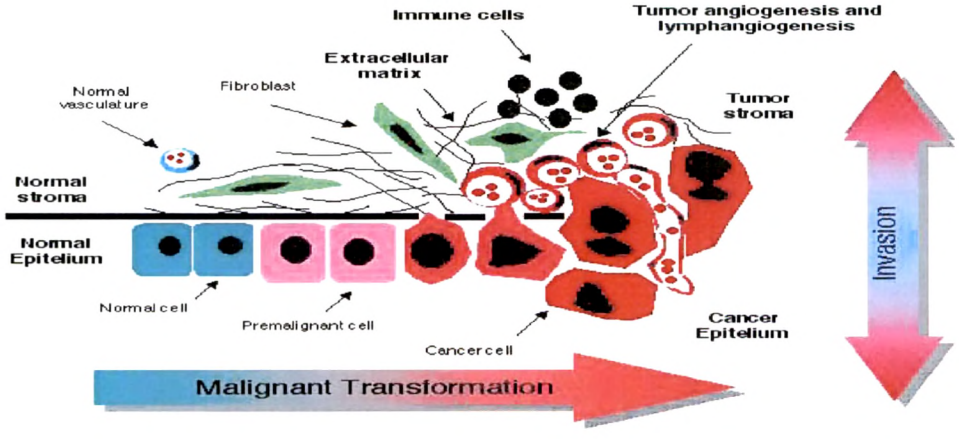
# Introduction

Cancer, a complex disease, represents the end product of a multistep biological process including growth and survival of neoplastic cells at the primary tumour site, invasion in to the host tissue, angiogenesis, intravasation and survival in the circulatory system, arrest and extravasation into a new tissue and finally growth at the secondary site (Alessandro et al, 2005). Although often classified as a genetic disease, it is functionally a proteomic disease. Genetic mutations can modify protein signaling pathways and thereby helping survival of the cells by ignoring the negative inhibitory signals or perpetually sending false positive signals. The pathogenic signaling pathways are not confined to the cancer cell, but extend to the tumour–host (cellular and ECM) cell interactions and also recognize that cancer is a product of proteomic tissue microenvironment and has important implications. Firstly, it shifts the emphasis away from therapeutic targets as an individual molecule. It might make more sense to target all the deranged signaling pathways both inside and outside the cancer cell. Second, the tumour–host communication system might involve enzymatic events and sharing of growth factors, so the microenvironment of the tumour-host interaction could be a source for biomarkers that could ultimately be shed into the serum proteome (**figure: 1**).

**Figure-1: Cancer: a disease of the tissue microenvironment**




*Adapted from http://cancergov/clinicaltrials/developments/anti-angio*

**2.1 Epidemiology and Incidence:** The estimates of the worldwide incidence, mortality and prevalence of 26 cancers in the year 2002 shows that there were 10.9 million new cases, 6.7 million deaths, and 24.6 million people living with cancer (within three years of diagnosis). MM, a malignant tumour of plasma cells in the bone marrow constitutes around 1% of all the cancers worldwide (86,000 new cases). MM comprises 10% of all haematological malignancies. Its incidence varies from 0.4 to 5 per 100,000 persons and is very rare in people under 40 years of age. The incidence of MM is high in North America, Australia, New Zealand, Northern and Western Europe compared with Asian countries. A slow increase in the incidence and mortality from myeloma is observed in most regions of the world, the reason for which is unknown. The exposure to ionizing radiation is the only well-established risk factor for MM, although some chemicals and occupational exposures have also been reported to be associated with an increased risk. The five-year survival rate for patients with MM is 15 to 20 % (Parkin et al, 2005).

In India, incidence of MM varies from 0.3 to 1.9 and 0.4 to 1.3 per 100,000 in men and women respectively (ICMR, 2005). The median age of presentation with MM is 55 years, a decade less than that in USA. The incidence of myeloma increases with age and men to women ratio is 1.5:1 (Kumar et al, 2006). This is reflected in the cancer registry data of GCRI, which equals to more than 100 cases per year (Annual Report, 2004).

Cervical cancer is the seventh in overall cancer in frequency. But it is the second most common cancer among women worldwide after breast cancer with an estimated 493,000 new cases and 274,000 deaths in the year 2002. Generally, it is more common in the developing countries. In developed countries, cervical cancer accounts for only 3.6% of new cancers.

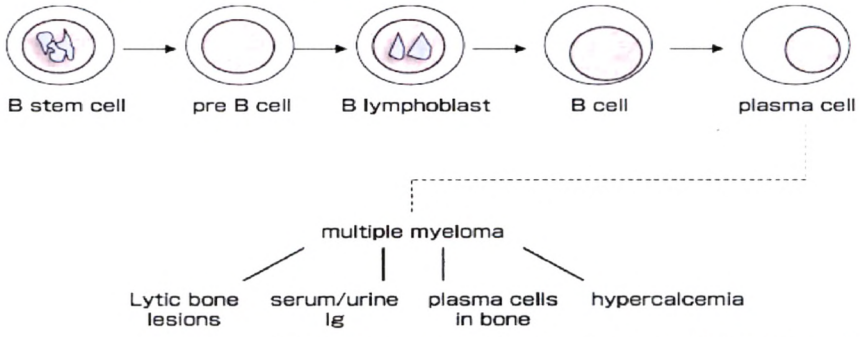
The ratio of mortality to incidence of cervical cancer is 55% worldwide. The survival rates vary between various regions. A good prognosis is seen in low-risk regions (74% in SEER and 63% in the European registries). Even in developing countries, where many cases present in relatively advanced stage,

the survival rates are fair. The major etiological factor is oncogenic subtypes of the HPV. Other cofactors [e.g. high parity, tobacco smoking (Zivaljevic et al, 2001), and use of oral contraceptives] modify the risk in women infected with HPV. As a result of these trends, cervical cancer has ceded its place as the leading cancer in developing countries to breast cancer (Parkin et al, 2005). India has one third of the world's population but also has two third of the world's cervical cancer burden (National cancer control programme, 2008). It is a leading cause of cancer mortality among women in developing country. According to the hospital based cancer registry, around 1200 new cases are registered every year (Annual Report, 2004).

## 2.2 Biology of Multiple Myeloma:

**Definition:** MM is a plasma cell dyscrasia characterized by the proliferation of monoclonal plasma cells. It is the most common malignancy involving plasma cells also called as plasma cell myeloma, myelomatosis or Kahler's disease (**figure: 2**).

**Figure-2: B cell development and multiple myeloma**

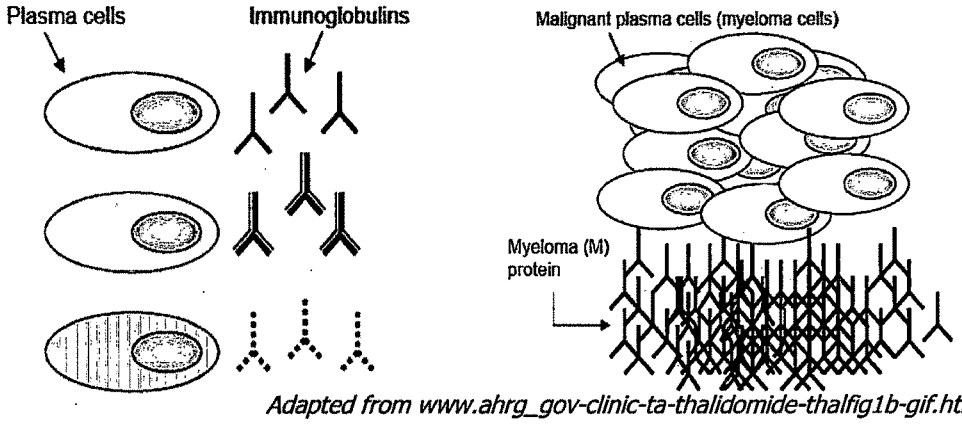



*Adapted from www.glycoforum.gr.jp/.../HA27/images/fig02.gif*

They represent a spectrum of diseases that are classified according to the degree of plasma cell proliferation and the presence of end-organ damage including bone pain, anaemia, hyper viscosity, recurrent bacterial infections, renal insufficiency, amyloidosis or hypercalcaemia. The disease begins with the mere presence of M-protein in the serum. The circulating M-protein may consist of an intact immunoglobulin (fraction of plasma proteins having antibody activity), the light chain or rarely the heavy chain only. The heavy

chain belongs to one of the immunoglobulin classes, while the light chain is either kappa (k) or lambda (λ) type and progressing through both asymptomatic and symptomatic forms of MM, ending with the presence of monoclonal plasma cells in peripheral blood (**figure: 3**). It can occur in any one of these forms, and they are capable of remaining stable or progressing further to more severe disease (Fentress et al, 2006). MM is an interesting and instructive disease from the point of view of diagnosis and pathophysiology.

**Figure-3: Production of the M-protein in multiple myeloma**




*Adapted from www.ahrg_gov-clinic-ta-thalidomide-thalfig1b-gif.htm*

**Risk Factors for Multiple Myeloma:** No specific etiologic factor for MM has been found. The predisposing factors of MM in human appear to be:

1. **Radiation exposure:** This increases the risk for MM.
2. **Chronic antigen stimulation:** Many M-proteins have been shown to be antibodies directed against specific antigens, such as microbial antigens, red blood cell antigens, lipoproteins, rheumatoid factors, neural antigens, and coagulation factors. Chronic antigenic stimulation (e.g. chronic osteomyelitis or cholecystitis) may predispose to the development of MM.
3. **Environmental exposure:** Exposure to benzene in the work place and the use of hair dye are associated with an increased incidence of MM.
4. **Human herpes virus- 8:** A new human herpes virus has been found in the non malignant bone marrow dendritic cells of patients with myeloma.

**Pathophysiology:** The Pathophysiology is mainly classified in two ways.

**(i) Basic physiology of MM**

In 80% of patients there is a paraprotein in the serum usually of the IgG or IgA class. The abnormal cells may also produce free light chain, which is excreted in the urine as Bence-Jones protein. In 20% of patients only free light chains are produced (Bence-Jones myeloma) with no M-protein in the serum. In 60% of patients, bone pain due to the number of plasma cells in the bone marrow is increased focally and osteoclasts are activated in the region of plasma cell foci causing bone resorption e.g. vertebral collapse, pathological fracture of a long bone. Bone destruction may result in hypercalcaemia (Samson, 2000). Renal failure is also a common problem in myeloma patients. 25–30% of patients have some degree of renal impairment and about 5% present with severe renal failure. Anaemia is another common presenting feature appears to be mediated by cytokines such as IL-1β rather than as a direct effect of marrow replacement. In some patients renal failure may also contribute to the anaemia due to deficient erythropoietin production (Devita, 2001a).

**(ii) New insights into the pathophysiology of MM**

Richardson and Anderson (2004) have reported the study of development of an orderly series of genetic changes that accumulate in the malignant plasma cell and the development of changes in the bone marrow microenvironment that support tumour growth, and a failure of the immune system. Numeric chromosomal abnormalities are present in virtually all myelomas (Avet-Loiuseau et al, 1999). The most frequent site of chromosomal deletion is in 13q. Primary translocations are thought to occur early in pathogenesis, whereas secondary translocations are involved in progression of the disease. During the pathogenesis of MM, most primary translocations are simple reciprocal translocations that juxtapose an oncogene and one of the immunoglobulin enhancers. Mainly they mediate errors in immunoglobulin heavy chain switch recombination, but sometimes also cause errors in somatic hypermutation during plasma cell generation in germinal centers (Bergsagel

et al, 2001). The incidence of heavy chain translocations increases with the stage of tumourigenesis. Light chain translocations are less common. Lamda chain of immunoglobulin translocations are identified in approximately 20% of advanced MM (Avet-Louseau et al, 2000; 2001a). Four partner chromosomes (4p16, 6p21, 11q13 and 16q23) and associated oncogenes that commonly become fused to immunoglobulin enhancers have the hallmarks of primary translocations, whereas additional infrequent but recurrent partner loci often have the characteristics of secondary translocations. The second group of gene is located on 4p16 and encodes two proteins namely MMSET (a putative histone methyltransferase involved in chromatin remodeling) and fibroblast growth factor receptor (FGF)-3 plus an oncogenic receptor tyrosinekinase. These are translocated in about 15% of MM. The third group of genes commonly involved in immunoglobulin gene translocations comprises the code for two β-zip transcription factors-c-Maf (encoded on 16q23) and MAFB (encoded on 20q11). These are translocated in about 10% of MM (Chesi et al, 1998a, 1998b; Hanamura et al, 2001). In MM, complex translocations deregulate c-myc as a late progression event that is associated with enhanced cell proliferation. It is frequent in the advance stages and is often heterogeneous among cells within the MM (Avet-Loiseau et al, 2001b). In addition, there is evidence that a number of other well-known tumour suppressor genes- p16, p18, PTEN- cellular phosphatase involved in the regulation of phosphatidylinositol phosphates (PIP's) and p53- are sometimes involved in the progression of MM. (Urashima et al, 1997; Kulkarni et al, 2002 ;Neri et al, 1993; Ge et al, 2000a). MM that overexpress fibroblast growth factor 3 as a result of a t(4;14) translocation can have activated mutations of ras or FGF receptor 3 but not both. This is consistent with constitutive activation of the mitogen, an activated protein kinase (MAPK) pathway downstream of both FGF receptor 3 and ras suggesting that it may be redundant to activation of both pathways. (Chesi et al, 2001). IL-6 is an important cytokine originally identified as a B-cell differentiation factor that causes proliferation of plasmablastic cells and induces terminal differentiation of B cells into antibody producing cells. There is increasing evidence that IGF-

1 is another paracrine factor that enhances tumour survival and growth. Both cytokines are thought to increase proliferation by activating the ras-MAPK pathway. An enhanced survival is mediated by different pathway: IL-6 activates a transcription factor namely STAT3; which increases the expression of anti-apoptotic genes including mcl-1, bcl-XL and pim-1 as well as c-myc; which presumably enhance cell proliferation (Catlett-Falcone et al, 1999; Puthier et al, 1999). STAT-3 also seems to activate the phosphatidylinositol PI3-kinase-Akt pathway (Hideshima et al, 2001). IGF-1 also activates PI3-kinase, which phosphorylates BAD, and Akt to inhibit apoptosis (Ge et al, 2000b). Mutations in the ras (MAPK pathway) or FGF receptor 3 enhance survival and proliferation, but do not always result in IL-6 independence.

**The bone marrow microenvironment:** The pathogenesis of most tumours includes complex and budding mutual interactions that affect the number and phenotypes of both the tumour cells and a variety of normal bone marrow stromal cells (BMSC) (Liotta et al, 2001). Normal long-lived plasma cells and all stages of intramedullary tumours are dependent on the bone marrow microenvironment for survival, growth, and differentiation (Shain et al, 2000). There are a number of major biological phenomena that are affected by these tumour-host interactions. It includes: (i) homing of the tumour to the bone marrow (ii) spread of the tumour via the blood stream from one site to a second site within the bone marrow (iii) angiogenesis (iv) osteoclastogenesis, osteolysis and inhibition of osteogenesis etc.

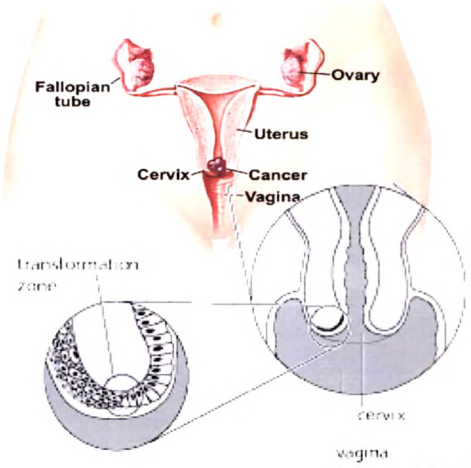
## 2.3 Cervical Cancer

Amongst all the cancers, only cervical cancer can reliably be prevented by an effective, inexpensive screening technique that allows detection and treatment of precancerous conditions. Cervical cancer was first identified by George Papanicolaou, a Greek Physician working at Cornell University, in 1928. Papanicolaou's previous research on the oestrous cycles of guinea pigs led to the discovery that female mammals have specific cytologic patterns

within the genital tract that correspond with their menstrual cycles (Vilos et al, 1998).

**Definition:** Cervical cancer is caused by neoplastic changes in the cells that line the wall of the cervix. These cells begin as normal cells and then gradually transforms to precancerous cells leading to lesions in the cervical wall. The cells may eventually become cancerous. Most cervical carcinomas arise primarily at the junction between columnar epithelium of the endocervix and squamous epithelium of the ectocervix. This transformation zone is the most common site for the development of cervical cancer (**figure: 4**).

**Figure-4: Anatomical view of cervical cancer**



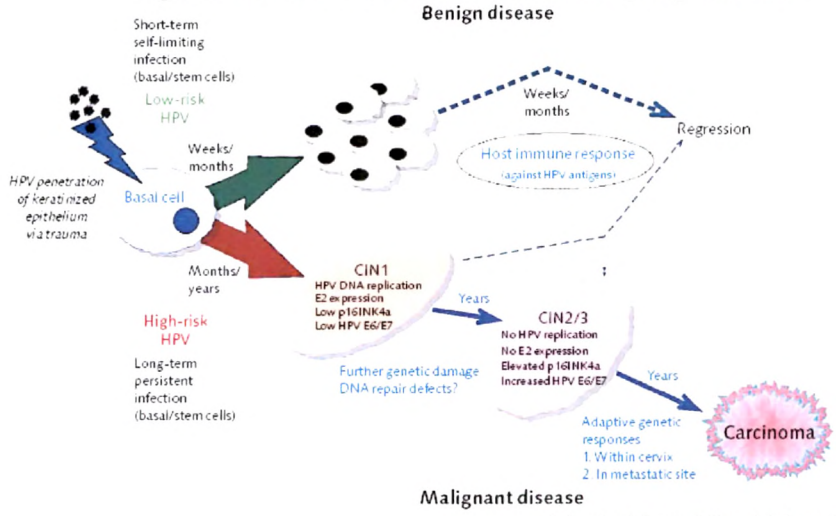

*Adapted from www.eontarionow.com*

**Risk Factors for Cervical Cancer:**

The most common risk factor for cervical cancer is an exposure to certain strains of the HPV. HPV infection is a sexually transmitted disease (STD), which may or may not be accompanied by symptoms. Women aged 30-60 are mostly at risk and the risk increases further with the age and irregular screening are also considered as risk factors for cervical cancer. Moreover, women who have early onset of sexual activity, multiple sexual partners (prostitutes), sexually transmitted diseases, or bearing children at young age, promiscuous sexual behavior in their male partners, prolong oral contraceptive use, immunodeficiency, vitamin A or C deficiency, cigarette smoking and socioeconomic status etc.

**HPV,** is a family of closely related viruses, each designated as a type, numbered in order of discovery. More than 100 HPV types are known to exist and at least 30 serotypes are detected in the anogenital tract. E6 has multiple activities including the inactivation of the tumour suppressor gene p53 by ubiquitin-mediated degradation and telomerase activation (high-risk viruses only), while E7 binds to and sequesters the tumour suppressor gene pRB110 resulting in cell-cycle stimulation. The effects of these two proteins permit long-term unrestricted cell proliferation with minimal checkpoint restriction to eliminate cells which might lead to mutations. This is ideal for the replication of HPV, as the infected host cells are induced into cycle. Each virus is assigned a number and the five types, most commonly associated with cervical cancer are 16, 18, 31, 33 and 45. HPV is a common virus that can pass through sexual activity. It usually causes no symptoms. In majority of women, the immune systems get rid of the infection (Devita, 2001b). In cervical cancer, HPV infection initially results in low-grade lesions termed cervical intraepithelial neoplasias (CIN: grade I and II). They are considered as pre-malignant and mostly regress spontaneously within 1–2 years. In CIN2 and CIN3, however, the onset of late events is retarded. Some of these can persist for longer period and may progress into malignant tumours (**figure: 5**).

**Figure-5: HPV and cervical cancer progression**




*Adapted from Microbiology Today, 2005*

**2.4 Central Dogma of Biology:** The cell is the structural and functional unit of the biology. DNA is the chemical basis of heredity and is organized into genes, the fundamental units of genetic information. Study of proteins presents an exciting challenge in this information rich age of genome biology. An organism is an integrated system of mutually dependent molecular and cellular processes. Although various investigations have yielded abundant information about individual proteins and an integrated understanding of biological system. The great promise of the human genome project is that a plethora of novel studies have been carried out to investigate the molecular mechanisms of the encoded genes. Transcriptome is the complete set of mRNAs that are transcribed from genome. Many changes in gene expression might not be rejected at the level of protein expression or function. Moreover, quantitative mRNA level is insufficient to predict actual protein expression because of post-transcriptional regulation and internal ribosome initiation of translation (Washburn et al, 2003). Finally, proteins are subjected to post-transnational modifications and their stability (turnover rate) is regulated under varying physiological conditions. Proteins are the final products manufactured in living cells according to the 'blue print' contained in the genome. Proteins catalyze a diverse range of chemical reactions, act as information sensors, transducer signals, provide structural scaffolding for cells and extracellular tissue components, control membrane permeability, recognize and covalently bind other molecules, cause motion and control gene function.

**Serum proteins:** Although there are different proteins in human serum, cancer associated protein markers for protein profiling can be contributed by the tumour microenvironment (e.g. surrounding host cells such as fibroblasts and macrophages). Some of these products are circulated in blood. Hence, serum has been the traditional and potential sample for studies of biomarkers. Several pathological conditions result into alterations in electrophoretic patterns which give rise to changes in the particular protein fractions.

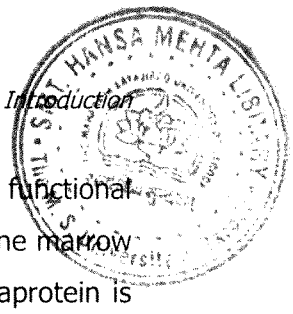
**Prealbumin:** Prealbumin or transthyretin is a hepatic secretory protein thought to be important in the evaluation of nutritional deficiency and nutrition support. The concentration of serum prealbumin is greatly decreased in protein energy malnutrition. It reflects the ability of the liver to synthesize protein and is a reliable index of liver function. Prealbumin is a negative acute phase reactant. Its concentration is more sensitive than albumin and transferrin for measuring protein and calorie intake. Serum levels of prealbumin decrease in various malignancies. (Myron Johnson et al, 2007).

**Albumin:** This is the major protein in the blood and is synthesized in liver. It binds with several substances like calcium, some hormones and certain drugs in the circulation and thus prevents their filtration by kidney. Albumin also acts to regulate the movement of water between the tissues and the bloodstream by attracting water to areas with higher concentration of salts or protein.

**Alpha globulins:** These proteins include alpha-1 and alpha-2 globulins. Alpha-1 globulin consists of alpha-1 protease inhibitor (alpha-1 anti trypsin, an enzyme produced by liver and lungs), alpha-1 acid glycoprotein (orosomucoid) and high density lipoprotein. Alpha-2 globulin, which includes serum hepatoglobulin, is a protein that binds free haemoglobin to prevent its excretion by kidney. Various other alpha globulins (alpha-2 macroglobulin, antithrombin III, ceruloplasmin) are produced as a result of inflammation, tissue damage, autoimmune disorders or certain cancers.

**Beta globulins:** These include low density substances involved in fat transport (lipoprotein), C-reactive protein, free hemoglobin, transferrin (principal component of the beta 1 subdivision), beta-2 microglobulin ($\beta_2$-M) and clotting system proteins (coagulation factors, plasminogen and complement).

**Gamma globulins:** All of the gamma globulins are antibodies produced by the immune system in response to infection, allergic reactions and graft vs. host disease. Quantification of immunoglobulin is clinically useful in detecting



and monitoring the hypogammaglobulinemia that results from functional impairment of the normal immunoglobulin producing cells of the bone marrow due to excessive expansion of malignant clone(s). Usually the paraprotein is all or part of an immunoglobulin molecule (O'Connell et al, 2005).

**M-protein:** "Paraprotein" term was first time coined in 1940 and described as the protein in blood and in urine produced by myeloma cells. So paraproteins were the first described as tumour markers. They are secreted by immunocytes and are important in diagnosis as well as monitoring of these diseases. Monoclonal immunoglobulins (paraproteins) are immunoglobulins or immunoglobulin fragments with homogeneous polypeptide chains unlike regular immunoglobulins which constitutes the primary structure. They are an identical product of a single clone of B-lymphocyte proliferating cells. Monoclonal immunoglobulin consists of two heavy polypeptide chains of identical antigenic type. Paraproteins can be formed from the parts of monoclonal immunoglobulin molecules. Later it was found that paraprotein production is not always connected with malignant immunocytoma. Various aberrations of immune response lead to production of homogenic immunoglobulin by a single B–cell clone. Monoclonal immunoglobulins are characterized electrophretically by a typical, distinctly defined gradient. Therefore, they are called M-proteins, M-gradients, M-components or M-fractions. This "M" means monoclonal, myeloma or macroglobulin components. The complete molecules of monoclonal immunoglobulin are detected in the following frequency IgG>IgA>IgM. IgD paraproteins are rare and IgE paraprotein is rarest of all. Serial measurements of serum and urine M-protein are also used to characterize response of treatment and for follow up studies (Adriena Sakalova et al, 2006).

**2.5 Post genomic era:** Proteome or protein index (translatome) is defined as the expressed set of proteins that are encoded by the genome. Proteomics combine share of two words: **PROTE** from PROTEIN and **OMICS** from GENOMICS. The term was coined in 1994 (Wilkins et al, 1996a, 1996b). Although the exact definition of proteomics varies, it is widely accepted that

proteomics is the study of all the protein forms expressed within an organism as a function of time, age, state, external factors, etc (Reynolds, 2002). In other words, proteomics characterizes the behavior of the system rather than the behavior of any single component. Essence of "proteomics" is the effort to realize the dynamics of protein function. By studying the proteome of a cell at various times or under different conditions, one can gain information about proteins that are relevant to certain processes like alterations in their post-translational modifications, expression and their interactions with other proteins that lead to the observed cellular phenotype (Patel et al, 2005a, 2005b ; Wu et al, 2002). This high throughput biochemistry is crucial for the emerging field of systemic biology contributing at a direct level to a full description of cellular function and biological phenomena. In particular, clinical biochemistry has found a new era from this holistic point of view. The interest in clinical proteomics is due in part to the scenario that a proteomics approach to disease investigations will overcome some of the limitations of other approaches. The field of proteomics is considered one of the most important post-genome era approaches applied in understanding cellular function and dysfunction, and has shown great promise in the field of cancer research.

**Cancer proteomics can be mainly divided into two categories:**

**(1) Expression Proteomics (2) Functional Proteomics**

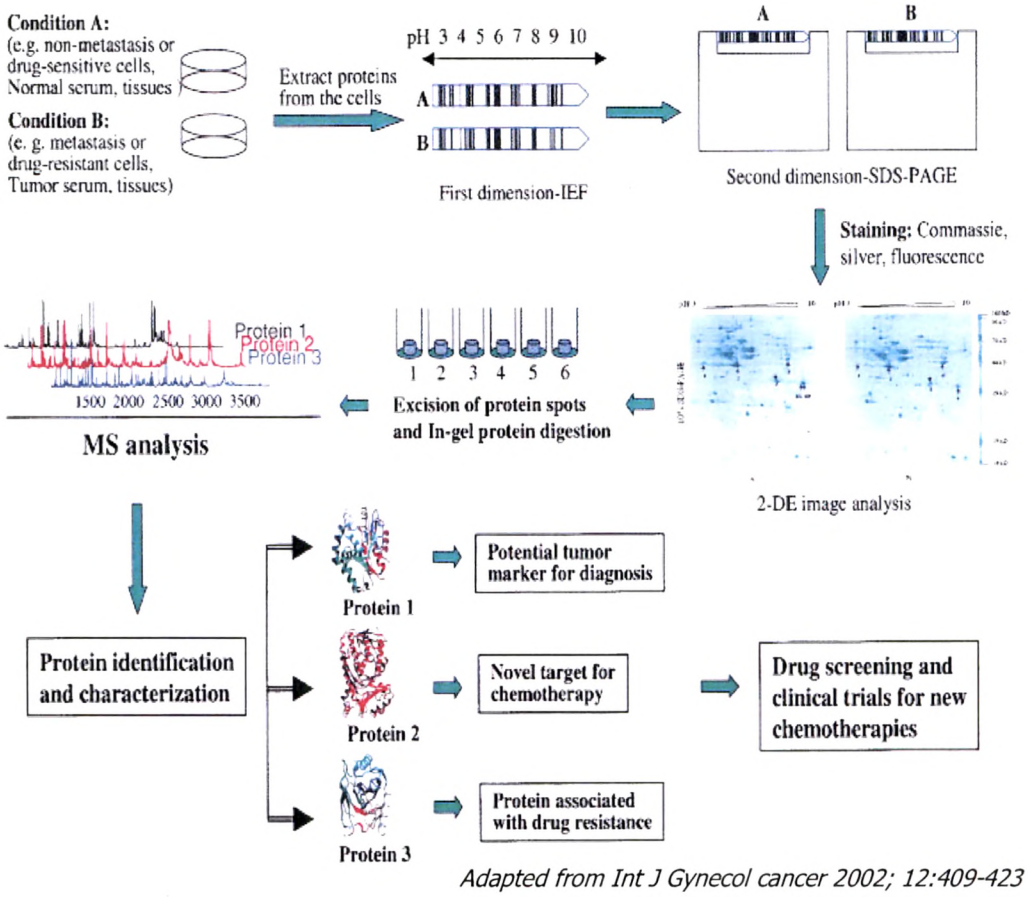
The tools of expression proteomics include mixture of "old" and "new" technologies.

The approach which include identification of proteins in a particular sample as a function of a particular state of the organism or cell (e.g. differentiation, developmental state or disease state) or as a function of exposure to a drug, chemical or physical stimulus. Expression profiling most commonly practiced as a differential analysis in which two states of a particular system are compared (i.e. normal and diseased cells or tissues can be compared to determine which proteins are expressed differently in one state compared to other) (Alaoui-jamali and Ying-jie, 2006; Liebler and Yates, 2002). Proteomics

use a combination of sophisticated techniques including 2D gel electrophoresis, image analysis, mass spectrometry, amino acid sequencing and bio-informatics. However, the central tool for displaying the proteome is 2D-PAGE to quantify and to characterize complex proteins (Patel et al, 2005a, 2005b; Westermeier and Naven, 2002). The proteomics approach for cancer research can reveal significant clinically useful data (**figure: 6**).

**Figure-6:**

**Basic experimental views of expression proteomics in cancer**




*Adapted from Int J Gynecol cancer 2002; 12:409-423*

**Separation of proteins using 2-D PAGE** (O' Farrell, 1975)**:** This technique sorts proteins according to two independent properties in two discrete steps: the first dimension step i.e. IEF separates proteins according to their Iso-electric points (pI) or charge. The second dimension step, SDS-PAGE, separates proteins according to their molecular weight. Each spot resulting on the two dimensional array corresponds to a single protein species in the

sample. Thousands of different proteins can be separated and quantitated. 2-D PAGE separates thousands of proteins, protein changes at post and co-transnational modifications, analysis of cell differentiation, early detection, disease markers, prognosis, monitoring therapies, drug discovery, purity checks and microscale protein purification in cancer research (Kavallaris and Marshall et al, 2005; Wu et al, 2002; Srinivas et al, 2001).

**Recent Studies on Proteome Based Approach in Cancer Research**

Various researchers have assessed the proteome based approaches in cancer research.

Using proteomic analysis Huang et al (2007) identified 6 differential protein spots. The results revealed that expressions of the squamous cell carcinoma antigen 1b (SCCA1b), KRT4 and annexin A1 were downregulated and triosephosphate isomerase (TPI1), heat shock protein 27 (HSP27) and manganese superoxide dismutase (MnSOD) were upregulated in esophageal cancer tissues as compared to normal tissues. The authors concluded that the identification of differentially expressed proteins could be helpful for screening the biomarker for early-stage diagnosis.

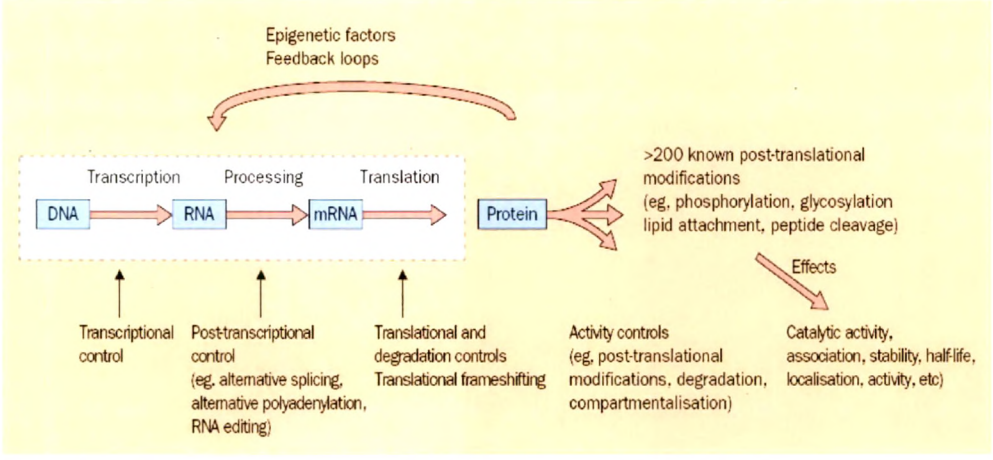
Hwa et al (2005) have studied tumour specific proteins from renal cell carcinoma and the normal kidney tissue with the 2D-PAGE and Matrix assisted laser desorption ionization-Time of flight-Mass spectrometers (MALDI-TOF-MS) analysis. The results showed that total 66 differentially expressed protein spots were found among which, 8 different proteins from 11 spots were identified by MALDI-TOF-MS. The expression of the following proteins were repressed; aminoacylase-1, enoyl-CoA hydratase, aldehyde reductase, tropomyosin-4 chain, agmatinase and ketohexokinase. Two proteins, vimentin and $\alpha$-1 antitrypsin precursor were dominantly expressed in renal cell carcinoma. The study concluded that the protein expression in renal cell carcinoma may help us to find tumour-specific proteins of these diseases. 2D-PAGE analyses of protein expression profiles in squamous cervical cancer patients were studied by Bae et al, (2005) in 50 tissue biopsies. 17 tissue

biopsies from non-tumour cervix tissues and 33 tissue biopsies from squamous cervical cancer and found that total 35 proteins were detected in squamous cervical cancer. Seventeen proteins were up-regulated and 18 proteins were down regulated. Among the proteins identified, 12 proteins (pigment epithelium derived factor, annexin A2 and A5, Keratin 19 and 20, HSP 27, smooth muscle protein 22 alpha, α-enolase, SCC 1 and 2, glutathione-S-transferase, apolipoprotein a1) were previously known proteins involved in tumour and 21 proteins were newly identified in this study. The study concluded that 2D offers further characterization of proteins that are differentially expressed. This can be useful to identify tumour specific diagnostic markers for squamous cervical cancer. Saito et al (2005) have analyzed urine samples collected from 40 patients with bladder cancers, 32 healthy volunteers, and 7 old volunteers with benign prostate hypertrophy were treated with gelatin-beads as a group-specific affinity carrier and found varying amounts of MMP-2, MMP-9 and fibronectin and their fragments. The proteomic approach with gelatin-affinity purification of urine samples was found to be useful for the preoperative diagnosis, tumour invasion and monitoring the recurrence in bladder cancer. Yu et al (2004) documented a study of proteome (2D-PAGE, MALDI-TOF-MS and peptide mass finger printing analysis) in primary cancer and hepatic metastatic tumour in colorectal cancer. They found that variations and hydrophobic protein expression in colorectal cancer initiation and hepatic metastasis are significant and can be observed with 2D-PAGE. The expression of calmodulin, ribonuclease 6 precursor and mannosidase-α is lost but the expression of proapolipoprotein is enhanced which is associated with colorectal cancer genesis and hepatic metastasis.

**2.6 Protein Post-translational Modifications:** Proteins undergo several types of covalent and noncovalent modifications. These include the cleavage of the N-terminal methionine, the formation of disulfide bridges between two cysteine residues, or cleavage of a precursor polypeptide region. Protein function is often regulated by post-translational modifications. As shown in

the **figure: 7**, more than one RNA can result from one gene through the process of differential splicing. Additionally, there are more than 200 post-translation modifications [(i.e. phosphorylation, lipid attachment, peptide cleavage and glycosylation (Mueller et al, 2007)]. Glycosylation, i.e. the attachment of monosaccharides or extended sugar chains to proteins, represents the most pronounced and most complex form of post-translational modifications. It is estimated that over 50% of all proteins are substituted with glycans attached to several sites (Pan et al, 2005). Carbohydrate have been found to participate in many biological processes, such as molecular recognition, inter and intracellular signaling, embryonic development, fertilization, immune defense, inflammation, cell adhesion and division processes, viral replication and parasitic infections. Therefore, alterations in various glycoproteins have long been associated with various malignancies.

**Figure-7: Pathway of protein post-translation modifications from DNA**




*Adapted from Banks et al, Lancet. 2000; 356:1749-56.*

**Cell Surface Glycoconjugates: An Overview**

Glycoconjugates are ubiquitous in nature and are found enriched in cell surfaces, internal membranes, and ECM such as basement membrane. Glycoconjugates comprise a heterogeneous group of compounds, all of which contain carbohydrate covalently attached to protein e.g. glycoproteins. Glycoproteins are proteins that contain oligosaccharide chains covalently

attached to either serine/threonine or aspargine residue in the polypeptide backbones. These oligosaccharide chains are often branched, and they do not contain repeating disaccharides. Most proteins in the blood are glycoproteins. The glycoconjugates serve as lubricant and protective agents, transport molecules, hormones, immunologic molecules, enzymes, cell attachment recognition site, interact with specific carbohydrates, receptors, folding of certain proteins and as structural components of the ECM (Murray et al, 2006). There is one class of glycoconjugate e.g. TSA, fucose and seromucoid fractions (MP and hexoses) or complex carbohydrates- equivalent terms used to denote molecules containing one or more carbohydrate chains covalently linked to protein to form glycoproteins. Glycoproteins are widely distributed in nature and found in bacteria to human.

The existence of sialic acid in nature was first recognized in 1927 by E. Walz and by P. A. Levene and K. Landsteiner (Gowda, 1984). Sialic acids have been found in the animal kingdom from the echinoderms upwards to humans. Sialoglycoconjugates are present on cell surfaces as well as in the intracellular membranes. In the higher animals they are also important components of serum and of mucous substances. The term "Sialic acids" represent a family of sugar molecules with an unusual and highly variable chemical structure that are found in terminal position of oligosaccharide chains on the surface of cells and molecules. Sialic acid is reflected in the variety of its biological functions like cellular and molecular recognition (e.g. immune system) process, binding and transport, attraction and repulsion phenomena, protective shield for the subterminal part of the molecule, to stabilize the correct confirmation of enzyme or cell membrane, masking effect of the cells and molecule (Traving and Schauer, 1998). Sialic acids play an important role in many pathological conditions, such as rheumatoid arthritis, cystic fibrosis, tuberculosis, diabetes mellitus, inflammatory process, acute myocardial infarction, autoimmune disease, trauma, prolonged bed rest, psychiatric disorders, oxidative stress and various malignancies (Nandave, 2005).

Fucose is a deoxyhexose that is present in the L-configuration of many N- and O-linked oligosaccharide structures of membrane as well as soluble glycoproteins and glycolipids produced by mammalian cells. The fucose is a component of the H, A, and B determinants of the ABO blood group and also is found in members of the Lewis series of histo-blood group antigens, including Lewis(X), Lewis(Y), Lewis (A), and Lewis (B). The trisaccharide antigens Lewis (A) and Lewis (X), each contain one fucose substituent. Characteristic of fucose is its almost exclusive presence at a preterminal or terminal position, i.e. not inserted in an oligosaccharide chain. Fucose can be alpha 1, 2 or alpha 1, 3 or alpha 1, 4 and alpha 1, 6 linked to the glycans of glycoconjugates (Listinsky. 1998; Becker et al, 2003). Fucose plays a crucial role in biological recognition events, such as cell-cell recognition and cell-matrix interactions, fucosylglycotopes, fucose-containing glycans, in many physiological processes, such as fertilization, embryogenesis, fetal development, neuron transmission, leukocytes adhesion, signal transduction, and apoptosis as well as in many diseases such as rheumatoid arthritis, atherosclerosis, cystic fibrosis, peptic ulcer disease, inflammatory process and neoplastic progression (Orczyk-Pawiłowicz, 2007).

Mucoproteins are proteins that contain carbohydrate. Mucoproteins, or mucoids, contain a mucopolysaccharide in firm chemical union with a peptide, and have a hexosamine content of over 4%. They include ovomucoid from eggs, seromucoid and seroglycoid from the $\alpha$-globulin fractions of blood serum, submaxillary and gastric mucoids, etc. They are glycoproteins in nature. The glycoprotein-rich seromucoid fraction is isolated by first precipitating most serum proteins with perchloric acid—seromucoid being precipitated from the filtrate with phosphotungstic acid. The solubility of certain glycoproteins in perchloric acid is assumed due to covalently bound hexose, hexosamine, fucose and sialic acid residues. Thus, seromucoid contains perchloric acid-soluble orosomucoid, Zn-$\alpha_2$-glycoprotein, haemopexin, the haptoglobins and $\alpha_1$-glycoprotein with total carbohydrate and sialic acid contents above 8.0 and 3.3%, respectively. The insoluble

glycoproteins, absent from seromucoid, include $\alpha_2$-macroglobulins, ceruloplasmin and transferrin with less carbohydrate content. Seromucoid also contains small amounts of co-precipitated albumin, pre-albumin and gamma - globulins which, when pure, are perchloric acid-insoluble (Anderson, 1965). Seromucoid fractions are normal constituents of cell membrane and architecture, connective tissue, ground substances, and basement membrane. The seromucoid fractions are involved in many pathological conditions, such as in diseases associated with inflammatory, neoplastic, degenerative, thrombotic, traumatic processes, diabetes mellitus, myocardial infarction, pneumonia, rheumatic fever and arthritis , trauma, hepatic disease, some endocrine dysfunctions, nephrosis and various malignancies.

**Aberrent glycosylation in cancer:** Altered glycosylation has been noted first by Hirtsfeld (1929) and Thomsen (1930). Alterations on the cell surface of the oligosaccharide portion of glycoconjugates, including glycoproteins and associated aberrant glycosylation are found both in membrane glycolipids and glycoproteins and in secreted proteins (Dabelsteen, 1996). Aberrant glycosylation includes several changes related to terminal carbohydrate structures, such as incomplete synthesis and modifications of normally existing carbohydrates, and changes in the carbohydrate core structures. The latter includes: (a) chain elongation of both glycolipids and proteins, (b) increased branching of carbohydrates in N-linked glycoproteins, and (c) blocked synthesis of carbohydrates in O-linked mucin like glycoproteins. Tumour-associated carbohydrate structures are not novel; by contrast, they often reflect a certain stage of cellular development, and most of the moieties are the structures normally found in other adult or embryonic tissues. In addition, there is no unique tumour carbohydrate structure, since certain structures that are tumour- related in one organ may be normal constituents of other tissues (Wang, 2006).

In general, the cell surface is concerned with many physiological properties related to neoplastic transformation and the metastatic spread of malignancy. These include cell shape, growth, division and differentiation, cellular

recognition, communication, adhesiveness, migration, contact inhibition of growth and immunological competence (Shah et al, 2008; Patel et al, 1996; 1997a, 1997b). Neoplastic changes in cell surface glycoconjugates and enzymes are expressed at or mediated through the cell membrane, leading to abnormal growth and behavior of malignant cells. Being the major constituents of cell membrane, various glycoprotein constituents are markedly elevated during malignant process. There is evidence that being attached to the surface of tumour cells, sialoglycoproteins affect various important functions. Altered levels of several glyco-conjugates e.g. TSA, fucose, seromucoid fractions and different glycoprotein regions including albumin, alpha, beta and gamma fraction glycoproteins are also implicated in malignant diseases.

**Glycosylation and Cancer**

A major problem in cancer is metastasis, the phenomenon where cancer cells leave their tissue of origin migrate through the bloodstream to some distant site in the body and grow there in an unregulated manner, with catastrophic results for the affected individual. Many cancer researchers have referred that alterations in the structures of glycoproteins and glycoconjugates on the surfaces of cancer cells are important in the phenomenon of invasion and metastasis in cancer research. Recently, Basoglu et al (2007) have reported that serum soluble intracellular adhesion molecule-1 and TSA levels were higher in patients with breast cancer and metastatic breast cancer as compared to the control group. Significant correlations between serum soluble intracellular adhesion molecule-1 and TSA may reflect the similar function of these molecules as adhesion molecules, and their roles in the carcinogenesis of breast cancer as well as metastasis. Krzeslak et al (2007) have documented that decrease in sialylation rather than increase is a characteristic feature of malignant transformation in the thyroid. It is documented that oxidative stress might be associated with the degree of sialylation of protein and graded changes in these parameters possibly unveil the pathogenic demarcation from benign to malignant condition of prostate (Goswami et al, 2007). Aurer et al

(2007) have studied both galactosylation and sialylation levels and found that multiple myeloma is characterized by aberrant immunoglobulin glycosylation. Sebzda et al (2006) have indicated that serum cathepsin B and TSA concentrations are sensitive markers for early detection of colorectal cancer. These markers with other clinical and biochemical criteria may play important metabolic roles in cancer progression. Rajpura et al (2005) have reported significant elevations in sialic acid levels in oral cancer patients and suggested potential utility of these parameters in diagnosis of oral precancerous and determining clinical stage of the malignant disease. Roy and Chakraborty (2005) have documented that the values, both in the serum and in the cervical tissue were increased gradually with the advancement of grading of frank malignancy. The elevation of sialic acid in the serum was slow to start but persisted for a longer period, whereas the concentration in local tissue was found to be increasing with the pathological process. Kossowska et al (2005) documented that extent of fucosylation could be a useful marker for estimation of the glycosylation status of serum proteins in small-cell and non-small-cell lung cancer patients and cluster analysis leads to the fucosylation status could serve as a predictive factor for patients' survival. Feijoo-Carnero et al (2004) have conducted study to evaluate the significance of preoperative serum sialic acid levels in the diagnosis and prognosis of colorectal cancer. The studies indicated that preoperative serum TSA/TP content could supply additional information to that provided by Dukes' stage about the prognosis of patients. Uslu et al (2003) have found that serum concentrations of bound sialic acid, free sialic acid, and alpha-1-acid glycoprotein were studied in laryngeal cancer patients. Bound sialic acid and alpha-1-acid glycoprotein but not free sialic acid showed correlation with the stage of laryngeal carcinoma. Mukhopadhyay, Giri and Dasgupta (2003) have reported that serum MP levels were found to be increased significantly in hepatocellular carcinoma. Previous reports from our laboratory reported that seromucoid fraction in breast cancer patients, MP and hexose levels were found to be significantly elevated in untreated patients with breast cancer when compared with the healthy participants and patients who had benign breast diseases, respectively. A

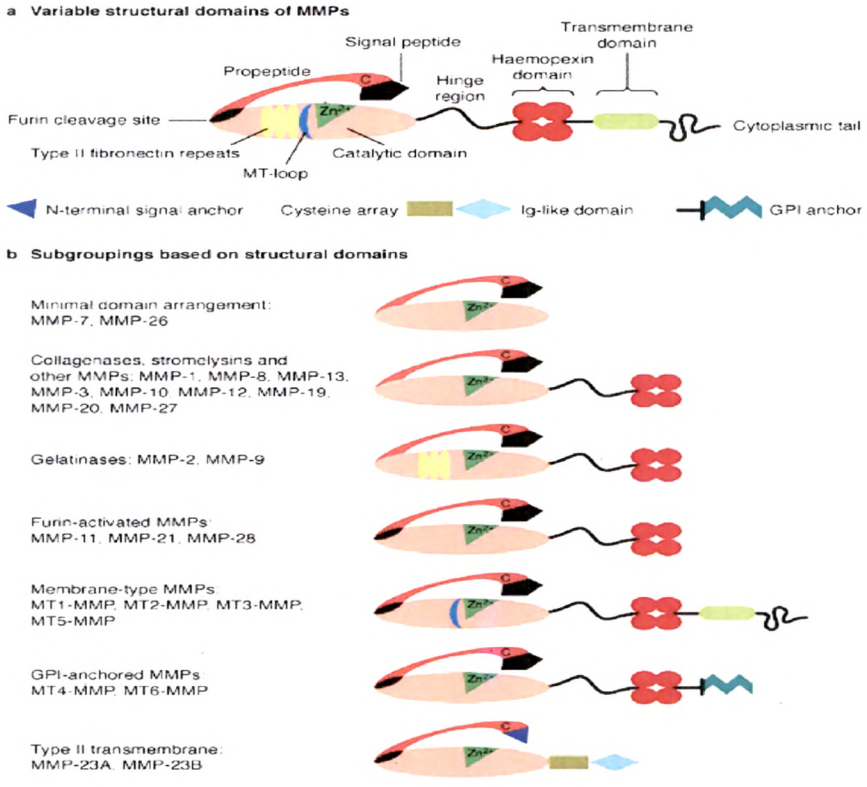
good correlation was observed between favorable treatment response and decline in serum marker levels. The markers in patients who did not respond to anticancer therapy remained stable or increased during follow-up. Seromucoid fraction can be a useful biochemical marker for breast cancer patients (Patel et al, 1998). Earlier research from our laboratory has also documented that the alterations in serum glycoprotein electrophoresis pattern (albumin, alpha, beta and gamma) may be useful for early detection and treatment monitoring of the upper aerodigestive tract cancer patients (Patel et al, 1997b).

## 2.7 Matrixproteins-Matrixmetalloproteinases (MMPs)

MMPs are a family of proteinases that were first described in 1962 when Gross and Lapiere identified an enzyme from a vertebrate source (tadpole tail) with proteolytic action capable of attacking collagen (Nagal et al, 1966; Gross and Lapiere, 1962). The initial MMPs were called interstitial collagenase because it cleaved collagen type I, II and III at a single site in the triple helix of the molecule. MMPs are also known as matrixins, (EC 3.4.24) form a family of structurally and functionally related calcium-dependent and zinc-containing endopeptidases found in eukaryotes from plants via hydra to humans, which are synthesized by endogenous connective tissue cells as well as other types of haemopoietic cells (Murphy, 1995). This multigene family of metal containing proteases shares several common characteristics including: (i) each degrade at least one component of the basement membrane; (ii) they are active at physiological pH; (iii) they require two $Zn^{++}$ ions/molecules in order to be active; (iv) they are inhibited by metal chelators and TIMPs; and (v) they are secreted as an inactive (latent) form, which is called a zymogen or a proMMP and require activation extracelluarly (Snoek-van Beurden and Von den Hoff, 2005). These latent MMPs require an activation step before they are able to cleave ECM *in vitro* and *in vivo* of degrading all kinds of ECM protein components such as interstitial and basement membrane collagens, proteoglycans and fibronectin (Woessner and Nagase, 2000; Brinckerhoff and Matrisian, 2002).

## Classification and Nomenclature:

**Figure-8: Domain structure of MMPs**




*Adapted from http://www.expertreviews.org*

To date, 25 different zinc-dependent MMPs have been discovered (Masanori et al, 2006). The family of human MMPs consists of 23 different forms, among them 20 MMPs which have been well characterized. (Hoekstra et al, 2001; Overall. 2002; Snoek-van Beurden and Von den Hoff, 2005). According to the organization of their peptide domains; their substrate specificity and sequence similarity are depicted in the **figure: 8**.

## Physiological and Pathological Role of Gelatinases:

Gelatinases have significant role in wide variety of physiological and pathological conditions. The gelatinases are required in invasive processes during reproduction, growth and development, leukocyte mobilization, inflammation, tissue remodeling and wound healing. Increased gelatinase

activity has been observed in a variety of pathological conditions including cancer, inflammation, infective diseases, renal, neurodegenerative and vascular diseases (Philippe et al, 2002; Van den Steen et al, 2002).

**Regulation of MMPs:** Regulation of the MMPs occurs on three levels as described below:

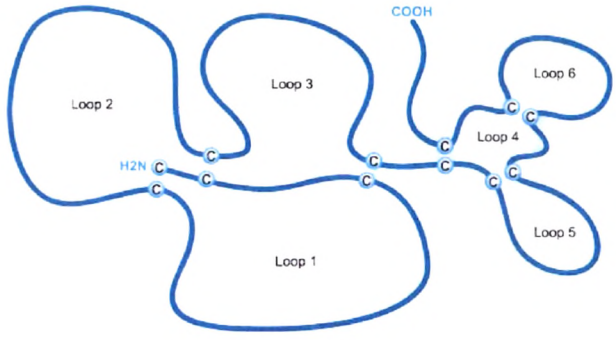
**(i) Alteration of gene expression:** MMPs are thought to be regulated by variety of cytokines, growth factors, steroid hormones, and phorbol esters. MMP regulation and transcriptional activation are not fully understood. These factors cause variable pattern of expression in different tissues and have variable effects on the different MMP family members, complicating the understanding of gene regulation of MMPs in both physiological and pathological states (Jhon and Tuszynski, 2001).

**(ii) Activation of MMPs:** Another level of MMP regulation is the activation of the zymogen/proenzyme secreted form of MMPs. MMPs can be activated by proteinases or *in vitro* by chemical agents, such as thiol-modifying agents (4-aminophenylmercuricacetate, $HgCl_2$, and *N*-ethylmaleimide), oxidized glutathione, SDS, chaotropic agents, and reactive oxygens. Low pH and heat treatment can also lead to activation. Proteolytic activation of MMPs is stepwise in many cases (Nagase, 1997).

**(iii) Inhibitors of matrixmetalloproteinases:** The third level of MMPs occurs through inhibition of enzyme activity. Various physiological agents have an inhibitory effect on MMPs including α2-macroglobulin and TIMPs, natural tissue inhibitors of metalloproteinases. The α2-macroglobulin molecule, a large molecular weight (780 kDa) serum protein can inhibit proteinases but its size prevents the molecule from entering into tissue spaces. The TIMPs are much smaller molecules and are expressed in various tissues and fluids. There are four members of the mammalian TIMP family; TIMP-1, TIMP-2, TIMP-3 and TIMP-4 (Brew et al, 2000). TIMP-1, TIMP-2 and TIMP-4 are secreted proteins whereas TIMP-3 is anchored in the ECM. The amino–terminal domain present in all TIMP molecules is responsible for the

MMP inhibitory activity (Nagase and Woessner 1999; Gomez et al, 1997). The TIMPs form high affinity, non-covalent complexes with all active MMPs in a 1:1 stoichiometric ratio (Jhon and Tuszynski, 2001). In addition, TIMP-1 and TIMP-2 can block the pro- forms of MMP-9 and MMP-2, respectively. The balance between protease and inhibitor is critical in determining net proteolytic activity.

**Figure -9: Schematic representation of TIMP-1 structure**




*Adapted from www.abcam.co.jp*

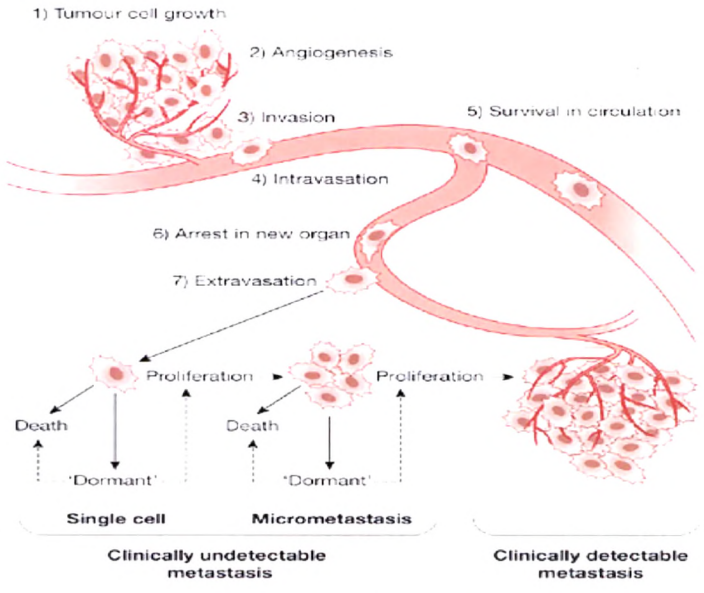
TIMP-1 contains 12 cysteine residues, which form six loop (**figure: 9**) structures through disulfide bonds. The N-terminus of TIMPs 1-4 binds to the catalytic domain of most activated MMPs and inhibit its function. The C-terminus of TIMP-1 and TIMP-2 binds to the hemopexin domain of proMMP-2 and proMMP-9, respectively; this binding regulates MMP function. TIMP-1 is a 28.5 kDa glycoprotein that has a wide variety of functions including growth factor activity, stimulating cell morphology changes, regulate apoptosis, amplify inflammation, inhibiting angiogenesis and regulate metastasis formation (Rhee et al, 2004). Increased TIMP-1 levels have traditionally been associated with reduced invasion and metastasis. TIMP-2 similar to TIMP-1, is associated with decreased metastatic potential. TIMP-2, a non glycosylated 21 kDa protein, suppresses tumour growth and invasion. In contrast to TIMP-1 and TIMP-2, TIMP-3 is associated with the ECM. The most recently identified molecule is TIMP-4, a 24 kDa protein. A small change in the balance could have a profound effect on proteolysis. Normally, the degradation potential of the MMPs is held in check mainly by the endogenous specific TIMPs. It is

important to consider the ratio of proteases, which is critical to invasive capacity. Disruption of this MMP-TIMP balance can result in tumour growth and cell invasion and metastasis (Bode and Maskos, 2003).

**Role of MMPs in Tumour Growth, Invasion and Metastasis:**

Although metastasis is the major cause of death in cancer patients, it is well established that metastasis formation is an inefficient process. The number of circulating tumour cells and tumour emboli correlate with the size and age of the primary tumour. However, the number of circulating tumour cells does not correlate with the clinical outcome of metastasis. The inefficiency of tumour cells in completing the metastatic cascade is the result of the fact that successful formation of metastatic foci consists of several highly complex and mutually dependent steps. Each step is rate limiting in that failure to complete any of these events completely disrupts metastasis formation (**figure: 10**).

**Figure-10: The steps of metastatic cascade**




*Adapted from EMBO reports. 2006; 7(11): 1084–1088.*

The steps involved in metastasis formation are thought to be similar in all tumours and are characterized as follows:

**(i)Tumourigenesis:** After the initial neoplastic transformation, the tumour cells undergo progressive proliferation that is accompanied by further genetic

changes and development of a heterogeneous tumour cell population with varying degrees of metastatic potential. The initial growth of the primary tumour is supported by the surrounding tissue microenvironment, which eventually becomes rate limiting for further growth.

**(ii) Angiogenic switch:** As the tumour grows and central tumour cells become hypoxic, the tumour initiates recruitment of its own blood supply by a process called the "angiogenic switch" which involves the secretion of various angiogenic factors and the removal or suppression of angiogenesis inhibitors.

**(iii) Clonal dominance of invasive phenotype:** Continued genetic alterations in the tumour cell population lead to selection of tumour cell clones with distinct growth advantage and acquisition of an invasive phenotype. Invasive tumour cells down regulate cell-cell adhesion, alter their attachment to the ECM by changing E-cadherin, integrin expression profiles, and by proteolytically altering the matrix accomplish stromal invasion. Tumour cells lose "contact inhibition" that prevents normal cells from continuing to divide when they contact their nearest neighbors. The survival of tumour cells and tumour cell emboli in the circulation in vascular or lymphatic compartments have a variety of hemodynamic and immunologic challenges.

**(iv) Tumour cell arrest in distant organs or lymph nodes:** This occurs by size trapping on the inflow side of the microcirculation or by adherence of tumour cells through specific interactions with capillary or lymphatic endothelial cells or by binding to exposed basement membrane.

**(v) Extravasation and growth at the secondary site:** Arrested tumour cells proliferate in response to paracrine growth factors or become dormant. The poor growth of tumour cells after extravasation from the circulation is a major factor contributing to the inefficiency of the metastatic process.

**(vi) Angiogenesis in metastatic foci:** Finally, continued growth of the metastatic foci is also dependent on angiogenesis. The development of this neovascular network at the metastatic site enhances the metastatic potential of these cells just as it does for the primary tumour.

**(vii) Evasion of immune response:** Tumour cell evasion of immune response in metastatic foci includes antigenic modulation and immunosuppression and prevents their eradication. Therefore, detachment of single cells or clumps of cells from the tumour mass may be directly related to a decreased level of cell adhesiveness in tumour populations are regulated by a variety of cell surface molecules such as the cadherins and integrins. Circulating tumour cells arrest at distant sites and by a repeated process of invasion may colonize a secondary site for growth. In some instances, this organ preference of metastasis can be explained simply in terms of the anatomical relationship of the organ with the site of primary tumour growth. Some cancers express organ preference that is not associated with a non-specific entrapment process, but rather with specific determinants, which actively promote the growth of the metastatic cells, thereby providing favorable soil. Fully metastatic behavior is attributable to the expression of only one or a few metastasis promoting genes to the loss of an equally small number of metastasis-suppressing genes.

After arriving in a secondary site metastatic cells begin proliferation, undergo apoptosis or remain as solitary dormant cells. The process of metastasis is extremely inefficient with the majority of the cells undergoing apoptosis and thus becoming clinically irrelevant. The cells that begin proliferation and dormant cells are responsible for cancer recurrence (Townson and Chambers, 2006). An event, termed as "angiogenic switch" characterized by an imbalance between pro- and anti-angiogenic factors, by the influence of two proteolytic systems, urokinase plasminogen activator and MMPs, often marks interruption of the dormant state, thus triggering invasive tumour growth (Indraccolo et al, 2006).

## MMPs and TIMPs in Cancer Research

Although extensive amounts of efforts on MMPs and TIMPs are being placed in determining the etiology of cancer, the evidence regarding the metastatic potential of tumours is still to be elucidated. Metastatic spread of cancer continues to be the greatest barrier to cancer cure and majority of basic

cancer research has been focused on unraveling the molecular mechanisms of tumourigenesis and metastasis. Numerous studies have shown that, higher the MMP expression in the tumour, more aggressive the cancer. The higher levels have been found to correlate with advanced stage, increased tumour progression and metastasis. Recently, Dragutinovic et al (2006) have found that overexpression of gelatinase B protein may serve as a marker for invasiveness and metastasis of gastric cancer. ProMMP-9 can be used for the detection of primary or recurrent cancer and for the estimation of tumour extent. Both MMP-2 and MMP-9 were increased in malignant tissues as compared to their benign counterparts of uterine cervix and colorectal cancer (Nair et al, 2003; Moran et al, 2005). Stankovic et al (2006) have reported that serum MMP-2 and MMP-9 levels in breast cancer patients showed significant clinical stage-dependent increased with higher level of MMP-9 associated with a worse overall survival rate. Earlier reports from our laboratory have documented that higher MMP-2 and MMP-9 levels in malignant tissues as compared to adjacent normal tissues of oral cancer patients (Patel et al, 2005). In colorectal cancer, no prognostic significance has been found to either of the gelatinases (Oberg et al, 2000; Sis et al, 2004; Moran et al, 2005). Kuittinen et al (2003) has reported that positivity for MMP-9 immunoreactive protein is an independent sign of an unfavorable prognosis in non-Hodgkin's lymphomas. This is not mediated through influences in tumour dissemination or neovascularization indicating it to carry other important biological functions. In Hodgkin's disease, MMP-2 expression may be a favorable prognostic factor and MMP-9 an adverse prognostic factor in Hodgkin's lymphoma (Kuittinen et al, 2002). An enhanced expression of MMP-2 and MMP-9 has been observed in cancers of breast, colon, lung, skin, ovary and prostate among others (Egeblad and Werb, 2002). Vacca et al (1999) have studied and reported that plasma cell extracts showed significantly higher levels of the angiogenic basic FGF-2 in the active MM patients than in nonactive MM and monoclonal gammopathy of undetermined significance patients respectively. Accordingly, neutralizing anti–FGF-2 antibody caused a significant inhibition of the biological activity exerted on

cultured endothelial cells. Finally, the bone marrow plasma cells showed that active MM patients express significantly higher levels of MMP-2 mRNA and protein when compared with non active MM and monoclonal gammopathy of undetermined significance patients, whereas MMP-9 expression was similar in all groups. Taken together, these findings indicated that progression of plasma cell tumours is accompanied by an increase of bone marrow neovascularization. This is paralleled by an increased angiogenic and invasive potentials of bone marrow plasma cells, which is dependent by FGF-2 and MMP-2 production. Induction of angiogenesis and secretion of MMPs by plasma cells in active disease may play a role in their medullary and extramedullary dissemination, raising the hypothesis that angiostatic/anti-MMP agents may be used for therapy of MM (Barille et al, 1997).

Interestingly, several factors known to regulate the gelatinase expression or activity in cancer have been shown to participate in the regulation of TIMP expression and inhibition of these proteases can be helpful for the prevention of cancer development and for inhibition of dissemination. Therefore, increased MMPs in malignant tumours would be accompanied by decreased TIMP expression. This may reflect an attempt to control the degradative potential of MMPs or it may indicate that TIMPs are multifunctional molecules (Jiang et al, 2002). Elevated pretreatment plasma levels of TIMP-1 predicted a decreased response to second line hormone therapy and reduced survival in women with metastatic breast cancer (Lipton et al, 2007). Ries et al (2007) have reported that in human acute monocytic leukemia cell line (THP-1), leukaemic cells endogenously produce a unique proMMP-9 variant exposed on the cell surface with reduced susceptibility to inhibition by TIMP-1. Adopting an immunological method specifically detecting this novel 82 kDa proMMP-9, found the enzyme to be expressed in different leukaemic cell lines, but not in normal white blood cells. Its detection in patient-derived leukaemic blast cells indicates that the 82 kDa proMMP-9 also occurs *in vivo*, supporting the importance of this enzyme species. Therefore, overexpression of TIMP-1, insensitive MMP-9 variant on the surface of malignantly transformed cells may

increase pericellular proteolysis and thereby promote cancer progression *in vivo*. Rhee et al (2004) have reported that TIMP-1 alters susceptibility to carcinogenesis. Nair et al (2003) reported that cervical tissue samples exhibited increased expression of MMP-2, MMP-9, TIMP-1 and TIMP-2 with the progression of cervical cancer from low-grade SILs to high grade SILs to SCC. Lein et al (2000) have studied MMP-2 MMP-9, TIMP-1 and TIMP-2 in plasma and tissue samples of patients with renal cell carcinoma from cancerous and non-cancerous parts of the same kidney. In tumour tissue, MMP-9 and TIMP-1 were significantly higher than the normal counterparts. TIMP-2 values could not be measured and plasma MMP-9 concentration was significantly higher in renal cell carcinoma patients than in healthy controls. MMP-2 and TIMP-2 concentration were higher in healthy controls and TIMP-1 concentrations were not different. TIMP-1 and TIMP-2 have been shown to be generally present and over-expressed in gynecological cancers (Huang et al, 2000; Sakata et al, 2000; Nair et al, 2003).

**2.8 Implication of current study:** Cancer is a group of over 100 devastating diseases that share a number of characteristics, a primary hallmark of which is uncontrolled growth. However, considering the genotypic and phenotypic characteristics, there are significant differences among these diseases that cause major difficulties in their management. Though the scientific community has been keenly interested for so many years to understand the biochemical events leading to the cancer transformation, understanding of the prospects for certain shared biochemical events for these diseases are emerging only recently. It has been argued that tumours undergo a Darwinian evolution in a multistep process with dynamic changes in the genome to proteome. That's why multiple routes to the development of cancer are emerging as because so many distinct metabolic and biochemical steps can be altered to give rise to uncontrolled cell growth. Current techniques have already yielded putative molecular targets, uncovered signaling pathway dominance and advanced early cancer detection. The co-evolution of genomics and proteomics as complementary approaches to

cancer will help us to move closer to the goals of early prevention, detection, and institution of a molecular-based tumour-specific approach to the treatment of individual patient. Although molecular-targeted therapies are still in their infancy, the addition of high-throughput drug resistance and toxicity monitoring will give us a comprehensive system for the prescribing and monitoring of molecular medicine. Current clinical and pathological markers poorly predict early diagnosis and response to treatment. Standard diagnostic methods, including tissue histopathology are now shifting rapidly toward molecular diagnosis because of rapid progress in proteomic technology. This powerful technology can identify all proteins and their protein post-translational modifications such as glycosylation. The alterations in the structures of glycoproteins and glycoconjugates on the surfaces of cancer cells are important in the event of invasion and metastasis. This will greatly accelerate the progress toward novel diagnostic and predictive marker in cancer research to track early disease and tailor treatments to specific patients. The current view is that gelatinases are needed at multiple stages during the tumour progression and different tumours may utilize different MMPs and TIMPs. The steps where MMPs are involved include the growth of the primary tumour, angiogenesis, intravasation of the tumour cells, migration and invasion of the metastatic cells in the secondary organ as well as initiation and support the tumour growth in the metastatic site.

The present study was carried out on MM and cervical cancer patients. In spite of the recent advancement in understanding the role of M-protein as diagnostic marker, in-depth research is essential. In MM, study was aimed to correlate the biomarkers between two groups: presence of M-protein (Group-I) and absence of M-protein (Group-II). In cervical cancer, there is lack of the basic research in carcinogenesis, tumourigenesis, angiogenesis, invasion and metastasis is lacking. Therefore, there is a need to explore for specific protein in cervical cancer especially those which play a role in progression of the disease.

The major **OBJECTIVES** of the study were as follows:

**MM patients:**

- Analysis of the changes in serum total protein fractions in untreated MM patients and controls.
- Serum immunoprofiling ( IgG, IgA, IgM) in MM patients and controls.
- Serum total protein profiling in MM patients and controls.
- Isolation of M-protein through 2D-PAGE approach and analysis of the 2D maps using PDQuest, the discovery series™ software.
- Evaluation of serum levels of glycoconjugates (TSA, fucose, MP and hexoses) in MM patients and controls.
- Serum total glycoprotein profiling in MM patients and controls.
- Analysis of serum MMP-2, MMP-9, TIMP-1 and TIMP-2 in MM patients and controls.

**Cervical cancer patients:**

- Serum total protein profiling in cervical cancer patients and controls.
- Evaluation of serum levels of glycoconjugates (TSA, fucose, MP and hexoses) in cervical cancer patients and controls.
- Serum total glycoprotein profiling in cervical cancer patients and controls.
- Analysis of serum MMP-2, MMP-9, TIMP-1 and TIMP-2 in cervical cancer patients and controls.

The above objectives were explored to evaluate clinical significance of the parameters in identification of high-risk group, early diagnosis, staging and prognostication of MM and cervical cancer.