© JLS 2013 J Life Science, 5(2): 133-138 (2013)
PRINT: ISSN 0975-1270 ONLINE: ISSN 2456-6306 DOI: 10.31901/24566306.2013/05.02.04

Proteases as Targets in Anticancer Therapy Using Their Inhibitors

Syed Rakashanda and Shajrul Amin*

Department of Biochemistry, University of Kashmir, Srinagar 190 006, Jammu and Kashmir, India

KEYWORDS Proteases. Protease Inhibitors. Antitumor. Oncogen

ABSTRACT Proteases, also known as proteolytic enzymes, are enzymes that catalyze the breakdown of proteins by hydrolysis of peptide bonds. Earlier proteases were considered as only protein degrading enzymes, however now dramatically the view has changed. Proteases are extremely important signalling molecules that are involved in numerous vital processes like apoptosis, cell growth and activation, adhesion, invasion, cell migration and metastasis, protein secretion, cellular interactions and signal transduction, phagocytosis and angiogenesis. Thus, show complete anticancer mechanism. And proteases from all six classes have been found to be involved in tumor growth and progression. Inhibitors of such proteases are emerging with promising therapeutic uses in the treatment of cancer. Protease inhibitor suppression of carcinogenesis is related to ability to effect the expression of certain oncogens and the levels of certain types of proteolytic activities. Protease inhibitors being found to be as special agents in anticancer therapy have been isolated from plants, bacteria and prepared synthetically. Under the same trend different protease inhibitors have been used and are being in clinical and pre-clinical stage. Thus, studying PIs as anticancer agents open a new field for treatment of cancer.

INTRODUCTION

From the literature available it is clear that all five major classes of proteases are involved in tumor growth and progression. Protease enzymes selectively catalyzing the hydrolysis of polypeptide bond are crucial for disease propagation and inhibitors of such proteases are emerging with promising therapeutic uses in the treatment of diseases like cancers. Inhibitors of these proteases can suppress several stages of carcinogenesis including initiation, promotion and progression although their mechanism of action is not yet fully clear. Studies have suggested that the protease inhibitors which prevent carcinogenesis affect processes in the early stages of carcinogenesis although they can be effective at long times period after carcinogen exposure in both in vitro and in vivo systems, while there is a strong evidence that these protease inhibitors can affect both the initiation and promotion stages of carcinogenesis and they have no effect on already transformed cells. Results have suggested that the first event in carcinogenesis is a high frequency epigenetic event and that a later event, presumably genetic, leads to the malignant state.

*Address for correspondence:
Dr. Shajrul Amin,
Department of Biochemistry,
University of Kashmir,
Srinagar, 190006,
Jammu and Kashmir, India
Telephone: +91-9419018174.
E-mail: shajrul@rediffmail.com

Effect of protease inhibitors on the following phenomenon are thought to be related to their anti carcinogenic activity:

1) Ability to effect the expression of certain oncogenes,
2) Ability to return carcinogen increased levels of certain proteolytic activities for example, Boc-val- pro- arg- Mca hydrolyzing activity to normal levels (Kennedy 1993).

Clawson, 1996 categorized the effects of Protease Inhibitors into three basic groups:

a) Signal transduction pathways
b) DNA repair processes
c) Nuclear proteases

From the studies it is simply clear that protease inhibitors can be used as drugs to treat the various tumor patients. Under the same studies different protease inhibitors have been used in clinical and pre-clinical stage. Many new ECP (Extra Cellular Proteases) inhibitors are currently under clinical investigation and a significant increase in new therapies based on protease inhibition can be expected in the coming years.

Protease Inhibitors and Antitumorigenesis

PI (Protease inhibitor) represent an important role in regulation of various cellular physiological and biological processes, including cell cycle, cell death, differentiation and the immune response (Fan et al. 2001; Buhling et al. 2006). Previous studies have shown the anticancer effect and probable mechanisms of the natural and chemical PI in vitro.

Many tumor promoters are inflammatory agents that stimulate the formation of oxygen radicals (O_2^-) and hydrogen peroxide (H_2O_2) in phagocytic neutrophils. The neutrophils use the oxygen radicals to kill bacteria, which are recognized by the cell membrane of the phagocytic cells causing a signal to mount the oxygen response. The tumor promoter from croton oil, 12-o-tetradecanoylphorbol-3-acetate TPA, mimics the signal, causing an oxygen radical release that is intended to kill bacteria; instead it injures cells in the host. Oxygen radicals cause single strand breaks in the DNA and modify DNA bases. These damaging reactions appear to be related to tumor promotion, as chemopreventive agents like protease inhibitors, suppress the induction of oxygen radicals in phagocytic neutrophils and suppress tumor promotion in skin cancer in mice. Protease inhibitor was a novel entry into the group of agents that suppress the oxygen response of phagocytes. Preotease inhibitors capable of inhibiting chymotrypsin show a greater suppression of the oxygen effect and are better suppressers of tumor promotion. Thus the Bowman-Birk soybean inhibitor, which is more powerful inhibitor of chymotrypsin than the Kuintz soybean inhibitor, showed 7-fold greater suppression of $\cdot O_2^-$ produced by TPA in neutrophils than the Kuintz inhibitor (Yavelow 1985). The most powerful inhibitor is the potato inhibitor 1 PI 1, which exclusively inhibits chymotrypsin (Walter 1989). Indeed Yavelow (1987) have identified a chymotrypsin-type enzyme in the cell membrane of 10T1/2 cell, which may act as a receptor for the localization of protease inhibitor with inhibitory properties to chymotrypsin. BBI the soybean derived proteases inhibitor that has been extensively studied as this is the proteases inhibitor that has risen to the human trial stage as a human cancer chemopreventive agent (Fernandes and Benerji 1997). Bowman-Birk Inhibitor concentrate shows promise to become an effective nontoxic chemopreventive agent based on results of extensive preclinical studies, and Phase I and Phase IIa clinical trials (Wan et al. 2002; Armstrong et al. 2003). Recently Bowman-Birk protease inhibitor isolated from *Vigna unguiculata* seeds is showing apoptosis and lysosome membrane permeabilization induction on breast cancer cell (Joinitti et al. 2010). Lunasin a bioactive cancer preventive agent in Soy Bowman Birk Inhibitor Concentrate (BBIC) is made bioavailable after oral administration by Bowman-Birk Inhibitor (Hseih et al. 2010).

Berkelic acid effectively inhibited MMP-3 in the micromolar range and caspase- 1 in the millimolar range. Berkelic acid was tested in the National cancer Institute (NCI) antitumor screen against 60 human cell lines. It showed selective activity towards ovarian cancer OVCAR-3 (Andrea 2006).

Cysteine proteases, in particular cathepsins B and L, have been implicated in tumor invasion and are thought to be important mediators of metastasis. H-59 carcinoma cells which are highly invasive and preferentially metastatic to the liver, express high levels of cathepsin L and lower levels of cathepsin B, whereas M-27 cells which are less invasive and only moderately metastatic to lung express cathepsin B only. Navab et al. (1997) studied the effect of cysteine proteinase inhibitor by using a reconstituted basement membrane matrigel invasion assay and found that E-64 blocked the invasion of H-59 cells under conditions which didn't affect cell viability. Minor but significant inhibitory effect up to 32% was also seen with the propeptide cathepsin B, implicating this enzyme in the invasion process. Treatment of H-59 cells with E-64 inhibited experimental liver metastasis formation by up to 90%. On the other hand invasion of M-27 cells could not be blocked by cysteine proteases inhibitors even under conditions which resulted in complete abrogation of intercellular enzymatic activity.

Beta- ursolic acid isolated from *Saliva officinalis* by Jedinak et al. (2006), significantly inhibited all tested proteases *in vitro* in the micromolar range. Beta-ursolic acid showed the strongest inhibition activity to urokinase IC_{50} = 12 μM and cathepsin B IC_{50}=10 μM as proteases included in tumor invasion and metastasis indicated possible anticancer affectivity. Beta- ursolic acid significantly decreased the number of B16 colonies in the lungs of mice at the dose of 50mg/kg $p< 0.05$. Another novel discovery was that of urinary trypsin inhibitor. It inhibits urokinase type plasminogen activator (UPA), a proteolytic enzyme mediating metastasis. UPA secreted by tumor cells can be bound to a cell surface receptor via a growth factor- like domain within amino-terminal fragment (ATF) of the uPA molecule with high affinity. Urinary trypsin inhibitor (UTI) efficiently inhibits the soluble and the tumor cell surface receptor- bound plasmin and subsequently reduces tumor cell invasion and the formation of metastasis (Hiroshi 1995).

A synthetic protease inhibitor FOY- 305 (FOYPAN) not only inhibited the skin tumoregenesis in mice but also suppressed the growth of autochthonous solid tumor in mice. Furthermore administration of FOY- 305 inhibited the metastasis of Lewis lung carcinoma and colon adenocarcinoma to the lung in mice, experimentally and spontaneously. Clinically, FOY- 305 prevented both recurrence and metastasis in the patients who has received many anticancer drugs. Ohkioshi (1981) suggested that there is possibility for applying a new type of chemotherapy using protease inhibitors. Oral administration of a synthetic protease inhibitor [N, N- dimethyl carbamylmethyl 4- 4- guanidinobenzoyloxyphenylacetate] methanesulfate, was used to challenge 3- methylcholanthrene induced carcinoma. Administration of more than 50.0 mg/kg of this protease inhibitor siginificantly inhibited tumor growth and prolonged the survival time of cancer harbouring animals (Ohkoshi 1995).

Widely distributed in human tissue, mammary serine protease inhibitor (maspin) is a member of the serine protease inhibitor superfamily (Khalkhali-Ellis 2006). Maspinis localized at chromatin 18q21.3, a region that often undergoes loss of heterozygosity in some forms of human cancers, suggesting its potential role in carcinogenesis (Schneider et al. 1995). In 1994, maspin initially was identified by Zou et al. as a putative tumor-suppressor gene in breast carcinoma. Since then, maspin has been reported sequentially as inversely related to cancer progression and metastasis in various cancers, such as prostate cancer, lung cancer, and oral squamous cell carcinoma. Maspin exhibited a metastasis-suppressive effect, which may be a consequence of the reversal of the malignant phenotype of EC109 cells (Cai et al. 2009). The switch of cellular metabolic phenotype to low glycolysis by the gain of maspin function may play a key role in the process. Evidence of the tumor metastasis-suppressive activity of maspin have been provided and may indicate a new direction for future studies of the mechanism of maspin (Maass et al. 2000; Cai et al. 2009).

A novel Kunitz-type serine proteinase inhibitor, termed PIVL, purified from the venom of the Tunisian snake *Macrovipera lebetina transmediterranea* specifically inhibiting trypsin activity, exhibited an anti-tumor effect and displays integrin inhibitory activity without being cytotoxic on human glioblastoma U87 cells (Morjen et al. 2013).

The development of small molecular weight inhibitors of MMPs (Matrixmetallo proteinases) offered the potential of preventing tumor invasion and angiogenesis thus inhibiting tumor growth and metastasis. Interference with the activity of MMPs through the expression of endogenous tissue inhibitors of MMPs TIMPS (Tissue inhibitor of metalloproteinases) has been shown to inhibit invasion *in vitro and in vivo* and can block tumor-induced neovacularization (Murphy et al. 1994). The use of novel, non-classical anticancer agents such as MMPs inhibitors represent a new and potentially effective approach. AG3340 Prinomastat is targeted against MMP1 with specificity directed against MMP2 and MMP-9 and this agent has been evaluated in two trials of patients with stage IIIB and IV NSCLC Shepherd, 2001. The MMP-1-PAR1 axis is involved in EOC invasion and at least partially mediates LPA-induced EOC invasion and also human glimos (Wang et al. 2010; Zang et al. 2010). Therefore, blocking MMP-1 or PAR1 may represent a new therapeutic option for metastatic EOC (Wang et al. 2010).

Tanomastat is a MMPs inhibitor that has preclinical activity in ovarian cancer models. It is a novel and specific non – peptidic biphenyl MMP inhibitor against several. MMPs implicated in tumor progression (Rowinsky et al. 2000). BMS-275291, an orally bio-available MMP inhibitor currently under development, has been shown to decrease the overall tumor burden in several animal models (Poulaki 2002). It is the newest MMP1 to be evaluated in lung cancer. It is broader spectrum with activity against MMPs 1, 2, 8, 9, 13, 14 and to some extent MMP3. MMP inhibitors MMPI are primarily considered to have anti-proliferative rather than direct cytotoxic effects.

Neovastat AE-941 is a shark cartilage extract, which has activity against MMP-2 and MMP-12. Clinical benefits observed upon Neovastat treatment rely on the presence of multiple angiogenesis inhibitors including inhibitors of MMP activities (Gingras et al. 2001). Neovastat specifically stimulates tPA-dependent plasmin generation through an increase in the affinity of the enzyme towards plasminogen apart from its stimulatory effect on tPA activity, neovastat also markedly stimulates tPA expression in endothelial cells through an increase in the transcription of the tPA gene (Gingras et al. 2004). Anginex is a potent inhibitor of EC adhesion, migration and

function by apoptosis (Griffioen et al. 2001). Marismastat is a broad- spectrum MMP inhibitor. It is currently in phase III clinical trials and is being evaluated for the treatment of invasive cancers and metastasis (Summers and Davidsen 1998). Recent advances in development of tetracycline derivatives as potential inhibitors of MMPs, which have shown promising preclinical and early clinical results (Acharya et al. 2004). BILA 2157BS is another potent rennin inhibitor with some selectivity towards cathepsin D (Simoneau et al. 1999). Brem et al. (1990) found that a mild penicillamine- induced copper deficiency greatly reduced the growth of the tumors and their invasiveness. The cyclic thiol MR889 has been investigated as a chemotherapeutic agent for lung cancer (Inadaet et al. 1997). With its low toxicity and good in vivo properties, it may soon enter human trials. Bortezomib PS-341, proteasome inhibitor, is the first inhibitor to undergo clinical testing, has demonstrated impressive antitumor activity and manageable toxicities in phase I and II trials both as a single agent and in combination with other drugs (Voorhees et al. 2003). Inhibitors of the proteasome impact on cells, in part, through down regulation of nuclear factor kB, but also through modulation of cell cycle proteins and other pro and anti apoptotic pathways. The development of specific and potent chemical inhibitors of the proteasome has sparked considerable excitement about the therapeutic potential of this class of drugs not only in cancer but other immune disorders (Mitchell 2003). MG-132, vinyl sulfone, epoxomicin, lactacystin and clasto-Lactacystin β-lactone are some of the representative classes of proteasome inhibitors.

ADAM (A Disintegrin And Metalloproteinase) activities are regulated by a group of physiological inhibitors called TIMPs. TIMPs have shown the inhibiting properties against the protease activity of certain ADAM molecules. TIMP-1 and TIMP-3 has been shown to inhibit the protease activity of ADAM-10, while only TIMP-3 has been demonstrated to be inhibitory towards ADAM-17/TACE (Amour et al. 1998; 2000). Furthermore, a number of selective ADAM inhibitors, especially against ADAM10 and ADAM17, have been shown to have anticancer effects (Duffy et al. 2011).

Batimastat mimics the substrate of the matrix metalloproteinases, specifically the peptide residues on one side of a principal cleavage site in the extracellular matrix molecule, collagen. This allows it to fit tightly in the active site of the enzyme. The hydroxamate group -CONHOH of the batimastat molecule binds to the zinc atom in the active site resulting in potent but reversible inhibition of the metalloproteinase. Other 'zinc chelating' groups have been employed in related inhibitors. These include thiol (Darlak 1990) and carboxyl groups. Modifications to other groups have led to the development of gelatinase-selective inhibitors. This work has been aided by the availability of X-ray crystallography data on the mammalian matrix metallo-proteinases (Bode 1994). Six month toxicology studies have been completed for batimastat in two species, without significant adverse effects in the intended pharmacokinetic range. This is an encouraging indication of the relative safety of MMPI therapy.

The proteasome is a ubiquitous enzyme complex that plays a critical role in the degradation of many proteins involved in cell cycle regulation, apoptosis and angiogenesis. Since these pathways are fundamental for cell survival and proliferation, particularly in cancer cells, the inhibition of proteasome is an attractive potential anticancer therapy. Bortezomib (Velcade), formerly PS-341 is an extremely potent and selective proteasome inhibitor that shows strong activity in in vitro and in vivo laboratory studies against many solid and hematologic tumor types. Moreover, bortezomib, mainly by inhibition of the NF-kappa B pathway, has a chemo sensitizing effect when administered together with other antitumoral drugs. Clinical phase I trials, showed good tolerance of bortezomib at doses that achieved a desired degree of proteasome inhibition. Phase II studies showed high response rates in refractory multiple myeloma patients, which led to the accelerated approval of bortezomib by the Food and Drug Administration FDA and the European Medicines Agency EMEA for this indication. A phase III trial comparing bortezomib to dexamethasone in refractory/relapsed multiple myeloma patients had to be halted due to a survival advantage in the bortezomib arm. Additional studies are focusing in the potential benefit of bortezomib in newly diagnosed multiple myeloma patients. In other solid and hematological malignancies, phase II studies with bortezomib alone or in combination are ongoing with encouraging results, particularly in lung cancer and lymphoma (Montagut et al. 2006). Old drug disulfiram a proteasome inhibitor which was used

for about 50 years in treatment of alcoholism have been successfully used to suppress hepatic metastasis originating from ocular melanoma. The inhibitory potency of disulfiram against the proteasome conforms to current anticancer strategies and represents a new, promosing approach to proteasome inhibition (Cvek 2008).

CONCLUSION

In the face of such very strong evidence for proteolytic enzymes being crucial mediators of mammalian physiology and disease, it is not surprising that there has been extensive research activity and resources targeted to the development of potent and selective protease inhibitors, particularly over the last 15 years. However what is perhaps surprising is that so few compounds have actually arrived in the marketplace to date and one important being bortzomib. Bortezomib may be the "backbone" for the development of more effective treatment strategies to improve patient outcome in multiple myeloma. Although many compounds have entered clinical trials over the past 5 years since, most have not progressed as expected. In other numerous studies, the role of natural and chemical PIs in cancer have been reported *in vitro* and *in vivo*, but there is little information about the use of these compounds in inhibiting cancer in humans and the mechanism of their action. Thus, it will be a wide open spectrum of biological and clinical studies.

ACKNOWLEDGEMENTS

The authors express gratitude to University of Kashmir, Srinagar for providing financial assistance and to the department of Biochemistry for the infrastructure.

REFERENCES

Acharya R, Venitz J, Figg D, Sparreboom A 2004. Chemically modified tetracyclines as inhibitors of matrix Metalloproteinases. *Drug Resistance Updates*, 7: 195-208.

Amour A, Slocombe PM, Webster A, Butler M, Knight CG et al. 1998. TNF-alpha converting enzyme TACE is inhibited by TIMP-3. *FEBS Letters*, 435: 39-44.

Amour A, Knight CG, Webster A, Slocombe PM, Stephens PE, Knauper V, Docherty AJP, Murphy G 2000. The in vitro activity of ADAM-10is inhibited by TIMP-1 and TIMP-3. *FEBS Letter,* 437: 275-279.

Andrea A, Stierle, Donald B, Kelly K 2006. Berkelic Acid, a novalspiroketal with selective anticancer activity from an acid mine waste fungal extremophile. *J Org Chem,* 17(14): 5357-5360.

Armstrong WB, Wan XS, Kennedy AR, Taylor TH, Meyskens FL Jr 2003. Development of the Bowman-Birk inhibitor for oral cancer chemoprevention and analysis of Neu immunohistochemical staining intensity with Bowman-Birk inhibitor concentrate treatment. *Laryngoscope*, 113(10): 1687-1702.

Bode W, Reinemer P, Huber R et al. 1994. The X-ray crystal structure of the catalytic domain of human neutrophil collagenase inhibited by a substrate analogue reveals the essentials for catalysis and specificity. *EMBO J*, 13: 1263-1269.

Brem SS, Zagzag D, Tsanaclis AM, Gately S, Elkouby MP, Brien SE 1990. Inhibition of angiogenesis and tumor growth in the brain, suppression of endothelial cell turn over by penicillamine and the depletion of copper, an angiogenic cofactor. *Am J Pathol*, 137: 1121-1142.

Buhling F, Groneberg D, Welte T 2006. Proteases and their role in chronic inflammatory lung diseases. *Curr Drug Targets*, 7: 751-759.

Cai Z, Yuan Z, Ting L, Jen-Fu C, Qing-Yu H 2009. Mammary serine protease inhibitor inhibits epithelial growth factor-induced epithelial-mesenchymal transition of esophageal carcinoma cells. *Cancer*, 115: 36-48.

Clawson GA 1996. Protease inhibitors and carcinogenesis: A review. *Cancer Invest*, 146: 597-608.

Cvek B, Dvorak Z 2008. The value of proteasome inhibition in cancer. can the drug, disulfirum, have a bright new future as a novel proteasome inhibitor? *Drug Discovery Today*, 13(15-16): 716-722.

Darlak K, Miller RB, Stack MS et al. 1990. Thiol-based inhibitors of mammalian collagenase. *J BiolChem*, 265: 5199-5205.

Duffy MJ, Mullooly M, O'Donovan N, Sukor S, Crown J, Pierce A, McGowan PM 2011. The ADAMs family of proteases: New biomarkers and therapeutic targets for cancer? *Clinical Proteomics,* 8: 9.

Fan XM, Wong BC, Wang WP, Zhou XM, Cho CH, et al. 2001. Inhibition of proteasome function induced apoptosis in gastric cancer. *Int J Cancer*, 93: 481-488.

Fernandes AO, Banerji AP 1997. Long term feeding of field bean protein containing protease inhibitors suppress virus- induced mammary tumors in mice. *Cancer Lett*, 116: 1-7.

Gingras D, Labelle D, Nyalendo C, Biovin D, DemeuleM, Barthomeuf C, Beliveau R 2004. The antiangiogenic agent Neovastat AE-941 stimulates tissue plasminogen activator activity. *Invest New Drugs,* 22: 17-26.

Gingras D, Renaud A, Mousseau N, Beaulieu E, Kachra Z, Beliveau R 2001. Matrix proteinase inhibition by AE-941, a multifunctional antiangiogenic compound. *Anticancer Res*, 21: 145-155.

Griffioen AW, van der Schaft DW, Barendsz-Janson AF, Cox A, Struijker Boudier HA et al. 2001. Anginex, a designed peptide that inhibits angiogenesis. *Biochem J*, 354: 233–242.

Hiroshi K, Junko G, Yasuyuki H, Michio F, Dan S, Toshihiko T 1995. Inhibitory effect of a conjugate between human urokinase and urinary trypsin inhibitor on tumor cell invasion in vitro. *The Journal of Biological Chemistry*, 270(14): 8361-8366.

Hsieh CC, Hernández-Ledesma B, Jeong HJ, Park JH, de Lumen BO 2010. Complementary roles in cancer prevention: protease inhibitor makes the cancer preventive peptide lunasin bioavailable. *PLoS One*, 26: 5.

Inada M, Yamashita J, Ogawa M 1997. Neutrophil elastase inhibitor ONO-5046-Na inhibits the growth of human lung cancer cell lines transplanted into severe combined immunodeficiency scid mice. *Res Commun Mol Pathol Pharmacol*, 97: 229-232.

Jedinak A, Muckova M, Kostalova D, Maliar T, Masterova I 2006. Antiprotease andantimetastatic activity of ursolic acid isolated from *Salvia officinalis*. *Z Naturforsch*, 61c: 777-782.

Joanitti GA, Azevedo RB, Freitas SM 2010. Apoptosis and lysosome membrane permeabilization induction on breast cancer cells by an anticarcinogenic Bowman-Birk protease inhibitor from Vigna unguiculata seeds. *Cancer Lett*, 293(1): 73-81.

Kennedy AR 1993. Potential mechanisms of antitumorigenesis by protease inhibitors. *Basic Life Sci*, 61: 301-7

Khalkhali-Ellis Z 2006. Maspin: The new frontier. *Clin Cancer Res*, 12: 7279-7283.

Maass N, Hojo T, Zhang M, Sager R, Jonat W, Nagasaki K 2000. Maspin-a novel protease inhibitor with tumor-suppressing activity in breast cancer. *Acta Oncol*, 39(8): 931-934.

Mitchell BS 2003. The proteasome - an emerging therapeutic target in cancer. *N Engl J Med*, 348(26): 2597-8.

Montagut C, Rovira A, Albanell J 2006. The proteasome: A novel target for anticancer therapy. *Clin Transl Oncol*, 8(5): 313-317.

Morjen M, Kallech-ziri O, Bazaa A, Othman H, Mabrouk K, Zouari-kessentini R, Sanz L, Calvete JJ, Srairi-Abid N, El Ayeb M, Luis J, Marrakchi N 2013. PIVL, a new serine protease inhibitor from Macrovipera lebetina transmediterranea venom, impairs motility of human glioblastoma cells. *Matrix Biology*, 32(1): 52–62.

Murphy G, Willenbrock F, Crabbe T et al. 1994. Regulation of matrix metalloproteinase activity. *Ann NY Acad Aci*, 732: 31-40.

Navab R, John S, Pnina B 1997. Inhibition of carcinoma cell invasion and liver metastases formation by the cysteine proteinase inhibitor E-64. *Clinical and Experimental Metastasis*, 15: 121-129.

Ohkoshi N 1995. Protease inhibitors as anticancer chemotherapy- experimental and clinical studies. *Gan To Kagaku Ryoho*, 22(4): 417-430.

Poulaki V 2002. BMS-275291 Bristol- Meyers Squibb. *Curr Opin Invest Drugs*, 3: 500-504.

Rowinsky E, Humphrey R, Hamod LA et al. 2000. Phase I and pharmacologic study of the specific matrix metalloproteinase inhibitor BAY 12- 9566 on a protracted oral daily dosing schedule in patients with solid malignancies. *J Clin Oncol*, 18: 178-186.

Schneider SS, Schick C, Fish KE et al. 1995. A serine proteinase inhibitor locus at 18q21.3 contains a tandem duplication of the human squamous cell carcinoma antigen gene. *Proc Natl Acad Sci USA*, 92: 3147-3151.

Shepherd FA 2001. Angiogenesis inhibitors in the treatment of lung cancer. *Lung Cancer*, 34: 81–89.

Simoneau B, Lavallee P, Anderson PC et al. 1999. Discovery of non-peptidic P2-P3 butanediamide rennin inhibitors with high oral efficacy. *Bioorg Med Chem*, 7: 489-508.

Summers JB, Davidsen SK 1998. Matrix metalloproteinase inhibitors and cancer. In: JA Bristol (Ed.): *Annual Reports in Medicinal Chemistry.* Michigan: Academic Press, l33: 131-134.

Voorhees PM, Caliredees, O'Neil B, Orlowski RZ 2003. The proteasome as a target for cancer therapy. *Clinical Cancer Res*, 9: 6316-6325.

Walter T 1989. Protease inhibitors interfere with the necessary factors of carcinogenesis. *Envi H Perspectives*, 81: 59-63.

Wan XS, Serota DG, Ware JH, Crowell JA, Kennedy AR 2002. Detection of Bowman-Birk inhibitor and anti-Bowman-Birk inhibitor antibodies in sera of humans and animals treated with Bowman-Birk inhibitor concentrate. *Nutr Cancer*, 43(2): 167-173.

Wang FQ, Fisher J, Fishman DA 2010. MMP-1-PAR1 axis mediates LPA-induced epithelial ovarian cancer EOC invasion. *Gynecol Oncol*, 120(2): 247-255.

Yavelow J, Caggana M, Beck KA 1987. Proteases occurring in the cell membrane: A possible receptor for Bowman- Birk type of protease inhibitors. *Cancer Res*, 47: 1598-1601.

Yavelow J, Colins M, Birk Y, Troll W, Kennedy AR 1985. Nanomolar concentration of Bowman- Birk soybean protease inhibitor suppress X- ray transformation in vitro. *Proc Natl Acad Sci US,* 82: 5395-5399.

Zhang Y, Zhan H, Xu W, Yuan Z, Lu P, Zhan L, Li Q 2010. Upregulation of matrix metalloproteinase-1 and proteinase-activated receptor-1 promotes the progression of human gliomas., *Pathol Res Pract*, 207(1): 24-29.

Zou Z, Anisowicz A, Hendrix MJ et al. 1994. Maspin, a serpin with tumor-suppressing activity in human mammary epithelial cells. *Science*, 263: 526-529.