INTRODUCTION

Cancer defines as a population of cells that have lost their normal controls of growth and differentiation and are proliferating without check. The 30 trillion normal cells of healthy body live in a complex, interdependent condominium, regulating one another’s proliferation. Indeed, normal cells reproduce only when instructed to do so by other cells in their vicinity. Such unceasing collaboration ensures that each tissue maintains a size and architecture appropriate to the body’s needs.

Cancer cells, in contrast, violate this scheme. They become deaf to the usual controls on proliferation and follow their own internal agenda for reproduction. They also possess an even more insidious property that is the ability to migrate from the site where they began, invading nearby tissues and forming masses at distant sites in the body. Tumours composed of such malignant cells become more and more aggressive over time and they become lethal when they disrupt the tissues and organs needed for the survival of the organism as a whole.

History

Cancer is the Latin word for crab. The ancients used the word to mean malignancy; doubtless because of the crab-like tenacity of a malignant tumour sometimes seems to show in grasping the tissues it invades. Cancer is a multi step process involving accumulation of genetic alterations, which results in loss of cell - cell interaction, increasing invasive migration, loss of control in cell division and abnormal matrix assembly. Cells which undergo rapid, abnormal and uncontrollable growth at the cost of remaining cells are called neoplastic cells .The growth resulting from the division of such cells are called malignant tumor. Cancer cells are very similar to cells of the organs from which they originated and have similar (but not identical) DNA and RNA. This is the reason why they are not very often detected by the immune system. Cancer cells usually have an increased ability to divide rapidly and their number of divisions is not limited by telomeres on DNA. This can lead to the formation of large masses of tissue and in turn may lead to disruption of bodily functions due to destruction of organs or vital structures.
Today, carcinoma is the medical term for a malignant tumour derived from epithelial cells. It is Celsus who translated carcinos into the Latin cancer, also meaning crab. Galen used "oncos" to describe all tumours, the root for the modern word oncology. Hippocrates described several kinds of cancers. He called benign tumours oncos, Greek for swelling, and malignant tumours carcinos, Greek for crab or crayfish. This name probably comes from the appearance of the cut surface of a solid malignant tumour with a roundish hard center surrounded by pointy projections, vaguely resembling the shape of a crab. He later added the suffix -oma, Greek for swelling, giving the name carcinoma. Since it was against Greek tradition to open the body, Hippocrates only described and made drawings of outwardly visible tumours on the skin, nose, and breasts. Treatment was based on the tumor theory of four bodily fluids (black and yellow bile, blood and phlegm). According to the patient's tumor, treatment consisted of diet, bloodletting, and/or laxatives. Through the centuries it was discovered that cancer could occur anywhere in the body, but tumor-theory based treatment remained popular until the 19th century, the discovery of cells were realized.

Nomenclature and classification
The type of cell that resembles the tumor classifies cancers and, therefore, the tissue presumed to be the origin of the tumor. The following general categories are usually accepted:

**Carcinoma:** Malignant tumours derived from epithelial cells. This group represents the most common cancers including the common forms of breast, lung, colon and cervical cancer.

**Lymphoma and Leukaemia:** Malignant tumours derived from blood and bone marrow cells.

**Sarcoma:** Malignant tumours derived from connective tissue, or mesenchymal cells.

**Mesothelioma:** Tumours derived from the mesothelial cells lining the peritoneum and the pleura.

**Glioma:** Tumours derived from glia, the most common type of brain cell.

**Germinoma:** Tumours derived from germ cells, normally found in the testicle and ovary.

**Choriocarcinoma:** Malignant tumours derived from the placenta.
Cancer is a complex disease occurring as a result of a progressive accumulation of genetic aberrations and epigenetic changes that enable escape from normal cellular and environmental controls (Weinberg, 1996). Neoplastic cells may have numerous acquired genetic abnormalities including aneuploidy, chromosomal rearrangements, amplifications, deletions, gene rearrangements and loss of function or gain-of-function mutations. Recent studies have also highlighted the importance of epigenetic alterations of certain genes that result in the inactivation of their functions in some human cancers. These aberrations lead to the abnormal behaviour common to all neoplastic cells: deregulated growth, lack of contact inhibition, genomic instability, lack of anchoring substances, invasiveness, cytoskeletal changes, propensity for metastasis etc.

Cancer is a class of genetic disease comprising more than two hundred life threatening conditions characterized by uncontrolled and abnormal cell growth. It is by now widely accepted that cancer arises as a consequence of deregulated growth-controlling pathways due to mutations (Hanahan and Weinberg, 2000) or epigenetic alterations (Feinberg et al., 2006) affecting genes coding for the protein components of such pathways.

The immediate cause of cancer must be some combination of insults and cellular accidents that induces normal cells in a healthy human body to turn malignant, growing like weeds and sprouting in every direction disrespecting other cellular boundaries and acquire several extraordinary skills to be malignant by various different regulatory systems perturbed in order for a normal cell to grow as cancer. Neoplasia represents a pathological disturbance of cell growth characterized by an excessive and continuous proliferation of cells. Some tumors are called benign because they grow very slowly and remain localized so that the patient usually experiences little difficulty from them. Others are called malignant as they tend to proliferate rapidly and spread throughout the body (Ziegler and Buonaguro, 2009). The degree of differentiation of malignant cells is variable. Cancer cells that closely resemble the tissue of origin are called well differentiated. Bizarre tumor cells bearing little similarity to the tissue of origin are termed undifferentiated or anaplastic. Undifferentiated or anaplastic malignancies are more aggressive in their growth and behaviour than well-differentiated types. Anaplastic tumors are characteristically
display pleomorphism. Some cells may be many times larger while others can be extremely small and primitive appearing. The nuclei can be deep staining (hyperchromatic) and show increased nuclear cytoplasmic ratio approaching 1:1 instead of the normal 1:4 or 1:6. Their chromatin is often coarsely clumped and distributed along with the nuclear membrane. Some cells display typical and bizarre mitotic figures sometimes producing tripolar, quadripolar or multipolar mitotic spindles. The nuclei are usually prominent. There are also intermediate degrees of differentiation. These tumors are classified as moderately differentiated.

How is Cancer formed?
Cancer cells are formed from normal cells due to a modification / mutation of DNA and / or RNA. These modifications / mutations can occur spontaneously or they may be induced by other factors such as nuclear radiation, electromagnetic radiation (microwaves, X-rays, gamma-rays, ultraviolet-rays, etc.), viruses, bacteria, fungi, parasites (due to tissue inflammation/irritation), heat, chemicals, water, food, mechanical cell-level injury, free radicals, evolution and ageing of DNA and RNA, etc. All these can produce mutations that may start cancer. Cancer can be called therefore "Entropic Disease" since it is associated with the increase of entropy of the organism to the point where the organism cannot correct this itself. External intervention is required to allow the organism to return to a stable entropic state.

Cancer cells are formed continuously in the organism (it is estimated that there are about 100,000 cancer cells at any given time in a healthy person). The question is why some of these results in macroscopic-level cancers and some don't. First, not all damaged cells can multiply and many of them die quickly. Those, which have the potential to divide and form cancer, are effectively destroyed by the various defence mechanisms available to the immune system. This process takes place continuously. Therefore cancer develops if the immune system is not working properly and / or the amount of cells produced is too great for the immune system to eliminate. The rate of DNA and RNA mutations can be too high under some conditions such as: unhealthy environment (due to radiation, chemicals, etc.), poor diet (unhealthy cell environment), people with genetic predispositions to mutations and people of advanced age (above 80).
Cells are the structural units of all living things. Each of us has trillions of cells, as does a growing tree. Cells make it possible for us to carry out all kinds of functions of life: the beating of the heart, breathing, digesting food, thinking, walking, and so on. However, all of these functions can only be carried out by normal healthy cells. Some cells stop functioning or behaving as they should, serving no useful purpose in the body at all, and become cancerous cells (Fig.1.1).

Cytogenetic visualization technologies have traditionally played an important role in cancer research. Both the chromosomal number changes or aneuploidy, and the telomeric deficient mediated chromosomal breakage fusion-bridge cycle has long been linked to the cancer phenotype and chromosomal instability. Many chromosomal aberrations, particularly translocations or inversions are closely associated with a specific morphological or phenotypic subtype of leukemia, lymphoma or sarcoma (Rowley, 1998).

A great deal of attention has been focused on clonal chromosomal changes in the identification of both primary and secondary abnormalities. These clonal abnormalities, particularly if complex, are significant to neoplasia. As a result, these chromosomal visualization methods have served as an important tool for both cancer research and diagnosis. Extensive research has been performed with molecular probes targeting specific regions of the genome for detecting gene deletions and amplifications. With the development of live images as well as the maturation of FISH related technologies, more direct visualization approaches are available to cancer biology (Heng et al., 1997; Heng et al., 2001; Ibora et al., 2003; Zink et al., 2003).

In higher eukaryotes, controlled cell proliferation and differentiation is required for normal growth and development. Deregulation of cell division pathways can lead to increased cell division, tumour formation and carcinogenesis. Understanding the causes of increased cellular division is pivotal to understanding the development of human cancer. Two fundamental biochemical processes which control cell division are protein phosphorylation, mediated by the cyclin-dependent kinases (CDKs) and protein degradation mediated by the ubiquitin-dependent proteolytic pathway.
Cancer arises from a loss of normal growth control. In normal tissues, the rates of new cell growth and old cell death are kept in balance. In cancer, this balance is disrupted. This disruption can result from uncontrolled cell growth or loss of a cell's ability to undergo cell suicide by a process called" apoptosis." Apoptosis, or "cell suicide," is the mechanism by which old or damaged cells normally self-destruct.

Adopted from http://www.cancer.gov/cancertopics/what-is-cancer
Deregulated CDK activity is intimately involved in the development of human cancers due to increased cell division. Since increased activity of these molecules can lead to cancer, an important issue relates to understanding the molecules and growth pathways targeted by CDKs. These targets are the effectors molecules that mediate the adverse effects of deregulated CDK activity. To address these issues, novel methods have been developed to systematically identify the targets of CDKs and in due course numerous targets have been discovered. Current research involves characterising these newly identified CDK targets. In addition, a major focus relates to understanding how ubiquitin-conjugating and ubiquitin ligase enzymes control proteolysis and cell division. These studies will lead to an increased understanding of the molecular mechanisms of cell division providing insight into the development of human cancer (Fig.1.2).
Progression through the different cell cycle phases and cell division is controlled by the activation of cyclin/CDKs, which phosphorylate critical substrate target proteins, as well as degradation of key proteins by the ubiquitin-mediated proteolytic pathway. Mutations resulting in the increased or deregulated activities of cyclin/CDKs or components of the ubiquitin pathway can promote uncontrolled cell proliferation leading to cancer development.

Cancer invasion and metastasis

Invasion and metastasis occur within a tumour-host microecology, where stroma and tumour cells exchange signals that modify the local extracellular matrix, stimulate migration, and promote proliferation and survival (Hanahan and Weinberg, 2000). In general the genetic variability of cancer cells leads to the manifestation of six essential alterations that collectively dictate malignant and most likely also metastatic and invasive tumour growth: self-sufficiency in growth signals, insensitivity to growth inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis, and tissue invasion and metastasis.

The understanding of the molecular mechanisms of tumour growth and metastasis is one of the most important issues in cancer research. It is necessary to identify genetic but also epigenetic determinants which govern systemic tumour dissemination. Since cancer is attributed to genetic alterations it is indispensable to identify those genes whose alterations accumulate during tumour progression. Over the past two decades, a number of these genes have been identified. In 1980, identification of oncogenes opened the way to specifically search for genetic alterations in human cancers (Bishop, 1991). In the late 1980s and 1990s, isolation of tumour suppressor genes further accelerated the understanding of the genetic network. In the early period restriction fragment length polymorphism analysis (RFLP) of tumour suppressor genes (Cavenee et al., 1983) identified that loss of heterozygosity (LOH) – a hallmark of gene inactivation occurs frequently at multiple chromosomal loci in a variety of human cancers (Ponder, 1988). The presence of multiple genetic alterations in human cancers strongly indicated that alterations accumulate during tumour progression. Comparative analysis of these genetic alterations in early and late stage tumours led to the hypothesis of a multistage carcinogenesis in colorectal cancer progression which now is a widely accepted genetic tumour progression model (Kinzler and Vogelstein, 1996). A similar progression model has recently been postulated for pancreatic cancer, where an adenoma-carcinoma sequence is proposed (Brat et al., 1998). Based on the concept of multistage carcinogenesis, the metastatic activity of cancer cells should be defined by those genes whose alterations accumulate predominantly in late stage cancer cells.
Several controversial theories exist to explain the metastatic specificity. Among them, the homing theory suggests that organs distant to sites of primary malignancy actively attract malignant cells via expression of adhesion receptors or by secretion of soluble chemotactic factors (Muller et al., 2001). Identification of molecular addresses or adhesion receptors on endothelial cells in vascular beds of distal organs that specifically trap circulating malignant cells supports the active arrest view of the homing theory (Borsig et al., 2002). In contrast, the fertile soil theory proposes that different organ environments provide variable growth conditions for specific circulating cell types. It seems likely that distinct mechanisms/molecules might govern a malignant cell's journey to an ectopic tissue, separate from those regulating its growth and/or survival once its destination has been achieved (Hanahan and Weinberg, 2000). Tumor invasion encompasses the process of tumour cell penetration or infiltration into adjacent tissue. This event is also central and related to the development of metastasis. Loss of junctional contact between adjacent epithelial cells and cell-extracellular matrix association are essential prerequisites for tumour cell detachment from the primary tumour site (Tawil et al., 1996). It is postulated that migration and invasion of cancer cells into surrounding stroma are prevented by cell-cell and cell-matrix adhesion molecules. Disruption of these adherive connections leads to increased motility of tumour cells, which detach from the primary lesion. Therefore, adhesion molecules on the cell surface play an important role in tumour cell migration and regulate the potential for epithelial cells to metastasis.

All forms of cancer spread with the help of a matrix dissolving mechanism. To reproduce and spread to other parts in the body, cancer cells degrade the extracellular matrix (ECM) by secreting various matrix metalloproteinases (MMPs), which have been correlated with the aggressiveness of tumor growth. With the help of these collagen-dissolving enzymes, cancer cells can bulldoze their way through the ECM and capsule enclosing the tumor and through an adjacent blood vessel wall, to be carried to other sites where the cancer cells can invade other organs. Cancer metastasis normal cells become cancerous cells, which secrete MMPs. MMPs destroy collagen in the ECM, enabling cancer cells to escape and spread to distal organs through the blood stream (Fig.1.3).
Cancer begins as a cluster of abnormal cells. Over time, the abnormal cells continue to change in appearance and multiply, evolving into non-invasive (in situ) cancer or, eventually, invasive cancer. Invasive cancer can spread to other areas of the body through the bloodstream or the lymphatic system. 

Adapted from Mayo Foundation for Medical Education and Research (MFMER). 1998-10
The observation that angiogenesis occurs around tumours was made nearly 100 years ago (Goldman, 1907; Ide et al., 1939; Algire et al., 1945). The hypothesis that tumours produce a diffusible ‘angiogenic’ substance was put forward in 1968 (Greenblatt and Shubik, 1968; Ehrmann and Knoth, 1968). Folkman proposed that tumour growth and metastasis are angiogenesis-dependent, and hence, blocking angiogenesis could be a strategy to arrest tumour growth. This possibility stimulated an intensive search for pro- and anti-angiogenic molecules (Folkman, 2000).

Gullino showed that cells in pre-cancerous tissue acquire angiogenic capacity on their way to becoming cancerous. He proposed that this concept be used to design strategies to prevent cancer (Gullino, 1978), a hypothesis later confirmed by genetic approaches (Hanahan and Weinberg, 2000). It is now widely accepted that the ‘angiogenic switch’ is ‘off’ when the effect of pro-angiogenic molecules is balanced by that of anti-angiogenic molecules and is ‘on’ when the net various signals that trigger this switch have been discovered. These include metabolic stress (for example, low \( pO_2 \), low pH or hypoglycaemia), mechanical stress (for example, pressure generated by proliferating cells), immune/inflammatory response (for example, immune/inflammatory cells that have infiltrated the tissue), and genetic mutations (for example, activation of oncogenes or deletion of tumour-suppressor genes that control production of angiogenesis regulators (Kerbel, 2000; Carmeliet, 1999). How the interplay between environmental and genetic mechanisms influences tumour angiogenesis and growth is a complex and largely unresolved matter. Pro- and antiangiogenic molecules can emanate from cancer cells, endothelial cells, stromal cells, blood and the extracellular matrix (Fukumura et al., 1998). Their relative contribution is likely to change with tumour type and tumour site. It is also likely to change with tumour growth, regression and relapse. The challenge now is to establish a unified framework incorporating quantitative data on the magnitude and temporal sequence of the generation of these molecules (Ramanujan et al., 2000). This should help develop effective therapeutic strategies; balance is tipped in favour of angiogenesis (Hanahan and Weinberg, 2000; Bouck et al., 1996).

Invasion and metastasis are biological hallmarks of malignant tumours. They are the major cause of cancer related morbidity and mortality. Pathologically, metastasis is a pattern of tumour cell behaviour, which results from failure of some of the most
fundamental regulatory processes controlling body organization. Metastatic cascade can be divided into many phases (Bidard et al., 2008)

- Detachment of the tumour cells from its adjacent tumour cells
- Anchor aging of tumour cells to matrix components
- Degradation of extracellular matrix
- Invasion of extracellular matrix and vascular dissemination
- Homing of tumour cells in distant sites developed

Acquiring the capabilities needed to emigrate to another tissue is therefore a key event in the development of a cancer. To metastasize successfully, cancer cells have to detach from their original location, invade a blood or lymphatic vessel, travel in the circulation to a distant site and establish a new cellular colony (Nikitenko, 2009). To spread within the tissue, tumour cells use migration mechanisms that are similar, if not identical, to those that occur in normal, non-neoplastic cells during physical processes such as embryonic morphogenesis, wound healing and immune-cell trafficking. To migrate, the cell must modify its shape to interact with the surrounding tissue structures. Hereby, the ECM provides the substrate, as well as barrier towards advancing cell. Cell migration through tissues results from a continuous cycle of interdependent steps. Cell protrusions that initiate ECM recognition and binding can be quite diverse in morphology and dynamics. These termed lamellipoda, filopoda, pseudopods or invapods (Artym et al., 2009). These different cell protrusions contain filamentous actin, as well as varying sets of structural and signaling proteins and lead to dynamic interactions with ECM substrates.

**Molecular basis of cancer**

Number of theories on neoplasia have gained acceptance. One modifies the standard paradigm by postulating a dramatic increase in the accumulation of random mutations throughout the genomes of precancerous cells. Two other theories focus on the role of aneuploidy: large scale aberrations in the chromosomes. Aneuploidy could lead to genomic instability early and later mutate known cancer genes or it may form tumours through an almost infinite variety of genetic changes. Well over 100 genes have been found to be frequently mutating in one kind of cancer or another. According to the standard paradigm, the proteins normally produced by these tumor suppressor genes
and oncogenes are organized into complex biochemical circuits that control the reproduction and survival of cells. Mutations that cause parts of the circuitry to fail or become hyperactive prompt cells to multiply into tumours. But the sheer number of cancer genes has frustrated attempts to deduce which ones are necessary and sufficient to cause the disease. Two gene classes together constitute only a small proportion of the full genetic set play a major role in triggering cancer. In their normal configuration, they choreograph the life cycle of the cell, the intricate sequence of events by which a cell enlarges and divides. Proto-oncogenes encourage cell growth (mitosis); whereas, tumor suppressor genes inhibit it (Gronbaek and Guldberg, 2006). Collectively these two gene classes account for much of the uncontrolled cell proliferation seen in human cancers. When mutated, proto-oncogenes can become carcinogenic oncogenes that drive excessive multiplication. Oncogenes are genes within the cell that may initiate the cell’s transformation from normal to cancerous. Examples of positively acting oncogenes are quite rare in human tumors. So called tumor suppressor genes probably play a more important role. Well known tumor suppressor gene include the p53 gene and retinoblastoma gene (Rb) (Corney et al., 2008). These genes are involved in controlling the cell cycle. It is believed that p53 acts by sensing DNA damage within the cell and preventing the cell from reproducing until the DNA damage is repaired. If the damage can be repaired, p53 allows the cell to divide. This action prevents cells with DNA damage from dividing and therefore, suppresses potential tumors. If the DNA damage is too extensive, p53 causes the cell to die without dividing. This programmed cell death is called apoptosis (Whibley et al., 2009). However, if a mutation occurs in the p53 gene, then its function may be impaired and cells with genetic damage may replicate and malignant transformation may occur. The mutations may cause the proto-oncogene to yield too much of its encoded growth stimulatory protein or an overly active form of it. Tumor suppressor genes, in contrast, contribute to cancer when they are inactivated by mutations. The resulting loss of functional suppressor proteins deprives the cell of crucial brakes that prevent inappropriate growth. For a cancerous tumor to develop, mutations must occur in half a dozen or more of the founding cells growth controlling genes. Altered forms of yet other classes of genes may also participate in the creation of a malignancy, by specifically enabling a proliferating cell to become invasive or capable of spreading (metastasizing) throughout the body.
For many years, oncogenes and tumor suppressor genes held center point in understanding of the molecular basis of carcinogenesis. But now the genes that regulate the apoptosis also play a role in oncogenesis. It is known that mutation in several signalling pathways found in tumours include deregulated expression of the survival factors-Insulin like growth factor (IGF)-1 and IGF-II (Kim et al., 2009), activating mutations of Akt, a serine/threonine kinase that induces a strong survival signal (Vogt et al., 2009) and loss of the suppressor of Akt function (Denley et al., 2008). The anti-apoptotic oncoproteins Bcl-2 and Bcl-XL, which exert their principal effects through stabilization of the mitochondrion, are found over-expressed in several tumour types (Zhang et al., 2008). Recent analyses have exhibited that loss of Apaf-1 is a relatively frequent event in malignant melanoma that presumably confers resistance to apoptosis (Zermati et al., 2007). Loss of ARF is a link between deregulation of oncoproteins such as Ras, Myc and E2F, and consequently p53 activation, permitting cells to proliferate and survive with oncogene deregulation. The mechanisms of loss of ARF include methylation of ARF promoter and amplification of genes such as Bmi-1, Twist and TBX2, which encode repressors of ARF expression (Sreeramaneni et al., 2005).

Vital clues to how mutated proto-oncogene and tumor suppressor genes contribute to cancer came from studying the roles played within the cell by the normal counterparts of these genes. Many proto-oncogenes code for proteins in molecular “bucket bridges” that relay growth stimulating signals from outside the cell deep into its interior. The growth of a cell becomes deregulated when a mutation in one of its proto-oncogenes energizes a critical growth stimulatory pathway, keeping it continuously active when it should be silent.

Cancer cell genotypes is a manifestation of six essential alterations in cell physiology that collectively dictate malignant growth self-sufficiency in growth signals, insensitivity to growth-inhibitory (antigrowth) signals, evasion of programmed cell death (apoptosis), limitless replicative potential, sustained angiogenesis and tissue invasion and metastasis. Each of these physiologic changes novel capabilities acquired during tumor development represents the successful breaching of an anticancer defence mechanism hardwired into cells and tissues. Six capabilities are shared in common by most and perhaps all types of human tumours. This multiplicity
of defences may explain why cancer is relatively rare during an average human lifetime. Acquired GS autonomy was the first of the six capabilities in large part because of the prevalence of dominant oncogenes. Three common molecular strategies for achieving autonomy are evident, involving alteration of extracellular growth signals, of transcellular transducers of those signals, or of intracellular circuits that translate those signals into action (Hanahan, and Weinberg, 2000).

While most soluble mitogenic growth factors (GFs) are made by one cell type in order to stimulate proliferation of another the process of heterotypic signalling many cancer cells acquire the ability to synthesize GFs to which they are responsive, creating a positive feedback signalling loop often termed autocrine stimulation (Fedi et al.,1997). Clearly, the manufacture of a GF by a cancer cell obviates dependence on GFs from other cells within the tissue. The production of PDGF (platelet-derived growth factor) and TGFα (tumour growth factor a) by glioblastomas and sarcomas, respectively, are two illustrative examples (Fedi et al.,1997). The cell surface receptors that transducer growth stimulatory signals into the cell interior are themselves targets of deregulation during tumor pathogenesis. GF receptors, often carrying tyrosine kinases activities in their cytoplasmic domains, are over expressed in many cancers. Receptor over expression may enable the cancer cell to become hyper responsive to ambient levels of GF that normally would not trigger proliferation (Fedi et al., 1997). Six essential alterations in cell physiology that collectively dictate malignant growth are given in Fig.1.4.

These pathways within a cell receive and process growth-stimulatory signals transmitted by other cells in a tissue. Such cell-to-cell signaling usually begins when one cell secretes growth factors. After release, these proteins move through the spaces between cells and bind to specific receptors. Receptors span the outer membrane of the target cells, so that one end protrudes into the extracellular space and the other end projects into the cell’s interior, its cytoplasm. When a growth stimulatory factor attaches to a receptor, the receptor conveys a proliferative signal to proteins in the cytoplasm. These downstream proteins then emit stimulatory proteins, in a chain that ends in the heart of the cell, its nucleus. Within the nucleus, proteins known as transcription factors respond by activating a cohort of genes that help to usher the cell through its growth cycle.
Fig. 1.4

Acquired capabilities of cancer

Six essential alterations in cell physiology that collectively dictate malignant growth

Adapted from Hanahan, and Weinberg (2000)
The cell cycle is the decision maker of the cell whether to divide or not. These events take place in the nucleus. The cell cycle composed of four stages.

1. In the G\(_1\) (gap 1) phase, the cell increases in size and prepares to copy DNA.
2. The copying of DNA continues in the second stage S (synthesis) and enables the cell to duplicate precisely its complement of chromosome.
3. After chromosomes are replicated a second gap period, termed G2, follows during which the cell prepares itself for M (mitosis) - the time when the enlarged parent cell finally divides in half to produce its two daughters, each of which is endowed with complete set of chromosomes.
4. The new daughter cells immediately enter G1 and may go through the full cycle again. Alternatively they may stop cycling temporarily or permanently.

The cell cycle clock programs this elaborate succession of events by means of a variety of molecules. It’s two essential components, cyclins and cyclins-dependent kinases (CDKs), associate with one another and initiates entrance into the various stages of the cell cycle. In G\(_1\), for instance, D-type cyclins bind to CDKs 4 or 6, and the resulting complexes act on a powerful growth inhibitory molecule known as pRB. This action releases the breaking effect of pRB and enables the cell to progress in to late G\(_1\) and then into S (DNA synthesis) phase.

Abnormalities in the different compartments of cell cycle regulatory machine have been found in several types of human cancers. There are evidences to show that cell cycle regulators are disrupted in human cancers. Breast cancer cells often produce excesses of cyclin D and cyclin E. In many cases of melanoma, skin cells have lost the gene encoding the breaking protein p16. Half of all types of human tumors lack a functional p53 protein. The cyclin exerts its effects by binding to and activating a series of specific Cyclin-Dependent kinases (CDKs). The phosphorylation and dephosphorylation of CDK proteins further modulate this process by protein kinases, phosphatases and by a series of CDK inhibitor proteins (CDIs). Among them are p15 and p16, both of which block the activity of the CDK partners of cyclin D, thus preventing the advance of the cell from G\(_1\) into S. Another inhibitor of CDKs, termed p21, can act throughout the cycle. p21 is under control of a tumor suppressor protein,
p53 that monitors the health of the cell, the integrity of its chromosomal DNA and the successful completion of the different steps in the cycle.

Impairments in the functioning of these components involved in the regulation of cell cycle leads to increased proliferation of cells, the basis of tumour formation. The protein products of many oncogenes and tumour suppressor genes (TSG) regulate the activity of CDKs, responsible for the initial steps of the presynthetic phase (G₁) and transition of G₁ in to S phase of DNA synthesis (CyclinE-Cdk2). Some proto-oncogenes and TSG regulate activity of complexes of Cyclin A-Cdk2 (required for DNA replication) and Cyclin B-Cdk1 (responsible for the transition of G2 phase to mitosis). The passage through restriction point and entry in to S-phase is controlled by Cyclin dependent protein kinases (CDKs) which are regulated by cyclins D, E and A.

Most of the cells in multicellular organisms are in contact with an intricate meshwork of interacting, extracellular macromolecules that constitute the extracellular matrix. Tumor cells must interact with the ECM at several stages in the metastatic cascade. Local cells, especially fibroblasts, which are widely distributed in the matrix, secrete the macromolecules that constitute the extracellular matrix. In specialized matrix structures, such as cartilage and bone, these macromolecules are secreted locally by more specialized cells like chondroblasts from cartilage and osteoblasts from bone.

**Extra cellular matrix**

Extra cellular matrix is the relatively stable structural components that lies under epithelia and surrounds connective tissue cells. It is created by cells on mere scaffolding on or in which cell reside. It consists of proteins such as fibronectin, laminin and many non-collagenous extracellular proteins-polysaccharides complexes.

Most normal vertebrate cells cannot survive unless they are anchored to the extra cellular matrix. This anchorage dependence is often lost when a cell turns cancerous. To perform many of the functions, cells must bind to other cells or to the molecules surrounding them that comprise the scaffold upon which tissue and organ structure is built called extra cellular matrix (Baltimore _et al._, 1995). The components of extra cellular matrix play a key role in the onset of cancer. The matrix whose constituents are secreted by the cells themselves helps in cell adhesion, cell-cell communication
and cell-matrix interactions. The extra cellular macromolecules that make up the matrix are polysaccharide chains of the class called glycosaminoglycans (GAG’s), which are usually found covalently linked to proteins in the form of proteoglycans and fibrous proteins of two functional types; mainly structural (collagen and elastin) and adhesive (fibronectin & laminin). Four main groups of GAG’s have been distinguished by the type of sugar residues, the type of linkage between these residues and the number and location of sulfate groups.

A) Chondroitin sulfate and dermatin sulfate
B) Heparin sulfate and heparin
C) Keratin sulfate
D) Hyaluronan

Among all these, hyaluronan / hyaluronic acid / hyaluronate is gaining a lot of importance due to its usual but extraordinary properties that has profound influence on cellular behaviour (Toole, 2000).

**Hyaluronic acid (HA)**

Hyaluronic acid is a highly negatively charged, high molecular weight GAG found predominately in the ECM and it is the simplest of all GAG’s. It is a straight chain GAG polymer composed of repeating units of the disaccharides [D-Glucouronic acid (β1-3) and N-Acetyl glucosamine (β1-4)]. The structure of hyaluronic acid is shown in Fig.1.5.

Hyaluronic acid was first isolated from vitreous body of the eye by Meyer (Meyer, 1934). For decades, only few investigators were working on this polymer and the field was not competitive. Now the situation has been changed in the last 10 years. Interest in the hyaluronan has intensified in cell-biology, molecular biology, pathology, immunology, after it was shown that cells have receptors that can specifically recognise its pure polysaccharide. HA is a member of the glycosaminoglycan (GAG) family, but it differs in many ways from other GAGs. First, it is huge, usually with a molecular weight between $10^3$ and $10^4$ kDa and an extended length of 2–25 mm. Second, unlike other GAGs, HA contains no sulfate groups or epimerized uronic acid residues. Third, the mechanism of synthesis of HA
Fig. 1.5
Structure of hyaluronic acid
Adapted from www.rejuvenation-science.com/hyaluronic-acid
is unique, HA is made at the inner side of the plasma membrane rather than in the golgi apparatus; it is most likely elongated at the reducing rather than the non reducing terminus during synthesis; and it is not covalently linked to a protein backbone during synthesis (Fig. 1.6). After many years of frustration, characterization of the enzymes responsible for HA synthesis, i.e., the HA synthases (Has’s), has progressed rapidly over the past several years (Weigel et al., 1997, Spicer and McDonald, 1998).

Hyaluronan is synthesized by a class of integral membrane proteins called hyaluronan synthases (HAS), of which have three types: HAS1, HAS2, and HAS3 (Weigel et al., 1997; Itano et al., 1999) These enzymes lengthen hyaluronan by repeatedly adding glucouronic acid and N-acetylglucosamine to the nascent polysaccharide as it is extruded through the cell membrane into the extracellular space with products of varying chain length.

HA is degraded in vivo by hyaluronidases (HAases), a family of six different enzymes that each differently cleave HA chains by hydrolysis into shorter fragments. These enzymes enable physiologic turnover of HA, which occurs to the extent of nearly 33% of bodily HA content per day (Stern, 2004). However, the extent of HA degradation that occurs under pathological conditions may be greatly enhanced, as well as the size distribution of generated HA fragments significantly altered. Under both physiologic and pathologic scenarios, extremely short-sized (<20 monomers) HA fragments termed “HA oligomers” can by temporarily generated as products of HA degradation by a hyaluronidase isoform HYAL-1 (West et al., 1985). Production of these oligomers can be enhanced quite dramatically at sites of injury, inflammation and within tumours, due to increases in HA content itself or synthesis and availability of receptors for HA and HAases (Slevin et al., 2004), sometimes upon generation of reactive oxygen and nitrogen species (Noble, 2002).

Unlike native long-chain HA, shorter HA fragments, particularly HA oligomers, are highly bioactive and serve to interact with and influence cellular phenomena (including angiogenesis) in a manner that native long-chain HA does not and cannot (Rooney et al., 1995).
Fig.1.6
Hyaluronic acid biosynthesis

Adapted from Fraser and Laurent, 1989
The biologic roles and cellular interactions of HA with cells, are highly dependent on HA chain length. Native high molecular weight (HMW) HA (aka long-chain HA) is a largely bioinert molecule, that physically contributes to unique cellular microenvironment and mechanics of tissues, although it can also indirectly influences cell behaviour. In tissues, long-chain HA serves to maintain a highly hydrated environment, regulate osmotic balance, acts as a shock-absorber and space-filler, and as a lubricant. Simultaneously, with its numerous functional groups for binding, and highly anionic nature, HMW can sequester and release growth factors and other biologic signalling molecules to impart potent but localized influence on cell behaviour (Lai et al., 2006). HMW HA has been shown to inhibit extracellular proliferation and disrupt newly-formed extracellular monolayer (West et al., 1989). However, HMW HA can also sheath cells to prevent their interaction with other cells, and biologic signalling molecules with their cell-surface receptors.

Compared to HMW HA, shorter HA fragments and oligomers are more bioactive. Both the long-chain and oligomeric forms of HA are capable of binding to cell-surface HA receptor glycoproteins, such as cluster determinant molecule-44 (CD44), although the former is capable of multivalent interactions, while the latter interacts only monovalently. While HMW HA promotes cell quiescence upon such binding, oligomers can cluster and thus activate these receptors to trigger associated intracellular signalling cascades (Evanko, 2001). In this manner, HA oligomers can induce inflammatory cytokine release by inflammatory cells, and thereby activate non-inflammatory cell types to proliferate, migrate and trigger wound healing or tissue remodelling. (Noble, 2002) The glycoprotein receptors that mediate the cellular response to HA fragments are CD44, Receptor for Hyaluronan-Mediated Motility (RHAMM) and Toll-Like-Receptor-4 (TLR-4). Through interaction with one or more of these receptors, and the consequent effects of promoting extracellular proliferation and migration, HA oligomers can induce neo-angiogenesis and sprout formation, which are inherent and critical to cell/tissue viability, growth and to tissue repair and remodelling following injury/trauma.

This large polymer have great deal of biological functions, as a glue it participates in lubricating joints, space filling, anti angiogenic, immunosuppressive (Feinberg and Beebe, 1983; Delmage et al., 1986; McBride and Bard, 1979) and that impede
Hyaluronan has remarkable hydrodynamic characteristics, especially in terms of its viscosity and its ability to retain water. It therefore has an important role in tissue homeostasis and biomechanical integrity. Hyaluronan polymers are very large and can displace a large volume of water. This property makes them excellent lubricators and shock absorbers.

Hyaluronan also forms a multivalent template for interactions with proteoglycans and other extracellular macromolecules that are important in the assembly of extracellular and pericellular matrices (Toole, 2004). These properties of hyaluronan help to regulate the porosity and malleability of these matrices which are important factors in determining whether cells invade tissues during development, tissue remodelling and cancer progression. This function of hyaluronan and of pericellular matrices contributes to the ‘permissive’ or ‘landscaping’ role of the microenvironment in which cancer cells proliferate and metastasizes (Kinzler and Vogelstein, 1998; Hanahan and Weinberg, 2000).

The co-incidence of HA oligosaccharides and angiogenesis in various diseased states strongly suggests a cause-effect relationship between the two. The etiology of a lesion composed of an abnormal growth of cells resulting in unregulated proliferation (tumour), is thought to originate from the genetic mutation of one or more cells. The growth of a solid tumour beyond 2 mm in diameter is “angiogenesis-dependent” (Folkman, 1971) with growth arresting with translocation into a nonvascular region of the body. (Folkman et al., 1966) In many tumour types, long-chain hyaluronan has been shown to be crucial to the progression of cancer either by stimulating malignancy of tumour cells and their poor differentiation, and providing a loose
matrix for cell migration, (Folkman, 1971) while HA breakdown products, including HA oligosaccharides promote tumour spreading by stimulating angiogenesis and creation of a microvascular network (Rooney et al., 1995; Koyama et al., 2007; West and Kumar, 1989). The metastasis of cancer is attributed to HA-cell interactions in mammary carcinoma and other cancers where the formation of blood vessels to nourish the growing tumour also distribute cancer cells to distal body parts. (Itano et al., 1999) Although the HA fragment sizes that contribute to the various mechanisms via which tumours initiate, grow and metastasize are not clearly defined at present, despite the large body of work in the field. It is generally agreed that the HA oligomers/fragment containing between 4–25 disaccharide units are angiogenic and enhance tumour invasiveness (Lokeshwar et al., 1997). Others have showed that tumours contain a specific variant of HYAL, a hyaluronidase, which corresponded with generation of HA oligomers and tumour invasiveness and metastasis (Hautmann et al., 2001; Paiva et al., 2005). This was subsequently confirmed in various cancer types. As mentioned previously, the angiogenic effects of HA oligomers is accomplished by its interaction with CD44 and RHAMM on ECs and subsequent stimulation of EC proliferation, motility and tubule formation. Despite the complex involvement of HMW HA in lipid accumulation, and inflammatory cell recruitment and vascular cell migration within plaques, again, it is the shorter HA fragments that contribute to intra-plaque angiogenesis. It has also become evident recently that HA is an active modulator of proliferation and inflammation in atherosclerotic plaques (Bot et al., 2008). Such neo-vascularization of the neointima occurs by sprouting of blood capillaries from vessels in the adventitia, and helps nourish cellular metabolism with the plaque and sustain its growth. Though some studies attribute the presence/buildup of angiogenic HA fragments to enhanced expression of HAS-1 and -3 within the plaque (Bot et al., 2008). Others suggest that they are instead generated by enzymatic, oxidative or nitrative degradation of long-chain HA in the inflammatory microenvironment within plaques, to then stimulate new vessel sprouting, plaque growth and rupture. Likewise, in other diseases such as diabetic retinopathy too, long-chain HA is broken down into HA oligomers to locally induce angiogenesis within the normally unvascularized vitreous, and result in hemorrhage and blurred vision (West et al., 1988).
Hyaluronan (HA) in carcinogenesis

Hyaluronan is one of the important components of the extracellular matrix particularly in rapidly remodeling tissues undergoing regeneration and repair. Hyaluronan is also enriched in the pericellular matrices of many malignant human tumours and manipulations of hyaluronan interactions have strong effects on tumour progression in animal models. Most solid tumours contain elevated levels of hyaluronan (Knudson et al., 1989, Knudson, 1996). Hyaluronan levels have been found to be elevated in lung tumours, Wilma’s tumour and breast carcinomas (Knudson et al., 1989). Hyaluronan involvement during tumour progression is well documented. High levels of hyaluronan expression correlate with poor differentiation in ductal carcinomas of human breast (Auvinen et al., 1997) and with poor survival rates in human colorectal adenocarcinomas (Ropponen et al., 1998). Several studies have reported a relation between hyaluronan content and invasiveness and a greater enrichment of hyaluronan in the stroma that surrounds tumours than in parenchymal regions (Toole et al., 1979; Bertrand et al., 1992) Other studies have shown that hyaluronan production by stromal cells is stimulated by interactions with tumour cells (Knudson et al., 1984; Asplund et al., 1993) but that synthesis is also increased in malignant tumour cells themselves (Kimata et al., 1983; Zhang et al., 1995; Calabro et al., 2002). High levels of stromal hyaluronan are also associated with malignancy in patients who have non-small-cell lung adenocarcinomas (Pirinen et al., 2001) and prostate cancer (Posey et al., 2003; Lipponen et al., 2001). Hyaluronan is predictive of malignancy in breast and prostate carcinomas (Toole et al., 2002). Hyaluronan is associated with tumour cells and stromal hyaluronan are linked with cancer progression (Boregowda et al., 2006). Levels of parenchymal hyaluronan also correlate with malignancy in patients with gastric and colorectal cancers (Toole, 2004). However, hyaluronan levels do not correlate with progression in melanomas (Karjalainen et al., 2000) or in some epidermal carcinomas (Karvinen et al., 2003). Hyaluronan also constitutively regulates Erb2 phosphorylation and signaling complex formation in colon and mammary carcinoma cells (Ghatak et al., 2005). Differential hyaluronan expression in all human tumour progression explains the positive association of increased stromal hyaluronan expression with invasive nature of tumours irrespective of their origin (Boregowda et al., 2006).
These genes cluster in two tightly linked triplets on human chromosomes 3p21.3 (HYAL1, HYAL 2 and HYAL 3) and 7q31.3 (HYAL 4, PH 20 and HYALP1) (Csoka et al., 1999). It is reported that hyaluronidase is similar to the one on the human sperm PH20 but not on the spleen, ovary and liver (Gmachl et al., 1993). It is expressed by metastatic human melanoma, colon carcinoma and glioblastoma cell lines. This type of hyaluronidase is also demonstrated in tumour biopsies from patients with colorectal carcinoma, but not in normal colonic tissues. More over angiogenesis is induced by hyaluronidase positive tumour cells but not hyaluronidase negative tumour cells (Dacai et al., 1996). It is known that proteoglycans bind to growth factors (Ruoslahti et al., 1991). It is postulated that binding of growth factors to proteoglycans in the extracellular matrix can concentrate or protect growth factors from degradation. Digestion of extracellular matrix by tumour hyaluronidase may, therefore, free growth factors, stored in the microenvironment, thus enhancing their availability for target cells. Some of these factors may be essential for angiogenesis.

HA plays a critical role in dynamic structural changes within extracellular matrix during development and tissue remodeling, as well as maintenance of mechanical properties and homeostasis of many tissues (Toole et al., 2002). Increased synthesis of HA is associated with wound repair, tumour invasion and immune recognition (Toole, 1982; Iozzo, 1985). Further, HA has been proposed to regulate cell locomotion and cytodifferentiation (McClay and Ettensohn, 1987). Hyaluronan constitutively regulates activation of multiple receptor tyrosine kinases in epithelial and carcinoma cells (Suniti et al., 2006).

Circulating hyaluronan

It was clear from the foregoing that nearly all the body’s hyaluronan content lies within the tissues, where its turnover was thought to occur until it was shown that the lymphatic vessels carry considerable amounts of HA to the bloodstream (Laurent and Laurent, 1981). In densely structured tissues such as bone and cartilage, some 20–30% of HA turnover occurs by local metabolism, and the rest is removed by the lymphatic pathways. The tissue half-life of HA ranges from half a day to 2 or 3, regardless of its route of elimination. On reaching the blood stream about 85–90% is eliminated in the liver by receptor-facilitated uptake and catabolism in the hepatic sinusoidal.
endothelial cells. The kidneys extract about 10% but excrete only 1–2% in urine. In some species, the spleen also has a high avidity for HA and an equally rapid capacity for its metabolism but its contribution depends on its relative size and circulation. The normal fractional turnover of plasma HA in humans is about 15–35% per minute, which explains the low plasma levels in the face of the lymphatic input. (Fraser and Laurent, 1989; Laurent and Fraser, 1991). The lymph nodes have been found to have a considerable capacity for extraction and catabolism of HA (Fraser et al., 1996). Comparison of lymph before and after passage through the nodes shows an extraction of as much as 90%, which occurs in the lining cells of the lymphatic sinuses, comparable to those of the blood vessels in liver and spleen. Studies of peripheral lymph have also revealed:

1. That its content of HA is still much lower than that of the tissues from which it is derived;
2. That it contains, nevertheless, very large polymers similar to those in the tissues, which suggests that they are displaced from the tissues hydro dynamically rather than by diffusion and unlike collagens and larger proteoglycans, do not require any prior degradation; and
3. That these polymers are preferentially eliminated in the lymph node, consistent with the higher affinity for larger polymers demonstrated in experiments on the hepatic HA receptor.

It should be noted that specificity of the metabolic receptor for HA in the liver is shared with chondroitin sulphate. Infusion studies in blood stream and lymph node confirm that both share the same catabolic pathways, but no clinical significance has yet been attached to this fact. All the major factors that affect the plasma levels of HA can now be anticipated. In the elimination pathways, they include the functional capacity of the specialized liver endothelial cells and the fractional distribution of cardiac output through the hepatic sinusoids, with possibly some effects from impaired renal function; significant disturbances in HA metabolism by lymph nodes have not yet been identified. From the input side, plasma HA can be elevated by an increased synthesis of HA in a great variety of diseases. It can be raised or lowered by variation in the many circumstances that alter the flux of fluid between the lymph and blood stream and the displacement of HA that accompanies it (Fraser and Laurent, 1997) (Fig. 1.7).
Fig. 1.7

Hyaluronam and the lymphatic system

Adapted from www.glycoforum.gr.jp/.../HA28/images/fig_01
In addition, it has been shown that hyaluronan levels are increased in the urine of patients with bladder carcinomas (Lokeshwar et al., 2002), and in the saliva of patients with head and neck cancer (Franzmann et al., 2003). As mention earlier, tissue HA enters the circulation via the lymph and is rapidly extracted and catabolised by the liver (Laurent and Fraser, 1986), resulting in a normal serum HA level of 10-100µg. The level of HA can rise in cirrhosis (Laurent et al., 1985) and rheumatoid arthritis (Laurent and Hallgren, 1985) and in end stage renal failure (Hallgren et al., 1987). There have been reports of raised serum levels of HA in advanced cancer; (Delpech et al., 1985), HA levels are increased in the serum of patients with various malignant tumors, such as multiple myeloma, (Dahl et al., 1999) breast cancer, (Delpech et al., 1990) bladder cancer (Hautmann et al., 2001), epithelial ovarian tumours (Hiltunen et al., 2002) and in oral cancer patients (Xing et al., 2008).

**Hyaluronan binding proteins (HABPs)**

The hyaluronan-binding proteins (HABPs) or hyaladherins, are a family of macromolecules whose various members serve as structural components of extracellular matrices or as receptors that bind hyaluronan to the surface of cells (Toole, 1990). Many hyaladherins contain a common structural domain of ~100 amino acids in length, termed a Link module, that is involved in ligand binding (Day, 1999). However, a growing number of hyaladherins lack this domain and are unrelated to each other at the primary sequence level. The widespread occurrence of HA-binding proteins indicates that the recognition of HA is important to tissue organisation and the control of cellular behaviour (Fig.1.8).

The hyaladherin family would include structural matrix HA-binding proteins as well as cell-surface HA receptors that exhibit high affinity binding of HA. Most well characterized hyaladherins have structurally similar hyaluronan binding domains with sequence homologies of 30-40%. These domains are called link modules or proteoglycan tandem repeats, form disulfide bonded loops and, in many hyaladherins, two modules are arranged in tandem array. Two link modules form the hyaluronan-binding region of link proteins and the aggregating proteoglycans (Ioizzo, 1998), where as only a single-link module is found in the hyaluronan-binding domains of CD44 (Sherman et al., 1994) and TSG-6 (Lee et al., 1992). Some hyaladherins, such as RHAMM, do not have link modules. However, mutation and sequence-swapping
Fig. 1.8
Structure of hyaluronic acid binding protein in complex with hyaluronic acid
Adapted from vkery.com/professional_background.htm
studies with RHAMM showed a possible hyaluronan-binding motif that is present not only in RHAMM, but also within or adjacent to the link modules of above explained hyaladherins. The motif is B (X7) B, where B is arginine or lysine and X is any non-acidic amino acid. Variations of this motif, e.g., B (X8) B, also bind to hyaluronan with significant affinity, and clearly clustering of basic amino acids within and around the motif is the key aspect that determines binding (Yang et al., 1994). Examples for the hyaluronan binding proteins which contain the B(X7)B and related sequence are ICAM (McCourt et al., 1994), hyaluronan synthases (Weigel et al., 1997), mammalian hyaluronidases (Zhu et al., 1994), Cdc37, a hyaluronan binding cell cycle regulatory protein (Grammatikakis et al., 1995), and P32, a hyaluronan-binding protein that associates with splicing factors (Deb and Datta, 1996) and 1HABP4, an intracellular hyaluronic acid binding protein (Huang et al., 2000).

**Hyaluronan binding proteins in cancer**

Hyaluronan receptors have been widely implicated in tumorigenesis. CD44 and RHAMM are established signal-transducing receptors that influence cell proliferation, survival and motility, and are known to be relevant to cancer. Other cell-surface hyaladherins such as lymphatic-vessel endothelial hyaluronan receptor 1 (LYVE1) and TOLL4, might also have roles in cancer pathogenesis. Recent evidences suggest that CD44 mediated events can enhance (Sy et al., 1991, Gunthert et al., 1991; Iida and Bourguignon, 1997) or inhibit (Takahashi et al., 1995; Schmits et al., 1997) tumour progression in different types of tumours. CD44 is a widely distributed cell surface glycoprotein that is encoded by a single gene but expressed as numerous isoforms as a result of alternative splicing. It is evidenced that CD44 gene, particularly the 80-90 kDa protein, plays a role in metastasis of certain human tumour cell lines when implanted in nude mice. It was shown that variant isoform of glycoprotein CD44, is required for metastatic behaviour of rat pancreatic adenocarcinoma cells (Gunthert et al., 1991).

Although hyaluronan is the main ligand for CD44, several other molecules interact with this protein, many of which bind to carbohydrate side groups that are attached to the ‘spliced-in’ regions. Among these other ligands, fibroblast growth factors, osteopontin and matrix metalloproteinases (MMPs) are particularly important in terms of relevance to cancer (Ponta et al., 2003). CD44 also mediates the cellular uptake
and degradation of hyaluronan, which in turn affects growth regulation and tissue integrity (Kaya et al., 1997; Teder et al., 2002). Interactions of hyaluronan with CD44 lead to numerous cellular responses, including those that involve tyrosine kinases, protein kinase C, focal adhesion kinase (FAK), phosphatidylinositol 3-kinase (PI3K), mitogen-activated protein kinase, nuclear factor-κB and RAS, as well as cytoskeletal components (Turley et al., 2002; Ponta et al., 2003; Bourguignon et al., 2001; Thorne et al., 2004).

Hyaluronan-RHAMM interactions have also been implicated in tumour cell behaviour in vitro and in vivo. Intracellular RHAMM interacts with several signalling proteins and cytoskeletal components, including SRC, extracellular signal regulated kinase 1 (ERK1), actin and microtubules (Turley et al., 2002; Hall and Peters, 1996). RHAMM is involved in the Ras and ERK signalling pathways and associates with the cytoskeleton (Turley et al., 2002). Hyaluronan-RHAMM interaction induces transient phosphorylation of p125FAK in concert with turnover of focal adhesions in ras-transformed cells, thus leading to initiation of locomotion (Hall et al., 1995). Suppression of this interaction inhibits cell locomotion and proliferation in vitro and leads to inhibition of tumour growth in vivo whereas, over-expression of RHAMM leads to enhanced tumour growth and metastasis (Turley et al., 2002; Hall et al., 1995). It has been shown that, over expression of cell surface RHAMM causes fibroblasts to become tumorigenic (Hall et al., 1995). Treatment of fibroblasts with a soluble form of RHAMM causes cells to get arrested in G2/M, due to inhibition of Cdc2 and cyclin B1 expression, and inhibits fibrosarcoma growth and metastasis in vivo (Mohapatra et al., 1996).

Finding a common antigen, which could detect any tumour cell derived from any source of human body for the evaluation of potentially metastatic cells and ultimately for therapeutic application is a general aim in oncological research. The monoclonal antibody IVd4 (mAb IVd4) (Banerjee and Toole, 1991) recognized hyaluronic acid binding proteins (HABP) of different molecular size - 30, 50, 70, and 90 kDa, and its cDNA encodes a protein homologous to Cdc 37, an essential cell cycle regulator that was previously characterized in yeast and Drosophila (Ferguson et al., 1986; Cutforth and Rubin, 1994). Cdc37 has affinity to the hyaluronan and other glycosaminoglycans which are intimately associated with extracellular matrices, cell surface, cytoplasm and the nucleus (Toole, 1991; Ishihara et al., 1986; Ripellino et al., 1989; Hiscock et al., 1994).
It is shown that Cdc37 is required for the association of Cdc28 with at least two cyclin subunits, the G1 cyclin Cln2 and the mitotic cyclin Clb2. Formation of active Cdc28-Cln complexes is necessary for cells to progress through G1 (Forsburg and Nurse, 1991; Gerber et al., 1995; Reed, 1992). It is observed that defects in progression from G1 to S phase in Cdc37-1 mutant cells, and analysis of Clb2 suggests that Cdc37 is required for passage through mitosis (Gerber et al., 1995).

Three approaches that have been used to manipulate endogenous hyaluronan–protein interactions are overexpression of soluble hyaladherins, administration of hyaluronan oligosaccharides, and treatment with antibodies that block hyaluronan–CD44 binding. Soluble hyaladherins competitively displace hyaluronan from its endogenous cell surface receptors, for example, CD44 or RHAMM, thus inhibiting putative downstream events. Several studies have demonstrated inhibition of tumour progression by treatment with soluble forms of CD44 (Sy et al., 1992; Bartolazzi et al., 1994). Overexpression of soluble CD44 in mouse mammary carcinoma cells or in human malignant melanoma cells leads to inhibition in vivo of growth, local invasion, and metastasis (Yu et al., 1997; Yu and Stamenkovic, 1999; Peterson et al., 2000; Ahrens et al., 2001). No significant effects were obtained if the soluble CD44 was mutated such that hyaluronan binding was eliminated. Soluble RHAMM, another hyaladherin, also inhibits metastasis (Mohapatra et al., 1996) and a hyaluronan-binding complex from cartilage, containing link protein and fragments of aggrecan, inhibits both tumour growth and metastasis (Liu et al., 2001). Hyaluronan oligomers compete for endogenous polymeric hyaluronan–receptor interactions, thus resulting in low-valency, low-affinity binding rather than polyvalent, high-affinity interactions with receptors (Underhill et al., 1983). Oligomers containing 6–18 sugar residues are effectively monovalent in their interaction with CD44 (Lesley et al., 2000). Thus displacement of endogenous polymeric hyaluronan with oligomers of this size could potentially lead to the loss of hyaluronan-induced signaling. In similar fashion to soluble hyaluronan-binding proteins, these oligomers inhibit growth of several tumour types in vivo (Zeng et al., 1998). Likewise, treatment with antibodies that block hyaluronan binding to CD44 inhibit tumour growth and invasion (Guo et al., 1994; Zahalka et al., 1995).
The surface of cancer cells differs in many respects from that of normal cells. Neoplastic transformations of a variety of cell types are associated with changes in the composition of membrane glycoproteins (Dwivedi et al., 1990). Cell surface adhesive glycoproteins are involved in cell adhesion and migration in inflammatory diseases and malignant disorders and identified together with specific aberrations in malignant diseases (Wollenberg et al., 1996; Velikova et al., 1998). Although not much investigated so far, soluble forms of cell adhesion molecules have been linked to the clinical behaviour of tumours. Positive and negative regulation of cell adhesion will influence the process as metastatic cells break away from the primary tumour, enter the circulation and then adhere to cellular and extracellular matrix elements in particular secondary sites. It has been suggested that several cell adhesion molecules may play a role in infiltrative growth and metastatic processes (Velikova et al., 1998).

It has been shown that, over expression of cell surface RHAMM causes fibroblasts to become tumorigenic (Hall et al., 1995). Treatment of fibroblasts with a soluble form of RHAMM causes cells to get arrested in G2/M, due to inhibition of Cdc2 and cyclin B1 expression, and inhibits fibrosarcoma growth and metastasis in vivo (Mohapatra et al., 1996). CD44 is present as a soluble form in extracellular fluids; sCD44 has been measured and partially characterized in human plasma (Lucas et al., 1989). In a first report, sCD44 has been proposed to be a valuable indicator of tumour growth in gastric and colorectal cancer (Guo et al., 1994). CD44s variant was found to be higher in the ovarian cancer patients (Zeimet et al., 1997) and cervical cancer (Gadducci et al., 1998).

Soluble CD44 is a potential marker for the early detection of head and neck cancer (Franzmann et al., 2007). Alternative splicing of CD44 and aberrant levels of soluble CD44 variants in the serum of cancer patients have been correlated to tumour progression and metastasis in different tumours including breast cancer (Mayer et al., 2008).

As cancer involves the transformation and proliferation of altered cell types that produce high levels of specific proteins and enzymes such as proteases, e.g., PSA and prostate-specific membrane antigen (PSMA) (Hugosson et al., 2003; Ghosh et al., 2005), it not only modifies the array of existing serum proteins (the serum
proteome) but also their metabolic products, i.e., peptides (the serum peptidome). It is well established that human serum contains thousands of proteolytically derived peptides (Richter et al., 1999; Tirumalai et al., 2003; Koomen et al., 2005), yet it remains unclear to date whether this complex peptidome may provide a robust correlate of some biological events occurring in the entire organism.

Within this field of research, interest continues to grow regarding a previously unexplored reservoir the array of existing proteins in a patient’s serum (coined as the serum proteome), particularly those of low molecular weight (LMW), as well as the metabolic products of these serum proteins (the serum peptidome, fragmentome, or degradome) (Fig. 1.9).

Blood-based tests may be good candidates as early cancer screening tools, since: (i) blood collection is simple and minimally invasive; (ii) tumour biomarkers may come not only from the tumour but from other organs and tissues and may represent the systemic response to tumour growth. As such, these proteins may be secreted into the bloodstream at the very early stages of tumourgenesis, when tumour itself is undetectable by conventional imaging methods. Presently, very few blood biomarkers have proven useful for diagnosing primary cancer.

Tumor markers have five potential uses in patient care: They can be used for screening, for diagnosis, for establishing prognosis, for monitoring treatment, and for detecting relapse. The value of a marker in a given setting depends on two marker-related characteristics—sensitivity and specificity. The value of a marker in a particular malignancy also depends on the effectiveness of therapy for the malignancy. Tumor markers have been used to screen for occult cancer but have proved to be valuable only in selected cancers. As diagnostic tools, tumor markers have limitations.

Screening tests require high sensitivity to detect early-stage disease; therefore an understanding of tumor markers is becoming increasingly important. These soluble molecules in the blood are usually glycoproteins which detected by monoclonal antibodies. Each tumor marker has a variable profile of usefulness for screening, determining diagnosis and prognosis, assessing response to therapy, and monitoring
Proteinases generate biomarker fragments. Circulating protein fragments generated in the diseased tissue microenvironment may serve as diagnostic protein markers. Proteolytic cascades within the tissue (a product of the interacting cellular ecology such as stromalepithelial interactions), immune cell MHC presentation, or apoptosis generate protein fragments that passively diffuse into the circulation. Shed LMW peptides are protected from kidney-mediated clearance by sequestration on abundant resident blood proteins such as albumin. According to the results presented by Villanueva et al diagnostic protein fragments can also be generated ex vivo by circulating enzymes derived from the diseased tissue microenvironment acting on exogenously derived peptides produced by serum collection methodology.

Adapted from Liotta and Petricoin, 2006
for cancer recurrence. To date, no tumor marker has demonstrated a survival benefit in randomized controlled trials of screening in the general population. Nevertheless, tumor markers can play a crucial role in detecting disease and assessing response to therapy in selected groups of patients. In monitoring patients for disease recurrence, tumor marker levels should be determined only when there is a potential for meaningful treatment.

"The blood contains a treasure trove of previously unstudied biomarkers that could reflect the ongoing physiologic state of all tissues" (Liotta and Petricoin, 2003), and the latter, therefore, appears to be more attractive. Many scientists have pointed to what they perceive to be a dried-up blood-borne cancer biomarker pipeline for disease detection since recent searches for a single, cancer-specific marker have not proved fruitful. However, the prospects of blood proteomics are challenged by the fact that blood is a very complex body fluid, comprising an enormous diversity of proteins and protein isoforms with a large dynamic range of at least 9–10 orders of magnitude (Anderson 2005). The abundant blood proteins, such as albumin immunoglobulin, fibrinogen, transferrin, haptoglobin and lipoproteins, may mask the less abundant proteins, which are usually potential markers (Omenn et al., 2005). Investigators began to use MS to explore the LMW component of the circulatory proteome in order to determine whether the LMW pool contained any disease-related biomarker candidates.

Appropriate biomarker-based screens should be minimally invasive and reproducible. A simple blood or urine test that detects molecules specific to tumor tissues would be ideal. In addition, screening technology must be sufficiently sensitive to detect early cancers but specific enough to classify individuals without cancer as being free of disease (Etzioni et al., 2003). Despite an intensive search during the past decade(s), only a very small number of identified cancer biomarkers, all plasma proteins (e.g., prostate-specific antigen [PSA], carcinoembryonic antigen [CEA], cancer antigen 125 [CA125], and thyroglobulin), have proven clinically useful, often in combination with other diagnostic tools, for the prognosis of response to therapy, relapse, and survival and for defining the rate of progression and monitoring of treatment, but they have been less useful for broad-based population screening (Sidransky, 2002; Bidart et al., 1999). Those proteins are typically present in plasma or serum at subnanomolar
concentrations and require individual immunoassays for detection and quantitation (Jortani et al., 2004; Watts, 1999). New and improved cancer biomarkers and facile detection methods are clearly in order but have so far eluded discovery and implementation.

Monoclonal antibodies are used to detect serum antigens associated with specific malignancies. These tumor markers are most useful for monitoring response to therapy and detecting early relapse. With the exception of prostate-specific antigen (PSA) (Catalona et al., 1994; Tchetgen and Oesterling, 1997) tumor markers do not have sufficient sensitivity or specificity for use in screening. Cancer antigen (CA) 27.29 (Chan et al., 1997; Gion et al., 1999) most frequently is used to follow response to therapy in patients with metastatic breast cancer. Carcinoembryonic antigen is used to detect relapse of colorectal cancer, and CA 19-9 (Steinberg, 1990) may be helpful in establishing the nature of pancreatic masses. CA 125 (Tuxen et al., 1995; Chen et al., 1988) is useful for evaluating pelvic masses in postmenopausal women, monitoring response to therapy in women with ovarian cancer, and detecting recurrence of this malignancy. Alpha-fetoprotein (AFP) (Johnson, 2001), a marker for hepatocellular carcinoma, sometimes is used to screen highly selected populations and to assess hepatic masses in patients at particular risk for developing hepatic malignancy. Testing for the beta subunit of human chorionic gonadotropin (β-hCG) (Fowler et al., 1982, Bosl et al., 2001) is an integral part of the diagnosis and management of gestational trophoblastic disease. Combined AFP and β-hCG testing is an essential adjunct in the evaluation and treatment of nonseminomatous germ cell tumors, and in monitoring the response to therapy. AFP and β-hCG also may be useful in evaluating potential origins of poorly differentiated metastatic cancer. PSA is used to screen for prostate cancer, detect recurrence of the malignancy, and evaluate specific syndromes of adenocarcinoma of unknown primary.
Table 1.1: Conditions Associated with Elevated Tumor Marker Levels

<table>
<thead>
<tr>
<th>Tumor Marker</th>
<th>Normal Value</th>
<th>Primary Tumor(s)</th>
<th>Additional Associated Malignancies</th>
<th>Benign Conditions</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA 27.29</td>
<td>&lt; 38 units per mL</td>
<td>Breast cancer</td>
<td>Colon, gastric, hepatic, lung, pancreatic, ovarian, and prostate cancers</td>
<td>Breast, liver, and kidney disorders, ovarian cysts</td>
<td>&gt; 100 units per mL</td>
</tr>
<tr>
<td>CEA</td>
<td>&lt; 2.5 ng per mL in nonsmokers &lt; 5 ng per mL in smokers</td>
<td>Colorectal cancer</td>
<td>Breast, lung, gastric, pancreatic, bladder, medullary thyroid, head and neck, cervical, and hepatic cancers, lymphoma, melanoma</td>
<td>Cigarette smoking, peptic ulcer disease, inflammatory bowel disease, pancreatitis, hypothyroidism, cirrhosis, biliary obstruction</td>
<td>&gt; 10 ng per mL</td>
</tr>
<tr>
<td>CA 19-9</td>
<td>&lt; 37 units per mL</td>
<td>Pancreatic cancer, biliary tract cancers</td>
<td>Colon, esophageal, and hepatic cancers</td>
<td>Pancreatitis, biliary disease, cirrhosis</td>
<td>&gt; 1,000 units per mL</td>
</tr>
<tr>
<td>AFP</td>
<td>&lt; 5.4 ng per mL</td>
<td>Hepatocellular carcinoma, nonseminomatous germ cell tumours</td>
<td>Gastric, biliary, and pancreatic cancers</td>
<td>Cirrhosis, viral hepatitis, pregnancy</td>
<td>&gt; 500 ng per mL</td>
</tr>
<tr>
<td>β-hCG</td>
<td>&lt; 5 mIU per mL</td>
<td>Nonseminomatous germ cell tumours, gestational trophoblastic disease</td>
<td>Rarely, gastrointestinal cancers</td>
<td>Hypogonadal states, marijuana use</td>
<td>&gt; 30 mIU per mL7</td>
</tr>
<tr>
<td>CA 125</td>
<td>&lt; 35 units per mL</td>
<td>Ovarian cancer</td>
<td>Endometrial, fallopian tube, breast, lung, esophageal, gastric, hepatic, and pancreatic cancers</td>
<td>Menstruation, pregnancy, fibroids, ovarian cysts, pelvic inflammation, cirrhosis, ascites, pleural and pericardial effusions, endometriosis</td>
<td>&gt; 200 units per mL</td>
</tr>
<tr>
<td>PSA</td>
<td>&lt; 4 ng per mL for screening Undetectable level after radical prostatectomy</td>
<td>Prostate cancer</td>
<td>None</td>
<td>Prostatitis, benign prostatic hypertrophy, prostate trauma, after ejaculation</td>
<td>&gt; 10 ng per mL12</td>
</tr>
</tbody>
</table>

CA = cancer antigen; CEA = carcinoembryonic antigen; AFP = alpha-fetoprotein; β-hCG = beta subunit of human chorionic gonadotropin; PSA = prostate-specific antigen.

Adapted from (Greg et al., 2003)
In accordance with our previous observations of the linear over-expression of H$_{11}$ antigen (HABPs) recognized by IVd4 hybridoma during tumour progression the investigation showed a decline in HA expression by the tumour cells while maintaining its presence in the tumour associated stroma. The discovery of H$_{11}$B$_3$C$_2$ protein is one more addition to the previously observed HA-binding proteins such as IHABP4, CDC37, The significant role of these proteins in vertebrates is well explained (Grammatikakis et al., 1995; Huang et al., 2000). Based on the previous evidence about HA and its receptors, in human cancer tissues, it was hypothesized that sequestered peptides may provide a potentially rich source of cancer-associated markers for clinical evaluation therefore, cancer serum samples are selected to carry out the research program.

In the present study it was decided to highlight the importance of the involvement of HA and HABPs (H$_{11}$ antigen) in human cancer serum.

**The main objectives of the present investigations are:**

- **Studies on screening of HABPs in surgical tissue samples and normal and cancer serum samples using biochemical techniques.**

- **Studies on expression of specific HABP (H$_{11}$ Antigen) in normal and cancer serum samples.**

- **Purification and biochemical characterization of HABP (H$_{11}$ Antigen) in normal and cancer serum samples using biochemical techniques.**