

## Review

# Role of proteases in cancer: A review

Syed Rakashanda, Farukh Rana, Shaista Rafiq, Akbar Masood and Shajrul Amin\*

Department of Biochemistry, University of Kashmir, Srinagar (J&K), 190006, India.

Accepted 19 July, 2012

**Proteases in normal cells are important in carrying out biological processes. In living systems, a balance between proteases and their anti-proteases occur, and disturbance of balance leads to many diseases like cancer. Steps starting from tumor initiation, growth, metastasis and finally invasion into some other site involve all five classes of proteases: serine, cysteine, aspartate, threonine and matrix metalloproteases. The activity of set of peptides in cancer progression is known as cancer 'degradome'. A great number of reports have shown a correlation between the activity of lysosomal cysteine proteases and tumor progression. Trypsin, one of the typical well-known digestive serine protease has also been found to be involved in various cancers and promotes proliferation, invasion and metastasis. The colorectal cancer with trypsin expression has poor prognosis and shorter disease free survival. Protease involvement in cancer suggests the use of protease inhibitors as anticancer drugs. In this review, we will focus on role of proteases in various processes of carcinogenesis and some protease inhibitor based drugs.**

**Key words:** Proteases, protease inhibitors, cathepsins, matrix metalloproteases, threonine proteases, trypsin, tumor.

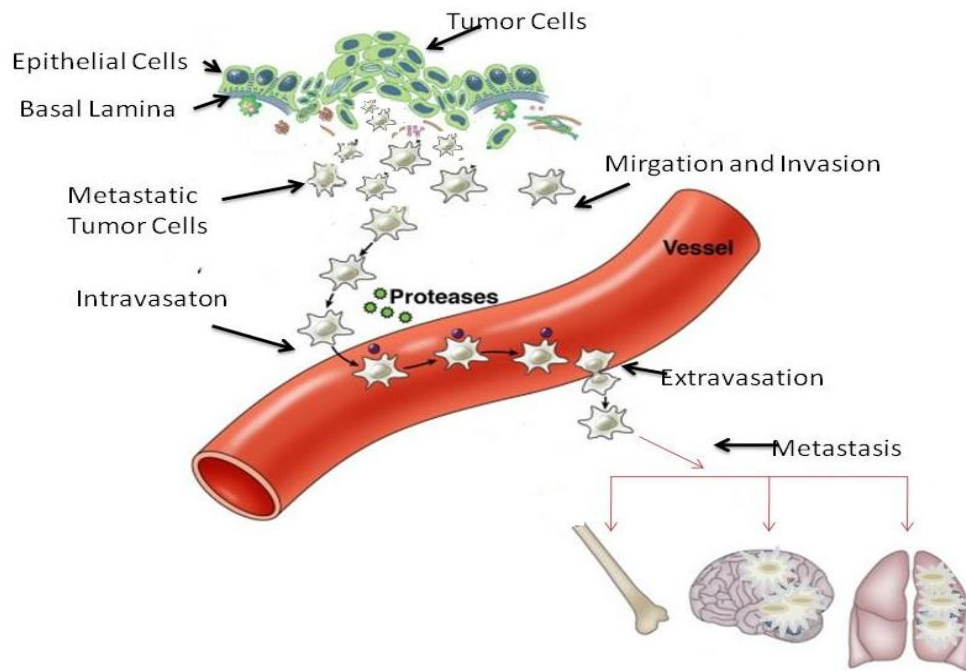
## INTRODUCTION

Proteolysis is one of the most important biological reactions. Proteolytic activity has been attributed to a class of enzymes called proteases. These enzymes are of wide distribution, and they perform significant biological processes. However, recent studies have shown that proteases are also involved in progression, and tumor growth both at primary and metastatic sites (Yang et al., 2009). A positive correlation between the aggressiveness of tumor and the secretion of various proteases has been found. Proteases in normal cells are very important in carrying out important biological processes, but transformed tumor cells are the cause of heavy destruction. The secretion of some specific proteases in tumor cells also make prognosis very difficult. However, proteases are not exclusively

expressed by cancer cells. In many instances, tumor cells induce the expression of proteolytic enzymes in non-neoplastic neighboring cells, hijacking their activity to favour tumor expansion (Zucker et al., 2000).

The recent availability of the genome sequence of different organisms has facilitated the identification of their entire protease repertoire, which has been defined as degradome (Lopez-Otin and Overall, 2002). The human degradome consists of at least 569 proteases and homologs grouped into 5 catalytic classes: 194 metallo, 176 serine, 150 cysteine, 28 threonine and 21 aspartic proteases; but not all of them have been linked with cancer (Punente et al., 2003; Quesada et al., 2009). When the normal cell undergoes multiple alterations and after various generations, the normal cell get transformed, leading to localized tumor and is finally able to invade other tissues and metastasize (Al-Mehdi et al., 2000; Steeg, 2006). Actually, a tumor formation is very complex process and involves many types of changes in the normal cell. Tumor progression involves successive

\*Corresponding author. E-mail: [shajrul@rediffmail.com](mailto:shajrul@rediffmail.com). Tel: +91-9419018174.



**Figure 1.** A general model of dissemination and colonization of tumor cells. Proteinases, may play a major role in this process by direct and indirect activation of other proteinase cascades and related factors.

rounds of mutation and natural selection. Cancer cell develops in slow stages from mildly aberrant cells. Changes like epigenetic changes that occur in normal epithelial cells (NEC) lead to tumor formation and growth.

Epithelial mesenchymal transformations also occur in the tumor cell at some time. Epithelial mesenchymal transition (EMT) involves the disruption of intercellular contacts and the enhancement of cell motility, thereby leading to the release of cells from the parent epithelial tissue. The resulting mesenchyme-like phenotype is suitable for migration and thus, for tumor invasion and dissemination, allowing metastatic progression to proceed. The other requirements for tumor to grow without limits are that the tumor cells must stimulate the development of blood vessels to bring the nutrients and oxygen. Formation of neovessel is stimulated where endothelial cells proliferate and invade towards the tumor site. Tumor vessels grow by various mechanisms:

1. The host vascular network expands by budding of endothelial sprouts or formation of bridges (angiogenesis).
2. Tumor vessels remodel and expand by the insertion of interstitial tissue columns into the lumen of pre-existing vessels (intussusception).
3. Endothelial cell precursors (angioblasts) home from the bone marrow or peripheral blood into tumours and

contribute to the endothelial lining of tumour vessels (vasculogenesis).

The tumor cells must arrive in circulation, arrest, extravasate, and invade the local environment and grow to set up distant metastasis (Figure 1). These metastasis steps occur through the interactions of tumor cell (TC), endothelial cell (EC), fibroblasts and invading inflammatory cells (IC), such as macrophages and the extracellular matrix (Koblinki et al., 2000; Xu et al., 2010). Macrophages play a dual role in tumor growth and angiogenesis (Chrystelle, 2006). Tumor-associated macrophages contribute to tumor progression by secreting growth factors instead of generating immune responses against them. They also participate in tumor progression by acting on endothelial cells and promoting the neovascularisation of the tumor.

In all these steps, starting from tumor initiation, growth and metastasis and finally invasion into some other site involve all five classes of proteases, that is, serine, cysteine, aspartic, threonine and matrix metalloproteases (Chambers et al., 1997; Lochter et al., 1997; Kim et al., 1998; Mook et al., 2004; Zhang et al., 2010; Deu et al., 2012). Studies have also shown that these enzymes target diverse substrates and regulate many processes that are essential for cell life and death in all organisms (Lopez-Otin and Hunter, 2010), which promotes tumor evolution, a fact that significantly altered the traditional

view of the role of proteases in tumor growth and progression (Lopez-Otin et al., 2007; Lopez-Otin and Bond, 2008; Mason and Joyce, 2011).

## PROTEASES INVOLVED IN THE TUMOR GROWTH AND METASTASIS

The activity of set of peptides (proteases) involved in cancer progression is collectively known as the cancer 'degredom'. Invasion and metastasis were initially considered as late event in cancer development and the process in which proteases were involved. However, studies have indicated that invasion and metastasis are not late events, but can occur during early stages as well. Moreover, other processes occurring in various stages of cancer progression are also protease dependent, such as (up regulation of) cell proliferation, (down regulation of) apoptosis, involvement of white blood cells, angiogenesis and induction of multi-drug resistance. Proteolytic activity in tumors is regulated in a complex manner, as both genetically unstable cells and stromal cells, such as fibroblasts, endothelial cells and inflammatory cells are involved. *In vitro* studies and studies using animal models have clearly shown protease dependency of many processes in carcinogenesis.

### Cysteine proteases

Mammalian cysteine proteases form a diverse group of proteolytic enzymes characterized by an active site cysteine residue. They can be localized in the lysosome (cathepsins B, L, H and S) or the cytosol (calpains), and are secreted in some cell types under pathological conditions. Cysteine proteases mediate general functions such as intracellular protein catabolism and specialized functions such as selective activation of signalling molecules (interleukin, enkephalin, protein kinase C) or extracellular protein degradation (bone resorption, macrophage function). A great number of reports have shown a correlation between the activity of lysosomal cysteine proteases and tumor progression. The family of cathepsin cysteine proteases can degrade both intracellular and extracellular matrix (ECM) proteins (Jedezsko and Sloane, 2004; Joyce et al., 2004; Mohamed and Sloane, 2006; Gocheva and Joyce, 2007). The action of cathepsins is regulated by the equilibrium between their endogenous inhibitors and activation of their inactive forms (Abrahamson et al., 2003). Cathepsins have been shown to function intracellularly as well as extracellularly, which puts them in a unique position and contrasts them with most other proteases such as metalloproteases or serine proteases. The extracellular activity of cathepsins allows cancer cells to

attack nearby tissues, blood and Lymph vessels and metastasize to outlying tissues (Vasiljeva and Turk, 2008; Matarrese et al., 2010). Consequently, cathepsins are considered to be promising targets for anti-cancer therapy (Jedezsko and Sloane, 2004; Gocheva et al., 2007).

Cathepsin B was the first lysosomal protease to be associated with breast carcinoma (Poole et al., 1978). Early reports indicating a link between cathepsin B and cancer, showed that the protease is released from malignant human breast tumor explants and is found in the serum of patients with neoplastic vaginal lesions. Cathepsin B has been actually shown to participate in the dissolution and remodeling of connective tissue and basement membrane in the processes of tumor growth, invasion, and metastasis (Schmitt et al., 1992; Sloane et al., 1994; Joyce and Hanahan, 2004) by podosome-mediated extracellular matrix degradation and invasion via secreted lysosomes (Tu et al., 2008). Increased levels of cathepsins B and L in tumors or in some extracellular fluids are associated with the disease-free and overall survival periods and may therefore serve as prognostic factors for cancer patients (Duffy, 1996; Kos and Lah, 1998). In addition, cathepsins are useful markers for identifying patients who are suffering from breast cancer (Kandalaf et al., 1993), colorectal cancer (Hirai et al., 1999), tongue carcinoma (Saleh et al., 2006) and pancreatic cancer (Michl, 2011). Kawasaki et al. (2002) have reported that the expression of cathepsins D and B was closely correlated with cancer invasion and progression of oral squamous cell carcinoma. Cathepsins B and L are more frequently overexpressed in chronic atrophic gastritis with dysplasia. Cathepsin B protein is also frequently overexpressed in laryngeal carcinoma (Macabeo-Ong et al., 2003). Also, the involvement of cathepsin in regulation of angiogenesis reveals another distinct role in tumor progression (Joyce et al., 2004).

Cathepsins can be regulated by the endogenous cysteine protease inhibitor, named cystatin, in normal tissues and cells (Turk and Bode, 1991; Turk et al., 1997). Cystatins are a group of reversible, tight-binding competitive inhibitors for cysteine peptidases such as cathepsins B, H, and L (Henskens et al., 1996). It was suggested that cysteine protease inhibitors can play a role in cancer, which are associated with alterations of the proteolytic system (Turk et al., 1997). Recent studies have shown cystatins can block invasion or metastasis of different cancers in experimental systems (Cox, 2009). Cystatin C inhibits motility and *in vitro* invasiveness of cancer cells, and can associate with the maintenance of cell differentiation (Troy et al., 2004). In inflammatory conditions or conditions with tissue breakdown, cystatin C free in the blood or other body fluid inhibits cysteine peptidases and thereby prevents tissue damage. The activity and concentration of cystatin seem varies in

different cancer tissues, but its interactions with cathepsin B has been widely investigated (Sokal and Schlemann, 2004). The incomparable levels between cathepsin B and its natural inhibitors suggest that it may contribute to the uncontrolled proteolysis and thus the malignant progression of tongue cancer (Saleh et al., 2006).

Elevated levels of other lysosomal proteases, such as cathepsins H, L, or D, have also been reported in many cancer types (Fujise et al., 2000). Cathepsin L2 (CTSL2) has been reported to be upregulated in a variety of malignancies like breast, lung, gastric, colon, head and neck carcinomas, melanomas, and gliomas (Lankelma et al., 2010) and also in endometrial cancer (Skrzypczak et al., 2012).

### Serine proteases

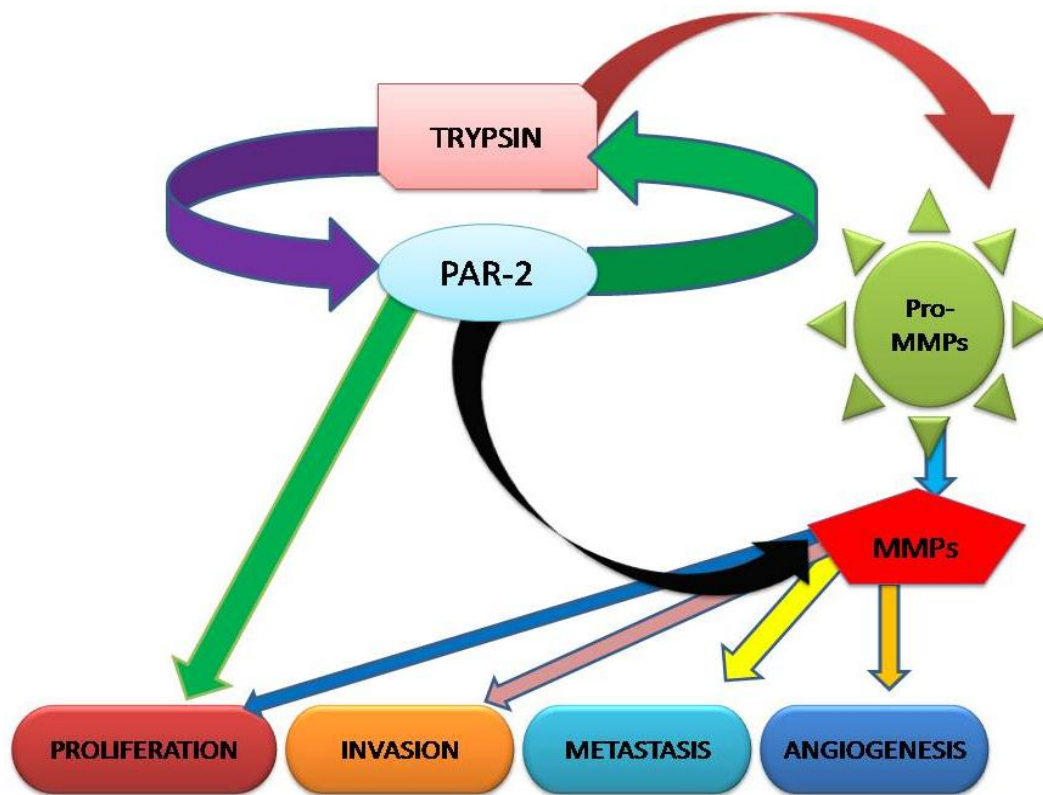
Serine proteases form a group of proteases which have close relationship with cell growth and differentiation. They often exist as zymogens and are activated by specific and limited proteolysis, which in turn regulate the enzyme activities. There also exist as physiological inhibitors inside cells, which regulate their activities. Normal regulation of serine protease activities is essential for physiological activities of the cell, and abnormal regulation of these protease activities can lead to pathological conditions. Urokinase-type plasminogen activators are one kind of serine proteases that have been well investigated for their relationship with tumor invasion and metastasis (Henneke et al., 2010). A number of studies have shown that their expression and enzyme activity regulation are closely related to malignant phenotype of tumors. Matriptase, a type II transmembrane serine protease is involved in angiogenesis, degradation of extracellular matrix, and in the progression of some epithelial cancers (Nakamura et al., 2009). But in normal cells, it is inhibited by hepatocyte growth factor activator inhibitor-1(HAI-1). During the progression of human prostate cancer (CaP), there is expression of matriptase and loss of HAI-1 which may be an important event. It has been suggested that the ratio of these two gene products may serve as a promising biomarker for CaP progression and a potential marker for establishing the efficacy of therapeutic and chemopreventive interventions (Saleem et al., 2006).

Trypsin is one of the best characterized serine proteases. These proteases play essential roles not only in many physiological processes (e.g. food digestion, blood coagulation, fibrinolysis, and control of blood pressure) but also in a wide range of important pathological processes e.g. atherosclerosis, inflammation and cancer (Borg, 2004). Previously, trypsin was known as a digestive enzyme produced primarily by pancreatic acinar cells. However, the presence of trypsin in patients

who had undergone pancreatectomy led to investigation of production in other sites in the human body (Itkonen et al., 1996). Currently, trypsin expression has been documented in epithelial cells of skin, esophagus, stomach, small intestine, colon, lung, kidney, liver, bile ducts, as well as in leukocytes, and splenic and neuronal cells (Koshikawa et al., 1998). Four different trypsinogen isoforms have been characterized in humans: trypsinogen-1, trypsinogen-2, trypsinogen-3 (found in various epithelial tissues), and trypsinogen-4 (found in the brain) (Wiegand et al., 1993). The different trypsinogens show great homology (>90%) at both nucleotide and protein levels.

Trypsin exhibits selective proteolytic activity against the peptide bonds in protein molecules that have carboxyl groups donated by the amino acids arginine and lysine. For physiological protection against premature activity, as known from pancreatic physiology, trypsin is secreted as an inactive zymogen (trypsinogen) in the pancreatic juice, and is activated by conversion to trypsin by an enteropeptidase in the alkaline milieu of the duodenal lumen. Secondly, trypsinogen may be activated into active trypsin by an enteropeptidase found in duodenal enterocytes (Imamura and Kitamoto, 2003). Interestingly, adenocarcinoma cells of the duodenum (Imamura et al., 2003), as well as other tissues expressing trypsin, have a trypsin-activating enteropeptidase (Miyata et al., 1999). Also, the antiprotease mediator pancreatic secretory trypsin inhibitor (PSTI) protects from premature activity. An imbalance in this 'protease-antiprotease-system' system plays a pathophysiological role in the development of pancreatitis (O'Keefe et al., 2005), and seems to pose an increased risk for developing pancreatic adenocarcinoma (Howes et al., 2004). Pancreatic secretory trypsin inhibitor (PSTI) is excreted by the mucosa of the normal gastrointestinal tract, where it serves to protect the cells from proteolytic breakdown. The same peptide is secreted by tumour cells, and is often referred to as 'tumour-associated trypsin inhibitor' (TATI), which is identical to PSTI (Stenman et al., 1991).

Trypsin is involved in colorectal carcinogenesis and promotes proliferation, invasion, and metastasis (Yamamoto et al., 2003; Soreide et al., 2006). Moreover, colorectal cancers with trypsin expression have a poor prognosis and shorter disease-free survival. Biological understanding of how trypsin causes cancer progression is emerging. It seems to act both directly and indirectly through a 'protease-antiprotease-system', and by activation of other protease cascades. Invasion of the basal membrane by cancer cells may be promoted directly by trypsin digestion of type I collagen. Trypsin activates, and is co-expressed with matrix metalloproteases (MMPs), which are known to facilitate invasion and metastasis (Nyberg et al., 2002). MMP-2, MMP-7, and MMP-9 are co-expressed together with



**Figure 2.** A model showing interaction of trypsin with proteinase-activated receptor 2 (PAR-2) and the matrix metalloproteinases (MMPs).

trypsin and seem to be of particular importance in proliferation, progression, and invasion. MMPs may play a role in both conversion from adenoma to carcinoma, and in the initiation of invasion and metastasis. Co-segregation of trypsin and MMPs within the tumour environment is important for the activation of MMPs, and may explain the deleterious effect of trypsin on prognosis in colorectal cancer. Trypsin and protease-activated receptor 2 (PAR-2) act together in an autocrine loop that promotes proliferation, invasion, and metastasis through various mechanisms, of which prostaglandin synthesis is important (Jahan et al., 2008; Han et al., 2011; Ramachandran et al., 2012; Suen et al., 2012) (Figure 2). PAR-2 activation, after site-specific proteolysis of the N-terminus by trypsin and presentation of the tethered ligand sequence (SLIGRL in mice) to extracellular domains of the receptor, suggested participation in tissue growth and differentiation, regeneration and repair, inflammatory response regulation and also in malignant transformation (Hansen et al., 2008; Adams et al., 2011).

The role of environmental body rate of proteases (including trypsin and trypsin like ones) and anti-proteases, resulting in a "certain" level of proteolytic activity influencing PAR-2 relative to tumour cells, has

also been investigated in an *in vitro* model of breast cancer (Matej et al., 2007). Stimulated by trypsin, both MMP and PAR-2 may activate the mitogenic MAPK-ERK pathway through activation of the epidermal growth factor receptor (Darmoul et al., 2004; Hirota et al., 2012). Experimental trypsin inhibition is feasible but not very effective, and trypsin as a target for clinical therapy is unlikely to be successful, owing to its universal distribution. However, as the pathways of trypsin and co-activated protein cascades emerge, biological understanding of colorectal carcinogenesis can be further elucidated and may pave the way for prognosticators, predictors, and novel targets of therapy. Likewise, the biological role of trypsin and its interactions may be a subject for investigation for the development of future (preventive) cancer therapies.

### Aspartate proteases

Aspartic proteases form a group of enzymes that consist of two lobes separated by a cleft containing the catalytic site made up of two aspartate residues. Cathepsin-D (Cath-D) is an aspartic endo-protease that is ubiquitously

distributed in lysosomes (Barrette and Cathepsin, 1970). It was considered for a long time that the main function of cath-D was to degrade proteins in lysosomes at an acidic pH. In addition to its classical role as a major protein-degrading enzyme in lysosomes and phagosomes, it has been shown that cath-D can also activate precursors of biologically active proteins in pre-lysosomal compartments of specialized cells (Diment et al., 1989). However, Cathepsin D has been studied over the last three decades, mainly from the perspective of its role in cancer development and as a suggested independent tumor marker. This research has also impacted the specification of Cath-D's physiological role and helped to discover new functionalities of Cath-D.

The aspartic protease cathepsin D (cath-D), a marker of poor prognosis in breast cancer (Westley and May, 1999; Rodriguez et al., 2005), is overexpressed and secreted at high levels by human epithelial breast cancer cells (Vashishta et al., 2009; Nicotra et al., 2010; Radiskt et al., 2010; Masson et al., 2011). Cath-D stimulates cancer cell proliferation, fibroblast outgrowth, angiogenesis and metastasis (Vashishta et al., 2007; Hu et al., 2008; Ohri et al., 2008). The direct role of cath-D in cancer metastasis was first demonstrated in rat tumor cells in which transfection-induced cath-D overexpression increased their metastatic potential *in vivo* (Liaudet et al., 1994; 2006). In that rat tumor model, the cath-D mechanism responsible for metastasis stimulation seemed to have a positive effect on cell proliferation, favouring the growth of micro-metastases, rather than increasing the invasive potential (Berchem et al., 2002).

Using an RNA antisense strategy, it was shown that cath-D was a rate limiting factor in the outgrowth, tumorigenicity and lung colonization of MDA-MB-231 breast cancer cells (Glondou et al., 2002). Procathepsin D (pCD), secreted from cancer cells, can act as a mitogen on both cancer and stromal cells and thus stimulates their pro-invasive and pro-metastatic properties. Numerous studies done showed that pCD/CD level represents an independent prognostic factor in a variety of cancers and is therefore under consideration as potential target of anti-cancer therapy. Studies dealing with functions of cathepsin D were complicated by the fact that there were several simultaneous forms of CD in a cell – pCD, intermediate enzymatically active CD and mature heavy and light chain CD. It thus became evident that these forms may differently regulate the aforementioned processes (Benes et al., 2008; Vetvicka et al., 2010). Other numerous studies also demonstrated that pCD secreted from cancer cells affects multiple stages of tumor progression, and inhibition of pCD secretion from cancer cells can inhibit cancer cell growth *in vitro* and *in vivo*, thus suggesting the possibility of using pCD suppression in clinical practice (Vetvicka and Fusek, 2012).

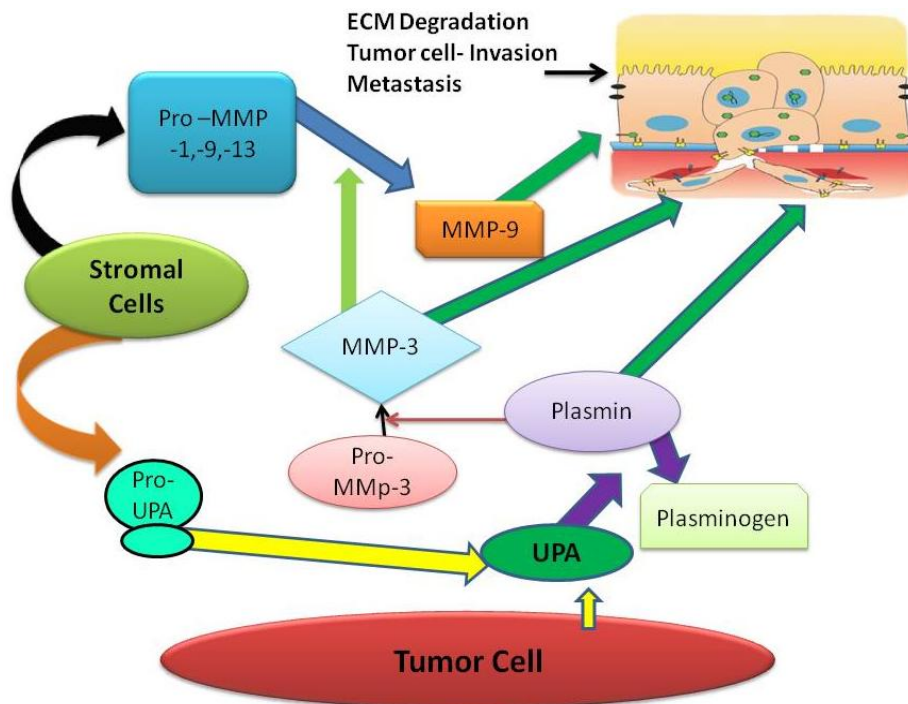
## Threonine proteases

Threonine proteases (proteasomes) have the task of eliminating cellular proteins, tagged for degradation through a complex modification termed polyubiquitination. It is a process of addition of a series of ubiquitin molecules to another protein, targeted for degradation (Mitchell, 2003). The 26S proteasome is a multicatalytic threonine protease with three distinct catalytic activities. It is responsible for intracellular protein turnover in eukaryotic cells, including the processing and degradation of short- and some long-living proteins required for regulation of various cellular functions ( Ciechanover, 1994; Hochstrasser, 1995; Orłowski and Dees, 2003; Adams, 2004). Since aberrant proteasome-dependent proteolysis appears to be associated with the pathophysiology of some malignancies, it was suggested that pharmacological inhibition of proteasome function may prove useful as a novel class of anticancer drugs. Thus, targeting key features of protein function responsible for the growth and progression of cancer has been the subject of intense investigation by many groups for drug discovery purposes.

Proteasome inhibition leads to the accumulation of pro-apoptotic proteins in tumorigenic cells but not normal tissue (Berenson et al., 2006; Kane et al., 2006). Bortezomib (Velcade, Millennium Pharmaceuticals, Inc., Cambridge, MA, and Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ) was the first proteasome inhibitor approved by the US FDA for the treatment of newly diagnosed multiple myeloma and relapsed/refractory multiple myeloma and mantle cell lymphoma (Kane et al., 2003; Zavrski et al., 2005; Kane et al., 2007; Chen et al., 2011). Although the mechanisms of its anticancer activity by proteasome inhibition are not fully elucidated, it is clear that multiple mechanisms are involved. Proteasome inhibition can promote degradation of anti-apoptotic proteins and prevent degradation of pro-apoptotic proteins, resulting in programmed cell death in malignant cells.

## Matrix metalloproteases

Matrix metalloproteases (MMPs) are a family of nine or more highly homologous  $Zn^{2+}$  endopeptidase that collectively cleave most of, if not all, the constituents of the extracellular matrix. The activity levels of proteases and MMPs are tightly controlled. This is logical since rampant proteolysis would not be efficient way to maintain homeostasis. In diseases setting, however, the expression levels of individual protease as well as number of different expressed proteases increases. MMP expression is raised in multiple tumor types, and mostly, the increase correlate with decreased survival. MMPs are



**Figure 3.** A representation of the role of matrix metalloproteinases in ECM (extracellular matrix) degradation, invasion and metastasis.

responsible for the turnover and remodeling of extracellular matrix proteins. Substrates for the enzymes range from the fibrillar collagens of bone, skin and interstitial, to the non-fibrillar collagens, laminina. As might be expected for enzymes with such degradative potential, the activity of MMPs is highly controlled. In addition to control through regulation of gene expression, matrix metalloproteases are secreted as latent pro-enzymes, requiring modification or removal of a 10 KDa amino terminal domain for the expression of enzyme activity (Kleiner and Stetler-Stevenson, 1993; Lijnen, 2001). Once activated, MMPs may be inhibited by general protease inhibitors, such as  $\alpha$ 2- macroglobulin, or by one of the group of specific inhibitors known as tissue inhibitor of metalloproteases [TIMPS] (Brew et al., 2000; Lambert et al., 2004; Deryugina and Quigley, 2006; Clark et al., 2007; Radisky and Radisky, 2010). This tight regulation of enzyme activity is essential in physiological situations where extensive extracellular remodeling must follow a well programmed course, such as morphogenesis or wound healing (Bullen, 1995).

The complexity of proteolytic systems operating in human tissues is impressive, as assessed by the finding that more than 500 genes encoding proteases or protease-like proteins are present in the human genome (Puente et al., 2003). However, among all the proteolytic enzymes potentially associated with tumour invasion, the

members of the MMP family have reached an outstanding importance due to their ability to cleave virtually any component of the ECM and basement membranes, thereby allowing cancer cells to penetrate and infiltrate the subjacent stromal matrix (Brinckerhoff and Matrisian, 2002; Bertucci and Birnbaum, 2009; Jemal et al., 2010). Over the last years, the relevance of the matrix metalloprotease (MMP) family in cancer research has grown considerably. These enzymes were initially associated with the invasive properties of tumour cells, owing to their ability to degrade all major protein components of the extracellular matrix (ECM) and basement membranes. Moreover, further studies have demonstrated the implication of MMPs in early steps of tumour evolution, including stimulation of cell proliferation and modulation of angiogenesis (Folgueras et al., 2004). MMPs allows local expansion of the tumor mass through the disruption of normal tissue structure and facilitates invasion of blood vessels and lymphatics by metastatic cells, thus promoting tumor spread.

Matrix metalloprotease secretion and activation seems to result from a specific interaction between tumour and stromal cells (Nielsen et al., 2001; De Wever and Mareel, 2003) (Figure 3). The breakdown of tissue architecture mediated by these activated enzymes allows the primary tumour to expand, invade the neighbouring blood vessels and spread to distant sites in the body. Invasive growth in

these secondary sites also appears to be facilitated by the action of matrix metalloproteases (Bonfil and Cher, 2011). MMP induction mechanisms appear to be different depending on the characteristics of the diverse cells, with ability to produce these enzymes. A wide variety of agents, including cytokines, growth factors and oncogene products, cause spatial and temporal variations of MMP expression (Westermarck and Kahari, 1999). Nevertheless, TNF- $\alpha$  (Tumor necrosis factor-  $\alpha$ ) and IL-1 (Interleukin-1) are regularly implicated in MMP gene induction in different tumours, whereas TGF- $\beta$  (Transforming growth factor-  $\beta$ ) or retinoids usually repress MMP transcription. However, there are several exceptions to this situation, since some family members such as Mmp11 or Mmp13 can be induced rather than repressed by these factors in diverse cell types (Farina et al., 1998; Kim et al., 2004; Munshi et al., 2004; Zhong et al., 2006; Lee et al., 2008; Sun et al., 2008; Kuo et al., 2009; Hsieh et al., 2010; Wiercinska et al., 2010). Also, possibilities of finding similarities among the signal-transduction pathways mediated induction of different MMPs, has been tried. And, it was found that the ERK and the p38 mitogen activated protein kinase pathways were relevant in a number of cases (Pan and Hung, 2002; Reunanen et al., 2002; Ruhul Amin et al., 2003; Tanimura et al., 2003; Giehl et al., 2007; Gomes et al., 2012).

Matrix metalloprotease inhibitors (MMPI) can inhibit the breakdown of extracellular matrix in the areas of proteolysis, and arrest tumour growth and metastasis. The potential application of TIMPs to block the MMP activity in cancer was initially supported by several studies demonstrating their ability to inhibit tumour growth in transgenic mouse models (Kruger et al., 1997; Martin et al., 1999). However, the possibility of using TIMPs in cancer therapy has technical difficulties, as it happens with other macromolecules (Overall and Lopez-Otin, 2002) which highlight the need for developing synthetic MMPIs that selectively target specific MMPs. The first series of synthetic inhibitors were pseudopeptides mimicking the cleavage sites of MMP substrates. Thus, Batimastat (BB-94), a broad-spectrum hydroxamate-based inhibitor, became the first MMPI to be tested in humans (Wojtowicz-Praga et al., 1996). However, clinical trials with intraperitoneally administered Batimastat did not show any significant responses, and it was replaced by Marimastat (BB-2516), another peptido-mimetic MMPI, but orally available. Marimastat inhibits the activity of many MMPs including MMP-1, -2, -3, -7, -9, -12, and -13. The number of distinct enzymes that this MMPI can target explains the musculoskeletal pain detected in patients after a sustained treatment with Marimastat (Nemunaitis et al., 1998). Despite this limitation, Marimastat is as effective as conventional therapy (gemcitabine) in the treatment of pancreatic carcinoma

patients (Bramhall et al., 2001). Furthermore, this inhibitor in combination with temozolomide, improved survival in patients with glioblastoma multiforme (Groves et al., 2002). Lastly, Marimastat increased the survival and time to disease progression in patients with advanced gastric cancer (Bramhall et al., 2002).

Recently, new series of non-peptidomimetics MMPIs with increased specificity and oral bioavailability and based on the 3D structure of MMP zinc-binding sites have been synthesized. Among them, BMS-275291 has special interest due to lack of musculoskeletal side effects, and it was evaluated in advanced lung cancer, prostate cancer and AIDS-related Kaposi's sarcoma (Lockhart et al., 2003). In addition, non-peptidic substances with inhibitory properties against MMPs, including tetracycline derivatives and bisphosphonates are being tested in clinical trials (Falardeau et al., 2001; Cianfrocca et al., 2002; Lacerna and Hohneker, 2003). In brief, despite initial problems with MMPIs, the stimulating results obtained with Marimastat on matrix metalloproteases in cancer are a proof of principle on the clinical value of these compounds for future cancer treatment.

## CONCLUSION

Numerous practical applications in the area of cancer research and the understanding that proteases are important targets for the drug design ultimately fuelled much research in this field. Presently, we know that increased expression, increased activity and altered localization of many proteases of all the five classes are associated with tumor progression and even secretion of some specific proteases in tumor cells makes prognosis very difficult. Cysteine proteases like cathepsin B participates in dissolution and remodelling of connective tissue and basement membrane in the process of tumor growth, invasion and metastasis. The redistribution of cathepsin B within tumor cells as well as the increased expression in tumor cells adjacent to the extracellular matrix suggests that proteases can be mobilized to regions of tumor cell invasion. Cathepsin D, an aspartate protease also plays role in cancer development. But among all these proteolytic events, the MMP family members have reached an outstanding importance because of cleaving virtually any component of ECM and basement membrane, thus allowing cancer cells to penetrate and infiltrate the subjacent stromal cells. Thus, involvement of proteases in cancer suggests the use of protease inhibitors (PIs) that can reduce the invasive and metastatic capabilities of tumor cells. The effect of protease inhibitors on tumor invasion could be direct due to inhibition of extracellular matrix proteolysis or indirect due to inhibition of activation of a proteolytic cascade. But the concept of using PIs is not so simple, as we know



that tumor cells are only one part of the tumor environment, extracellular matrix components and stromal cells are important contributors to the proteolytic activity of tumors.

Therefore, the need of the hour is the extensive study of proteases and their PIs to make target specific PI drugs for clinical use. Specific protease inhibitors, likely in combination with conventional anticancer agent will probably prove to have value for certain forms of cancer.

## ACKNOWLEDGEMENTS

The authors thank the contributing members of staff of the Department of Biochemistry, University of Kashmir, Srinagar, India, for their fruitful discussions and suggestions during the preparation of this review. The financial support from University of Kashmir, Srinagar, India is also acknowledged.

## REFERENCES

- Abrahamson M, Alvarez-Fernandez M, Nathanson CM (2003). Cystatins. *Biochem. Soc. Symp.* 70:179-199.
- Adams J (2004). The proteasome: a suitable antineoplastic target. *Nat. Rev. Cancer.* 4:349-360.
- Adams MN, Ramachandran R, Yau MK, Suen JY, Fairlie DP, Hollenberg MD, Hooper JD (2011). Structure, function and pathophysiology of protease activated receptors. *Pharmacol. Ther.* 130:248-282.
- Al-Mehdi AB, Tozawa K, Fisher AB, Shientag L, Lee A, Muschel RJ (2000). "Intravascular origin of metastasis from the proliferation of endothelium-attached tumor cells: a new model for metastasis." *Nature. Med.* 6:100-102.
- Barrett AJ, Cathepsin D (1970). Purification of isoenzymes from human and chicken liver. *Biochem. J.* 117:601-607.
- Benes P, Vetvicka V, Martin F (2008). Cathepsin D – many functions of one aspartate protease. *Crit. Rev. Oncol. Hematol.* 68(1):12-28.
- Berchem GJ, Glondu M, Gleizes M, Brouillet JP, Garcia M, Liaudet CE (2002). Cathepsin-D affects multiple steps of tumor progression: Proliferation, angiogenesis and apoptosis. *Oncogene* 51:5951-5955.
- Berenson JR, Yang HH, Sadler K, Jarutirasarn SG, Vescio RA, Mapes R, Purner M, Lee SP, Wilson J, Morrison B, Adams J, Schenkein D, Swift R (2006). Phase I/II trial assessing bortezomib and melphalan combination therapy for the treatment of patients with relapsed or refractory multiple myeloma. *J. Clin. Oncol.* 24:937-944.
- Bertucci F, Birnbaum D (2009). Distant metastasis: not out of reach any more. *J. Biol.* 8:28.
- Bonfil DR, Cher ML (2011). The Role of Proteolytic Enzymes in Metastatic Bone Disease. *IBMS BoneKey* 8(1):16-36.
- Borg TK (2004). It's the matrix! ECM, proteases, and cancer. *Am. J. Pathol.* 164:1141-1142
- Bramhall SR, Hallissey MT, Whiting J, Scholefield J, Tierney G, Stuart RC, Hawkins RE, McCulloch P, Maughan T, Brown PD, Baillet M and Fielding JW (2002). Marimastat as maintenance therapy for patients with advanced gastric cancer: a randomised trial. *Br. J. Cancer* 86:1864-70.
- Bramhall SR, Rosemurgy A, Brown PD, Bowery C, Buckels JA (2001). Marimastat as first-line therapy for patients with unresectable pancreatic cancer: a randomized trial. *J. Clin. Oncol.* 19:3447-3455.
- Brew K, Dinakarandian D, Nagase H (2000). Tissue inhibitors of metalloproteinases: evolution, structure and function. *Biochim. Biophys. Acta.* 1477:267-283.
- Brinckerhoff CE, Matrisian LM (2002). Matrix metalloproteinases: a tail of a frog that became a prince. *Nat. Rev. Mol. Cell Biol.* 3:207-214.
- Bullen EC, Longaker MT, Updike DL et al. (1995). Tissue inhibitor of metalloproteinases-1 is decreased and activated gelatinases are increased in chronic wounds. *J. Invest. Dermatol.* 104:236-240
- Chambers AF, Matrisian LM (1997). Changing views of the role of matrix metalloproteinases in metastasis. *J. Natl. Cancer Inst.* 89:1260-1270.
- Chen D, Frezza M, Schmitt S, Kanwar J, Dou QP (2011). Bortezomib as the First Proteasome Inhibitor Anticancer Drug: Current Status and Future Perspectives. *Curr. Cancer Drug Targets* 11(3):239-253.
- Chrystelle L, Michel A, Beat A (2006). Dual role of macrophages in tumor growth and angiogenesis. *J. Leukocyte Biol.* 80:705-713.
- Cianfrocca M, Cooley TP, Lee JY, Rudek MA, Ssadden DT, Ratner L, Pluda JM, Figg WD, Krown SE, Dezube BJ (2002). Matrix metalloproteinase inhibitor COL-3 in the treatment of AIDS-related Kaposi's sarcoma: a phase I AIDS malignancy consortium study. *J. Clin. Oncol.* 20:153-9.
- Ciechanover A (1994). The ubiquitin-proteasome proteolytic pathway. *Cell* 79:13-21.
- Clark JC, Thomas DM, Choong PF, Dass CR (2007). RECK-a newly discovered inhibitor of metastasis with prognostic significance in multiple forms of cancer. *Cancer Metastasis Rev.* 26:675-683.
- Cox JL (2009). Cystatins and cancer. *Front Biosci.* 1:463-474
- Darmoul D, Valerie G, Devaud H, Laburthe M (2004). Protease-activated Receptor 2 in Colon Cancer. *J. Biological. Chem.* 279(20):20927-20934.
- De Wever O, Mareel M (2003). Role of tissue stroma in cancer cell invasion. *J. Pathol.* 200:429-447.
- Deryugina EI, Quigley JP (2006). Matrix metalloproteinases and tumor metastasis. *Cancer Metastasis Rev.* 25:9-34.
- Deu E, Martijn V, Matthew B (2012). New approaches for dissecting protease functions to improve probe development and drug discovery. *Nature Structural Mole. Biol.* 19(1):10-16.
- Diment S, Martin KJ, Stahl PD (1989). Cleavage of parathyroid hormone in macrophage endosomes illustrates a novel pathway for intracellular processing of proteins. *J. Biol. Chem.* 264:13403-13406.
- Duffy MJ (1996). Proteases as prognostic markers in cancer. *Clin. Cancer. Res.* 2:613-618.
- Falardeau P, Champagne P, Poyet P, Hariton C, Dupont E (2001). Neovastat, a naturally occurring multifunctional antiangiogenic drug, in phase III clinical trials. *Semin. Oncol.* 28:620-625.
- Farina AR, Coppa A, Tiberio A, Tacconelli A, Turco A, Colletta G, Gulino A, Mackay AR (1998). Transforming growth factor-beta1 enhances the invasiveness of human MDA-MB-231 breast cancer cells by up-regulating urokinase activity. *Int. J. Cancer* 75:721-730.
- Folgueras AR, Pendas AM, Sanchez LM, Lopez-otin C (2004). Matrix metalloproteinases in cancer: from new functions to improved inhibition strategies. *Int. J. Dev. Biol.* 48:411-424.
- Fujise N, Nanashima A, Taniguchi Y, Matsuo S, Hatano Matsumoto Y, Tagawas Y, Ayabe H (2000). Prognostic impact of cathepsin B and matrix metalloproteinase-9 in pulmonary adenocarcinoma by immunohistochemical study. *Lung Cancer* 27:19-26
- Giehl K, Imamichi Y, Menke A (2007). Smad4-independent TGF-beta signaling in tumor cell migration. *Cells Tissues Organs.* 185:123-130.
- Glondu M, Liaudet-Coopman E, Derocq D, Platet N, Rochefort H, and Garcia M (2002). Down-regulation of cathepsin-D by antisense gene transfer inhibits tumor growth and metastatic potential of human breast cancer cells. *Oncogene* 21:5127-5134.
- Gocheva V, Joyce JA (2007). Cysteine cathepsins and the cutting edge of cancer invasion. *Cell Cycle* 1:60-64.
- Gomes RL, Leticia FT, Rosangela AMW, Leticia L, Mari CS (2012). TGF-b1 modulates the homeostasis between MMPs and MMP inhibitors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells. *BMC Cancer* 12:26.
- Groves MD, Puduvali VK, Hess KR, Jaecle KA, Peterson P, Yung WK, Levin VA (2002). Phase II trial of temozolomide plus the matrix metalloproteinase inhibitor, marimastat, in recurrent and progressive glioblastoma multiforme. *J. Clin. Oncol.* 20:1383-1388.

- Hansen KK, Oikonomopoulou K, Baruch, Ramachandran R, Beck P, Diamandis EP, Hollenberg MD (2008). Proteinases as hormones: targets and mechanisms for proteolytic signaling. *Biol. Chem.* 389:971-982.
- Henneke I, Greschus S, Savai R, Korfei M, Markart P, Mahavadi P, Ralph T, Schermuly RT, Wygrecka M, Jo R, Seeger W, Gunther A, Ruppert C (2010). Inhibition of Urokinase Activity Reduces Primary Tumor Growth and Metastasis Formation in a Murine Lung Carcinoma Model. *Am. J. Respir. Crit. Care Med.* 181:611-619.
- Henskens YMC, Veerman ECJ, Amerongen AVN (1996). Cystatins in health and disease. *Biol. Chem.* 377:71-86.
- Hirai K, Yokoyama M, Asano G, Tanaka S (1999). Expression of cathepsin B and cystatin C in human colorectal cancer. *Hum. Pathol.* 30:680-686.
- Hirota CL, Moreau F, Iablokov V, Dickey M, Renaux B, Hollenberg MD, MacNaughton WK (2012). Epidermal growth factor receptor transactivation is required for proteinase-activated receptor-2-induced COX-2 expression in intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.*, doi:10.1152/ajpgi.00358.2011.
- Hochstrasser M (1995). Ubiquitin, proteasomes, and the regulation of intracellular protein degradation. *Curr. Opin. Cell Biol.* 7:215-223.
- Howes N, Greenhalf W, Stocken DD, Neoptolemos JP (2004). Cationic trypsinogen mutations and pancreatitis. *Gastroenterol. Clin. North Am.* 33:767-787
- Hsieh HL, Wang HH, Wu WB, Chu PJ, Yang CM (2010). Transforming growth factor-beta1 induces matrix metalloproteinase-9 and cell migration in astrocytes: roles of ROS-dependent ERK- and JNK-NF-kappaB pathways. *J. Neuroinflammation* 7:88.
- Hu L, Roth JM, Brooks P, Luty J, Karpatkin S (2008). Thrombin up-regulates cathepsin D which enhances angiogenesis, growth, and metastasis. *Cancer Res.* 68:4666-4673.
- Imamura T, Kitamoto Y (2003). Expression of enteropeptidase in differentiated enterocytes, goblet cells, and the tumor cells in human duodenum. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 285:1235-1241
- Itkonen O, Stenman UH, Osman S, Koivunen E, Halila H, Schroder T (1996). Serum samples from pancreatctomized patients contain trypsinogen immune reactivity. *J. Lab. Clin. Med.* 128:98-102
- Jahan I, Fujimoto J, Mahfuzul S, Sato E Tamaya T (2008). Role of protease activated receptor-2 in lymph node metastasis of uterine cervical cancers. *BMC Cancer* 8:301.
- Jedieszko C, Sloane BF (2004). Cysteine cathepsins in human cancer. *Biol. Chem.* 385:1017-1027.
- Jemal A, Siegel R, Xu J, Ward E (2010). *Cancer Statistics, 2010.* CA Cancer J. Clin. 60:277-300.
- Joyce JA, Hanahan D (2004). Multiple roles for cysteine cathepsins in cancer. *Cell Cycle* 3:1516-1619.
- Joyce JA, Baruch A, Chehade K, Meyer-Morse N, Giraudo E (2004). Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. *Cancer Cell* 5:409-410
- Kandalaf P, Chang KL, Ahn CW, Traweck ST, Mehta P, Battifora H (1993). Prognostic significance of immunohistochemical analysis of cathepsin D in low-stage breast cancer. *Cancer* 71:2756-2763
- Kane RC, Bross PF, Farrell AT, Pazdur R. Velcade (2003). U.S. FDA approval for the treatment of multiple myeloma progressing on prior therapy. *Oncologist* 8:508-513.
- Kane RC, Dagher R, Farrell A, Ko CW, Sridhara R, Justice R, Pazdur R (2007). Bortezomib for the treatment of mantle cell lymphoma. *Clin. Cancer Res.* 13:5291-5294.
- Kane RC, Farrell AT, Sridhara R, Pazdur R (2006). United States Food and Drug Administration approval summary: bortezomib for the treatment of progressive multiple myeloma after one prior therapy. *Clin. Cancer. Res.* 12:2955-2960.
- Kawasaki G, Kato Y, Mizuno A (2002). Cathepsin expression in oral squamous cell carcinoma: relationship with clinicopathologic factors. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 93:446-454.
- Kim ES, Kim MS, Moon A (2004). TGF-beta-induced upregulation of MMP-2 and MMP-9 depends on p38 MAPK, but not ERK signalling in MCF10A human breast epithelial cells. *Int. J. Oncol.* 25:1375-1382.
- Kim J, Yu W, Kovalski K, Ossowski L (1998). Requirement for specific proteases in cancer cell intravasation as revealed by a novel semiquantitative PCR-based assay. *Cell* 94:353-62.
- Kleiner DE, Stetler-Stevenson WG (1993). Structural biochemistry and activation of matrix metalloproteases. *Curr Op Cell Biol.* 5:891-7
- Koblinski JE, Mamoun A, Bonnie FS (2000). Unraveling the role of proteases in cancer. *Clinica Chimica Acta* 291:113-135.
- Kos J, Lah TT (1998). Cysteine proteinases and their endogenous inhibitors: target proteins for prognosis, diagnosis and therapy in cancer. *Oncol. Rep.* 5:1349-1361.
- Koshikawa N, Hasegawa S, Nagashima Y, Mitsuhashi K, Tsubota Y, Miyata S (1998). Expression of trypsin by epithelial cells of various tissues, leukocytes, and neurons in human and mouse. *Am. J. Pathol.* 153:937-944.
- Kruger A., Fata JE, Khokha R (1997). Altered tumor growth and metastasis of a T-cell lymphoma in Timp-1 transgenic mice. *Blood* 90:1993-2000.
- Kuo YC, Su CH, Liu CY, Chen TH, Chen CP, Wang HS (2009). Transforming growth factor-beta induces CD44 cleavage that promotes migration of MDA-MB-435s cells through the up-regulation of membrane type 1-matrix metalloproteinase. *Int. J. Cancer* 124:2568-2576.
- Lacerna L, Hohneker J (2003). Zoledronic acid for the treatment of bone metastases in patients with breast cancer and other solid tumors. *Semin. Oncol.* 30:150-60.
- Lambert E, Dasse E, Hays B, Petitfrere E (2004). TIMPs as multifaceted proteins. *Crit. Rev. Oncol. Hematol.* 49:187-198.
- Lankelma JM, Voorend DM, Barwari T, Koetsveld J, Vander Spek AH, De Porto AP, Van Rooijen G, Van Noorden CJ (2010). Cathepsin L, target in cancer treatment. *Life Sci.* 86:225-233.
- Lee H, Lim C, Lee J, Kim N, Bang S, Min B, Park G, Noda M, Stetler-Stevenson WG, Oh J (2008). TGF-beta signaling preserves RECK expression inactivated pancreatic stellate cells. *J. Cell. Biochem.* 104:1065-1074.
- Liaudet E, Garcia M, Rochefort H (1994). Cathepsin D maturation and its stimulatory effect on metastasis are prevented by addition of KDEL retention signal. *Oncogene* 9:1145-1154.
- Liaudet-Coopman E, Mélanie B, Danielle D, Marcel G, Glondu-Lassis M, Laurent-Matha V, Christine P, Henri R and Françoise V (2006). Cathepsin D: newly discovered functions of a long-standing aspartic protease in cancer and apoptosis. *Cancer Lett.*, 237(2):167-179.
- Lijnen HR (2001). Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb. Haemost.* 86:324-333.
- Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ (1997). Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. *J. Cell Biol.* 139:1861-1872.
- Lockhart AC, Braun RD, Yu D, Ross JR, Dewhist MW, Humphrey JS, Thompson S, Williams KM, Klitzman B, Yuan F, Grichnik JM, Proia AD, Conway DA, Hurwitz HI (2003). Reduction of wound angiogenesis in patients treated with BMS-275291, a broad spectrum matrix metalloproteinase inhibitor. *Clin. Cancer. Res.* 9:586-593.
- Lopez-Otin C, Bond JS (2008). Proteases: multifunctional enzymes in life and disease. *J. Biol. Chem.* 283:30433-304
- Lopez-Otin C, Hunter T (2010). The regulatory crosstalk between kinases and proteases in cancer. *Nat. Rev. Cancer.* 10:278-292.
- Lopez-Otin C, Matrisian LM (2007). Emerging roles of proteases in tumour suppression. *Nat. Rev. Cancer.* 7:800-808.
- Lopez-Otin C, Overall CM (2002). Protease degradomics: a new challenge for proteomics. *Nat. Rev. Mol. Cell Biol.* 3: 509-519.
- Macabeo-Ong M, Shiboski CH, Silverman S, Ginzinger DG, Dekker N, Wong DTW, Jordan RCK (2003). Quantitative analysis of cathepsin L mRNA and protein expression during oral cancer progression. *Oral. Oncol.* 39:638-647
- Martin DC, Sanchez-Sweatman OH, Ho AT, Inderdeed DS, Tsao MS, Khokha R (1999). Transgenic TIMP-1 inhibits simian virus 40 T antigen - induced hepatocarcinogenesis by impairment of

- hepatocellular proliferation and tumor angiogenesis. *Lab. Invest.* 79:225-234.
- Mason SD, Joyce JA (2011). Proteolytic networks in cancer. *Trends Cell Biol.* 21:228–237.
- Masson O, Christine P, Danielle D, Aline M, Dray C, Danielle D, Didier Q, Philippe V, Catherine M, Liaudet-Coopman E (2011). Cathepsin-D, a Key Protease in Breast Cancer, Is Up-Regulated in Obese Mouse and Human Adipose Tissue, and Controls Adipogenesis. *PLoS ONE* 6(2):16452.
- Matarrese P, Barbara A, Laura C, Rosa V, Carlo L, Marco S, Anna MM, Caterina C, Marco GP, Walter M (2010). Cathepsin B inhibition interferes with metastatic potential of human melanoma: an *in vitro* and *in vivo* study. *Molecular Cancer* 9:207
- Matej R, Mandakova P, Netikova L, Pouckova P, Olejar T (2007). Proteinase-activated receptor-2 expression in breast cancer and the role of trypsin on growth and metabolism of breast cancer cell line MDA MB-23 1. *Physiol. Res.* 56:475-484.
- Michl P (2011). Targeting cathepsins: a new glimmer of hope for pancreatic cancer therapy? *Gut*, doi:10.1136/gutjnl-301816
- Mitchell BS (2003). The proteasome-an emerging therapeutic target in cancer. *NEJM Perspective*, 348:2597-2598.
- Miyata S, Koshikawa N, Higashi S, Miyagi Y, Nagashima Y, Yanoma S (1999). Expression of trypsin in human cancer cell lines and cancer tissues and its tight binding to soluble form of Alzheimer amyloid precursor protein in culture. *J. Biochem. (Tokyo)*, 125:1067-1076.
- Mohamed MM, Sloane BF (2006). Cysteine cathepsins: multifunctional enzymes in cancer. *Nat. Rev. Cancer.* 6:764-775.
- Mook OR, Frederiks WM, van Noorden CJ (2004). The role of gelatinases in colorectal cancer progression and metastasis. *Biochim. Biophys. Acta* 1705:69-89.
- Munshi HG, Wu YI, Mukhopadhyay S, Ottaviano AJ, Sassano A, Koblinski JE, Platanius LC, Stack MS (2004). Differential regulation of membrane type 1-matrix metalloproteinase activity by ERK 1/2- and p38 MAPK-modulated tissue inhibitor of metalloproteinases 2 expression controls transforming growth factor-beta1-induced pericellular collagenolysis. *J. Biol. Chem.* 279:39042-39050.
- Nakamura K, Hongo A, Kodama J, Abarzua F, Nasu Y, Kumon H, YUJI Hiramatsui Y (2009). Expression of Matriptase and Clinical Outcome of Human Endometrial Cancer. *Antic. Res.* 29:1685-1690.
- Nemunaitis J, Poole C, Primrose J, Rosemurgy A, Malfetano J, Brown P, Berrington A, Cornish A, Lynch K, Rasmussen H, Kerr D, Cox D, Millar A (1998). Combined analysis of studies of the effects of the matrix metalloproteinase inhibitor marimastat on serum tumor markers in advanced cancer: selection of a biologically active and tolerable dose for longer-term studies. *Clin. Cancer. Res.* 4:1101-9.
- Nicotra G, Castino R, Follo C, Peracchio C, Valente G (2010). The dilemma: does tissue expression of cathepsin D reflect tumor malignancy? The question: does the assay truly mirror cathepsin D mis-function in the tumor. *Cancer. Biomark.* 7:47-64.
- Nielsen BS, Rank F, Lopez JM, Balbin M, Vizoso F, Lund LR, Dano K and Lopez-otin C (2001). Collagenase-3 expression in breast myofibroblasts as a molecular marker of transition of ductal carcinoma in situ lesions to invasive ductal carcinomas. *Cancer. Res.* 61:7091-100.
- Nyberg P, Moilanen M, Paju A, Sarin A, Stenman UH, Sorsa T, Salo T (2002). MMP-9 Activation by Tumor Trypsin-2 Enhances *in vivo* Invasion of Human Tongue Carcinoma Cells. *J. Dent. Res.* 81(12):831-835.
- O'Keefe SJ, Lee RB, Li J, Stevens S, Abou-Assi S, Zhou W (2005). Trypsin secretion and turnover in patients with acute pancreatitis. *Am. J. Physiol. Gastrointest. Liver. Physiol.* 289:181-187
- Ohri SS, Vashishta A, Proctor M, Fusek M, Vetvicka V (2008) The propeptide of cathepsin D increases proliferation, invasion and metastasis of breast cancer cells. *Int. J. Oncol.* 32:491-498.
- Orlowski RZ, Dees EC (2003). The role of the ubiquitination-proteasome pathway in breast cancer: applying drugs that affect the ubiquitin-proteasome pathway to the therapy of breast cancer. *Breast. Cancer. Res.* 5:1-7.
- Overall CM, Lopez-Otin C (2002). Strategies for MMP inhibition in cancer: innovations for the post-trial era. *Nat. Rev. Cancer.* 2:657-672.
- Pan MR, Hung WC (2002). Nonsteroidal anti-inflammatory drugs inhibit matrix metalloproteinase-2 via suppression of the ERK/Sp1-mediated transcription. *J. Biol. Chem.* 277:32775-32780.
- Poole AR, Tiltman KJ, Recklies AD, Stoker TAM (1978). Differences in secretion of the proteinases cathepsin B at the edges of human breast carcinomas and fibroadenomas. *Nature* 273:545-547
- Puente XS, Sanchez LM, Overall CM, Lopez-Otin C (2003). Human and Mouse Proteases: A Comparative Genomic Approach. *Nat Rev Genet.* 4:544-558
- Quesada V, Ordóñez GR, Sanchez LM, Puente XS, Lopez-Otin C (2009). The Degradome database: mammalian proteases and diseases of proteolysis. *Nucleic Acids Res.* 37:D239–D243.
- Radisky ES, Radisky DC (2010). Matrix metalloproteinase-induced epithelial-mesenchymal transition in breast cancer. *J. Mammary Gland Biol. Neoplasia* 15:201-212.
- Ramachandran R, Noorbakhsh F, Defea K, Hollenberg MD (2012). Targeting proteinase-activated receptors: therapeutic potential and challenges. *Nat. Rev. Drug Discov.* 11:69-86.
- Reunanen N, Li SP, Ahonen M, Foschi M, Han J, Kahari VM (2002). Activation of p38 alpha MAPK enhances collagenase-1 (matrix metalloproteinase (MMP)-1) and stromelysin-1 (MMP-3) expression by mRNA stabilization. *J. Biol. Chem.* 277:32360-32368.
- Rodriguez J, Vazquez J, Corte MD, Lamelas M, Bongera M (2005). Clinical significance of cathepsin D concentration in tumor cytosol of primary breast cancer. *Int. J. Biol. Markers* 20:103-111.
- Ruhul Amin AR, Oo ML, Senga T, Suzuki N, Feng GS, Hamaguchi M (2003). SH2 domain containing protein tyrosine phosphatase 2 regulates concanavalin A-dependent secretion and activation of matrix metalloproteinase 2 via the extracellular signal-regulated kinase and p38 pathways. *Cancer. Res.* 63:6334-6339.
- Saleem M, Vaqar MA, Weixiong Z (2006). A Novel Biomarker for Staging Human Prostate Adenocarcinoma: Overexpression of Matriptase with Concomitant Loss of its Inhibitor, Hepatocyte Growth Factor Activator Inhibitor-1. *Cancer Epidemiol Biomarkers Prev.* 15:217-227
- Saleh Y, Wnukiewicz J, Andrzejak R, Trziszka T, Siewinski M, Ziolkowski P and Kopec W (2006). Cathepsin B and Cysteine Protease Inhibitors in Human Tongue Cancer: Correlation with Tumor Staging and *In Vitro* Inhibition of Cathepsin B by Chicken Cystatin. *J. Cancer. Mole.* 2(2):67-72.
- Schmitt M, Jänicke F, Graeff H (1992). Protease matrix degradation and tumor cell spread. *Fibrinolysis* 6:1-17.
- Skrzypczak M, Springwald A, Claus L, Julia H, Susanne S, Olaf O, Oliver T (2012). Expression of Cysteine Protease Cathepsin L is Increased in Endometrial Cancer and Correlates With Expression of Growth Regulatory Genes. *Cancer Invest.* 30:398–403.
- Sloane BF, Moin K, Lah TT (1994). Lysosomal enzymes and their endogenous inhibitors in neoplasia. In: Pretlow TG, Pretlow TP, ed. *Biochemical and molecular aspects of selected cancers.* New York: Academic pp. 411-466.
- Sokal JP, Schlemann WP (2004). Cystatin C antagonises transforming growth factor  $\beta$  signaling in normal and cancer cells. *Mol. Cancer Res.* 2:183-189.
- Soreide K, Janssen EA, Korner H, Baak JPA (2006). Trypsin in colorectal cancer: molecular biological mechanisms of proliferation, invasion and metastasis. *J. Pathol.* 209(2):145-156.
- Steeg PS (2006). Tumor metastasis: mechanistic insights and clinical challenges. *Nature Med.* 12(8):895-904.
- Stenman UH, Koivunen E, Itkonen O (1991). Biology and function of tumor-associated trypsin inhibitor, TATI. *Scand J. Clin. Lab. Invest. Suppl.* 207:5-9
- Suen JY, Barry GD, Lohman RJ, Halili MA, Cotterell AJ, Le GT, Fairlie DP (2012). Modulating human proteinase activated receptor 2 with a novel antagonist (GB88) and agonist (GB110). *British J. Pharmacol.* 165(5):413-1423.
- Sun L, Diamond ME, Ottaviano AJ, Joseph MJ, Ananthanarayan V, Munshi HG (2008). Transforming growth factor-beta 1 promotes matrix metalloproteinase - 9-mediated oral cancer invasion through

- snail expression. *Mol. Cancer Res.* 6:10-20.
- Tanimura S, Asato K, Fujishiro SH and Kohno M (2003). Specific blockade of the ERK pathway inhibits the invasiveness of tumor cells: down-regulation of matrix metalloproteinase-3/-9/-14 and CD44. *Biochem. Biophys. Res. Commun.* 304:801-806.
- Troy AM, Sheahan K, Mulcahy HE, Duffy MJ, Hyland JMP, O'Donoghue DP (2004). Expression of cathepsin B and L antigen and activity is associated with early colorectal cancer progression. *Eur. J. Cancer.* 40:1610-1616
- Tu C, Cesar FO, Gengsheng C, Norvin DF, Dora C, Bonnie FS, Vimla B, and Hamid B (2008). Lysosomal cathepsin B participates in the podosome-mediated extracellular matrix degradation and invasion via secreted lysosomes in v-Src fibroblasts. *Cancer. Res.* 68(22): 9147-9156
- Turk B, Turk V, Turk D (1997). Structural and functional aspects of papain-like cysteine proteinases and their protein inhibitors. *Biol. Chem.* 378:141-150.
- Turk V, Bode W (1991). The cystatins: protein inhibitors of cysteine proteinases. *FEBS Lett.* 285:213-219.
- Vashishta A, Ohri SS, Proctor M, Fusek M, Vetvicka V (2007). Ribozyme- targeting procathepsin D and its effect on invasion and growth of breast cancer cells: an implication in breast cancer therapy. *Int. J. Oncol.* 30:1223-1230.
- Vashishta A, Ohri SS, Vetvicka V (2009). Pleiotropic effects of cathepsin D. *Endocr. Metab. Immune. Disord. Drug Targets* 9:385-391.
- Vasiljeva O, Turk B (2008). Dual contrasting roles of cysteine cathepsins in cancer progression: apoptosis versus tumour invasion. *Biochimie.* 90:380-386
- Vetvicka V, Fusek M (2012). Procathepsin D as a tumor marker, anti-cancer drug or screening agent. *Anticancer Agents Med. Chem.* 12(2):172-5.
- Vetvicka V, Vashishta A, Saraswat-Ohri S, Vetvickova J (2010). Procathepsin D and cancer: From molecular biology to clinical applications. *World J. Clin. Oncol.* 10 1(1):35-40
- Westermarck J, Kahari VM (1999). Regulation of matrix metalloproteinase expression in tumor invasion. *FASEB J.* 13:781-92.
- Westley BR, May FE (1999). Prognostic value of cathepsin D in breast cancer. *Br. J. Cancer* 79:189-190.
- Wiegand U, Corbach S, Minn A, Kang J, Muller-Hill B (1993). Cloning of the cDNA encoding human brain trypsinogen and characterization of its product. *Gene* 136:167-175
- Wiercinska E, Naber HP, Pardali E, van der Pluijm G, van Dam H, Ten Dijke P (2010). The TGF-beta/Smad pathway induces breast cancer cell invasion through the up-regulation of matrix metalloproteinase 2 and 9 in aspheroid invasion model system. *Breast Cancer Res. Treat.* 128:657-666.
- Wojtowicz-Praga S, Low J, Marshall J, Ness E, Dickson R, Barter J, Sale M, Mccann P, Moore J, Cole A, Hawkins MJ (1996). Phase I trial of a novel matrix metalloproteinase inhibitor batimastat (BB-94) in patients with advanced cancer. *Invest. New Drugs* 14:193-202.
- Xu K, Rajagopal S, Klebba, Dong S, Ji Y, Liu J, Kuperwasser C, Garlick JA, Naber SP, Buchsbaum RJ (2010). The role of fibroblast Tiam1 in tumor cell invasion and metastasis. *Oncogene* 29 6533-6542.
- Yamamoto H, Iku S, Adachi Y, Imsumran A, Taniguchi H, Noshio K, Min Y, Horiuchi S, Yoshida M, Itoh F, Imai K (2003). Association of trypsin expression with tumor progression and matrilysin expression in human colorectal cancer.
- Yang Y, Hao Hong H, Yin Zhang Y and Weibo Cai W (2009). Molecular Imaging of proteases in cancer. *Cancer Growth and Metastasis.* 2:13-27.
- Zavrski I, Jakob C, Schmid P, Krebbel H, Kaiser M, Fleissner C, Rosche M, Possinger K, Sezer O (2005). Proteasome: an emerging target for cancer therapy. *Anticancer. Drugs* 16(5):475-481.
- Zhong J, Gencay MM, Bubendorf L, Burgess JK, Parson H, Robinson BW, Tamm M, Black JL, Roth M (2006). ERK1/2 and p38 MAP kinase control MMP-2, MT1-MMP, and TIMP action and affect cell migration: a comparison between mesothelioma and mesothelial cells. *J. Cell Physiol.* 207:540-552.
- Zucker S, Cao J, Chen WT (2000). Critical appraisal of the use of matrix metalloproteinase inhibitors in cancer treatment. *Oncogene* 19(56):6642-50.