarbons and are highly reactive in nature. Although terpenoids are widely used for medicinal purposes in many Asian countries, biogenesis and pleiotropic actions has not impacted on the practice of western medicines (Nanjoo et al., 1998).

Monoterpenes are nonnutritive dietary components found in the essential oils of citrus fruits, cherry, mint and herbs. They function physiologically as chemoattractants or chemorepellents (McGarvey and Croteau, 1995), and they are largely responsible for the distinctive fragrance of many plants. These 10 carbon isoprenoids are derived from the mevalonate pathway in plants but are not produced by mammals, fungi or other species. In citrus fruits (Chayet et al., 1977), peppermint and other plants, d-limonene is formed by the cyclization of geranylpyrophosphate in a reaction catalyzed by limonene synthase (Alonso et al. 1992; Kjonaas and Croteau, 1983). Limonene then serves as a precursor to
a host of other oxygenated monocyclic monoterpenes such as carveol, carvone, menthol, perillyl alcohol and perillaldehyde (Karp et al. 1990; McGarvey and Croteau, 1995). In addition, d-limonene is a prevalent flavoring agent for fruit juices, soft drinks, baked goods, ice cream and pudding. Orange oil, naturally consisting of 90–95% d-limonene, is a commercially available food flavoring agent. Furthermore, because of its pleasant citrus fragrance, d-limonene is commonly added to cosmetics, soaps and other cleaning products. Thus, human exposure to monoterpenes through the diet or environment is widespread.

VII TERPENOIDS IN CANCER THERAPY

A number of dietary monoterpenes have antitumor activity, exhibiting not only the ability to prevent the formation or progression of cancer, but to regress existing malignant tumors. Limonene has well established chemopreventive activity against many cancer types. Limonene has been shown to inhibit the development of spontaneous neoplasms in mice receiving 1200 mg/kg orally; dietary limonene also reduces the incidence of spontaneous lymphomas in p532/2 mice (Hurting et al. 1995). Furthermore, when administered either in pure form or as orange peel oil (95% d-limonene), limonene inhibits the development of chemically induced rodent mammary (Elegbede et al. 1984, Elson et al. 1988, Maltzman et al. 1989; Wattenberg 1983), skin (Elegbede et al. 1986b), liver (Dietrich and Swenberg 1991), lung and forestomach (Wattenberg et al. 1989 and 1991) cancers (reviewed in Crowell and Gould 1994; Elson and Yu 1994, Elson 1995). In rat mammary carcinogenesis models, the chemopreventive effects of limonene are evident during the initiation phase of 7-12-dimethylbenz [a]anthracene (DMBA)-
induced cancer (Elson et al. 1988) and during the promotion phase of both DMBA- and nitrosomethylurea (NMU)-induced cancers (Elson et al. 1988, Maltzman et al. 1989). Dietary limonene also inhibits the development of ras oncogene–induced mammary carcinomas in rats (Gould et al. 1994). There are many reports (Kawamori et al., 1996) that the development of azoxymethane-induced aberrant crypt foci in the colon of rats was significantly reduced when they were given 0.5% limonene in the drinking water. Caraway seed oil, and its principal monoterpenes, carvone, prevent chemically induced lung and forestomach carcinoma development when administered before the carcinogen (Wattenberg et al. 1989). In addition, carveol (Crowell et al. 1992a) and menthol (Russin et al. 1989) have chemopreventive activity against DMBA-induced rat mammary cancer when fed as 1% of the diet only during the initiation phase. Geraniol, an acyclic dietary monoterpenes, has in vivo antitumor activity against murine leukemia, hepatoma and melanoma cells (Shoff et al. 1991; Yu et al. 1995) when administered both before and after tumor cell transplantation. In addition, perillyl alcohol has promotion phase chemopreventive activity against chemically induced liver cancer in rats (Mills et al. 1995) and is very effective at preventing tumor recurrences or secondary tumors in animals treated in a chemotherapy regimen (Haag and Gould, 1994). Dietary monoterpenees have promising chemotherapeutic activity against established rodent pancreatic and mammary tumors. Both limonene (Elegbede et al. 1986a, Haag et al. 1992) and perillyl alcohol (Haag and Gould 1994) have chemotherapeutic activity against rat mammary tumors, causing the complete regression of 80% of established DMBA- or NMU-induced mammary carcinomas. Chander et al. (1994) reported that combination chemotherapy of NMU-induced rat mammary tumors with limonene and the aromatase
inhibitor 4-hydroxyandrostenedione was more effective than either drug alone. Perillyl alcohol has chemotherapeutic activity against pancreatic cancer at doses that cause little toxicity to the host (Stark et al. 1995). Perillyl alcohol reduced the growth of transplanted hamster pancreatic tumors to less than half that of controls. Moreover, a significant portion of perillyl alcohol–treated pancreatic tumors completely regressed, whereas none of the control tumors regressed (Stark et al. 1995). Phase I clinical trial testing of the cancer chemotherapeutic activity of limonene (McNamee 1993) and perillyl alcohol (Phillips et al. 1995) is in progress. The limonoids, limonin and nomilin are oxidized triterpenes, which are responsible for bitter taste of citrus fruits. They have shown to inhibit azoxymethane induced colon carcinogenesis in rats (Tanaka et al., 2000). Nomilin was found to inhibit benzo[a]pyrene (BP) induced neoplasia in fore stomach of ICR/1Ha mice. Ursolic acid is a pentacyclic triterpenes compound isolated from many types of medicinal plants are is present in human diet. It has been reported to possess a wide range of pharmacological properties and is one of the most promising chemopreventive agent (Rocha et al., 2004; Shih et al., 2004). Ursolic acid (3β-hydroxy-urs-12-en-28-oic acid) is a pentacyclic triterpenoid derived from berries, leaves, flowers, and fruits of medicinal plants, such as Rosemarinus officinalis, Eriobotrya japonica, Calluna vulgaris, Ocimum sanctum, and Eugenia jumbolana (Liu, 1995). Ursolic acid has been shown to suppress tumorigenesis (Huang 1994), and inhibit tumor promotion (Tokuda et al., 1986; Ohigashi et al., 1986; Nishino et al., 1988). Several of these effects of ursolic acid are mediated through suppression of the expression of lipoxygenase, COX-2, MMP-9, and iNOS (Simon et al., 1992; Najid et al., 1992; Ringbom et al., 1998 Subbaramaiah et al., 2000; Cha et al., 1998; Cha et al., 1996; Suh et al., 1998) all of which are genes regulated
by NF- \([\kappa]\) B. In addition, ursolic acid and its derivatives have been shown to induce apoptosis in a wide variety of cancer cells including breast carcinoma, melanoma, hepatoma, prostate carcinoma, and acute myelogenous leukemia (Es-Saady et al., 1996; Es-Saady et al., 1996 (b); Cho et al., 2000; Hollosy et al., 2000; Hollosy et al., 2001; Konopleva et al., 2002; Cho et al., 2000) through inhibition of DNA replication (Kimet al 2000), activation of caspases (Cho et al., 2000; Hollosy et al., 2001; Konopleva et al., 2002) inhibition of protein tyrosine kinases (Hollosy et al., 2000) and induction of Ca2+ release (Baek et al., 1997; Lauthier et al., 2000). Ursolic acid inhibits the cell proliferation of human lung cancer cell line A549 showed that the blocked cell cycle progression in the G1 phase (Hsu et al., 2004). It also decreased the protein expression of cyclin D1, D2, and E and their activating partner cdk2, 4, and 6 with concomitant induction of p21/WAF1. Ursolic acid is able to inhibit key steps of angiogenesis in vitro, including endothelial cell proliferation, migration, and differentiation. (Cardenas et al., 2004). Oleanolic acid and ursolic acid have known anti-inflammatory and anticarcinogenic activity (Deepak and Handa, 2000; Liu, 1995; Nanjoo et al., 1998).

A wide variety of natural compounds appear to possess significant anticancer as well as chemopreventive activity. Extracts of plants used in traditional medicine also have a similar property. Clearly, knowledge of the active principles associated with common plant products is of utmost importance. Many more screening studies are necessary using plant extracts and compounds isolated from them. Elaborate studies with such compounds with respect to their abilities to induce apoptosis and understanding their mechanism of action may provide valuable information for their possible application in
cancer therapy and prevention. Having the option to administer an agent through the diet or as a prescription drug is a major advantage.