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PROTEASES AS THERAPEUTIC TARGETS FOR CANCER TREATMENT

- **Parisha Garg**

ABSTRACT

In anatomic phases, proteases are fundamental in completing natural procedures, for example, quality articulation, separation, and cell passing. In any case, for belonging their capacity to debase extracellular network as well as proteins, they are emphatically connected to disease movement. A ton of group of proteases have been connected along expanding tumor progression and metastasis in various human diseases, recommending their focal capacities in the procedure of metastasis. The comprehension of the role of proteases in the tumor microenvironment is quickly expanding a direct result of a brought intrigue both up in protease and in new procedures that take into consideration complete examination of protease movement in physiologically important conditions. By and large, settled proteolytic systems comprise of various strides of enactment, a few key hubs through which majority of signals pass, and their inhibitors which can control action of various focuses in such type of systems. This type of a focal job found in a few flagging pathways, proteolytic enzymes speak to efficient medication focuses for an enormous arrangement of sicknesses, particularly for a chronic disease like CANCER. Protease inhibitors are mixes ready to square work of proteases assuming a key job in malignancy treatments. Be that as it may, their structure is a mind-boggling issue since various kinds of malignant growths utilize various proteases at the changing stages of disease advancement. Am important fact is that no single inhibitor can be utilized on all types of proteases. This paper will cover about the basic idea of proteases and how they can be used as therapeutic targets for cancer treatment. The paper will cover different types of cancer and how different proteases can act upon them. As proteases have an immense medical and pharmaceutical importance, they are used in the field of biotechnology.

Keywords: proteases, therapeutics, cancer, protease inhibitor, biomarker, tumor

INTRODUCTION

Proteases are said to be called as a set of proteolytic enzymes that rapidly increase the rate of the hydrolysis of peptide and isopeptide bonds and then joins the amino acids with the proteins. It is also called as peptidase, basically function to break down the proteins into amino acids and due to this breakdown energy is released which is used up by the body. Each protease stands alone embracing different types of functions shown by different kinds of proteases. Some of the functions that they inhibit are:[36]

- Blood clotting
- Reusing of proteins
- Cell division
- Strong Immunity

Proteases are easily found and can be visible in different stages of life and infections. They have shown many advanced functions and therefore different type of proteases are unique in its own way depending upon its role and activity that take place during biological and pathological processes.[36]

Function of proteases

The flow accomplishment of research right now old catalysts gets primarily from the enormous assortment of discoveries exhibiting their importance in the control of various natural procedures in every living being. Therefore, proteases direct the destiny, limitation, and action of numerous proteins, regulate protein-protein associations, make new bioactive atoms, add to the handling of cell data, and produce, transduce, and enhance sub-atomic signs. As an immediate consequence of these various activities, proteases impact replication of DNA and transcription, cell multiplication as well as separation, tissue processes and redesigning, heat stun and unfurled protein reactions, neurogenesis, angiogenesis, ovulation, wound fix, undifferentiated organism activation, hemostasis, aggravation, autophagy, blood coagulation, aging, and cell death pathway. Steady with these basic jobs of proteases in cell conduct and endurance and demise all things considered, modifications in proteolytic frameworks underlie different neurotic conditions, for example, cancer, neurodegenerative clutters, and provocative and cardiovascular infections. In like manner, numerous proteases are a significant focal point of consideration for the antibiotics business as budding medication targets and analytic and prognostic biomarkers. Proteases additionally assume key jobs in plants and add to the handling, development, or demolition of explicit arrangements of proteins in light of formative signs or to varieties in natural conditions. [22]

Types and Structure of proteases

Proteases are also called as peptidases because of peptide bond hydrolase. Peptidases are generally classified as endo and exopeptidases. The Endopeptidases separate peptide securities and focuses inside the protein and exopeptidases expel amino acids successively from N-or C-end.[30]

At present there are six types of proteases which are divided according to their catalytic action and these are:

1. **Serine Proteases**
   Serine proteases also called as serine endopeptidases are a set of peptidases which are portrayed by nearness of serine buildup inside the dynamic area of the protein. The prime most tribes found in the people incorporate the, alpha/beta hydrolase the subtilisin-like, the, chymotrypsin-like and sign peptidase actions. blood thickening insusceptibility, and aggravation, are some of the function which are performed by serine proteases. They have also contributed in to stomach related compounds in eukaryotes and prokaryotes. Serine exo- and endo- peptidases are of amazingly boundless event and differing capacity. The two of the biggest are subtilisin-like chymo-trypsin like families. Both the groups are recognized by a profoundly comparative game plan of synergist Ser His and Asp buildups in profoundly extraordinary α/β (subtilisin) and β/β (chymotrypsin) protein platforms. These serine proteases are similar to the induvial from the subsequent faction, are named as '**subtilases**', which happen in yeasts, microscopic organisms, growths. [21]

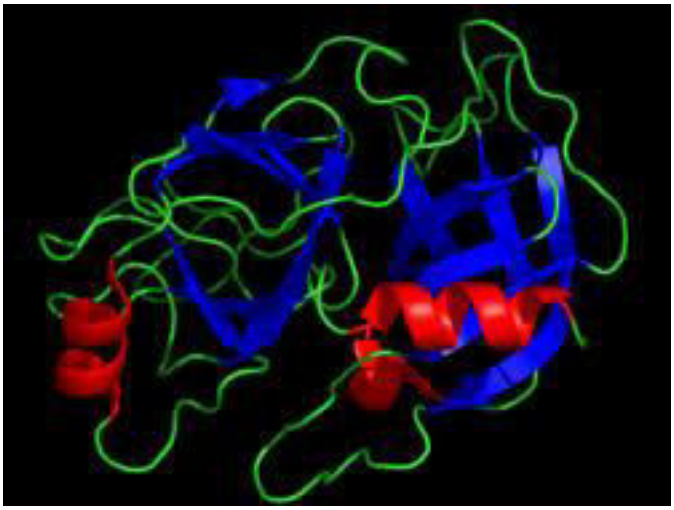


Figure 1.
Structure of trypsin a typical Serine Protease [35]

2. **Aspartate Proteases**

Aspartyl proteases otherwise called as aspartyl proteinases or corrosive proteases are mainly conveyed subset of proteolytic proteins having a place with the catalyst group of endo nucleases. Aspartyl proteases are said to exist in plants, vertebrates, plant infection, just as retroviruses. The underlying system of how these proteases cut the peptide-bond was planned for showing a covalent acyl transitional. Be that as it may, it has found to be certain that there is no covalent halfway in this group of proteases, in contrast to this firmly associated group of serine proteases. Aspartate proteases are a profoundly explicit proteases group - they will in general separate dipeptide bonds that have hydrophobic buildups just as a beta methylene bunch. Aspartyl proteases play a significant job in a few parts of our general wellbeing and physiology, including pulse, assimilation (chymosin and pepsin), and in the development of the (HIV I protease) called as Human Immunodeficiency Virus. Aspartic proteases have a place with the pepsin family.[21]

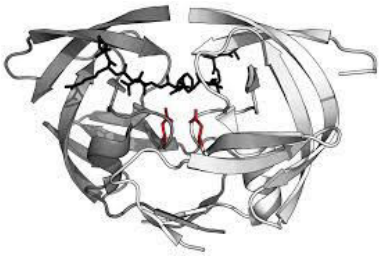


Figure 2. *Reteropepsin-HIV-1 Protease* [32]

3. **Cysteine proteases**

A group of proteases that possess an ordinary impulsive mechanism that entails a cysteine thiol which is present in a catalytic triad. It is imitated by removal of protons of a thiol present in the lively site by utilizing its neighbor amino group acid with a simple side chain, generally the histidine remnant. Further comes the nucleophilic assault with help of the deprotonated cysteine's sulfur (anionic) present on the carbonyl carbon. In this pace, a particle of the substrate is put-up along with an amino-terminus, the protease having the histidine remnant is reinstate to its deprotonated structure, and a thioester median connecting the brand new carboxy-terminus of the substrate with the cysteine thiol is established. The principal members of this team are calpains, caspases, cathepsins, and papain. The torrent of proteins involves several members of aspartate specific proteases.[21]

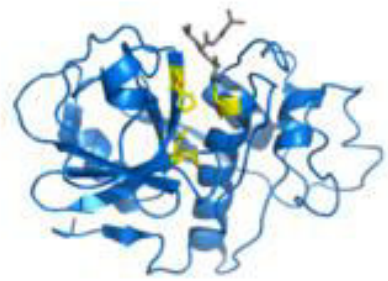


Figure 3. *crystal structure of Papain* [24]

4. **Metalloproteinase**

The metzincin superset, which relates to the metalloproteases, conceal an exceptionally preserved zinc-binding motif holding three histidine remnants that joins zinc, and a preserved methionine-revolve in the active-site helix. The metalloproteinases comprise of astacins, serralysins, adamalysins and matrix metalloproteinases (MMPs). Metalloproteinase comprises of metal ion like Ca^{2+} or Zn^{2+} into their active place. The ion generally serves to interrelate two or 4 aspect chains and it is necessary for the exercise of the enzyme.[21]

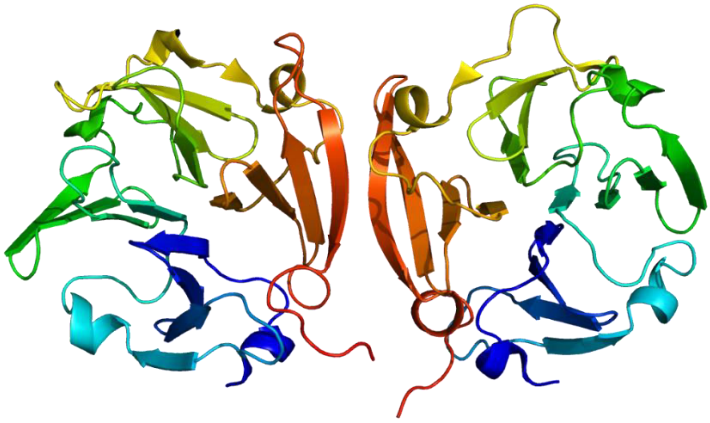


Figure 4. *MMP 9* [13]

5. **Threonine Proteases**

The proteasome hydrolases represent a special household of these type of proteases. A preserved N-terminus threonine is present in catalytic mechanism at every active place.

The three catalytic sub number which are harmonized as pre-defined proteins. Catalytic threonine proteases are uncovered at the luminal periphery. At present 29 threonine proteases have been established in rat degradome that consist of minimum of 626 homologs and proteases.[21]

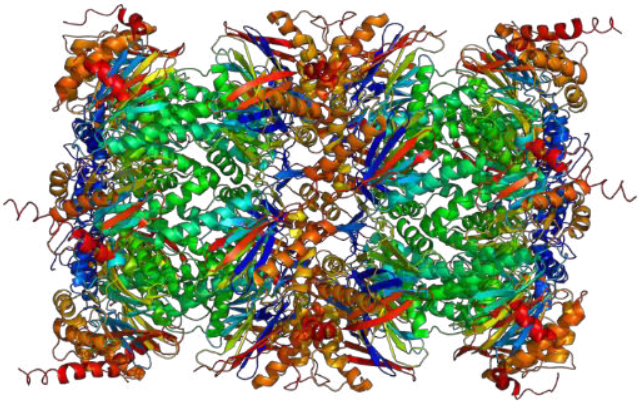


Figure 5. *Proteasome alpha 1* [14]

6. **Glutamic proteases**

lately the glutamic family of proteases has been re classified as the 6th type of proteases or family G1 as stated in the MEROPs database. Recent analogy of the molecular shape and catalytic mechanism has recognized these enzymes as an innovative protease family, the Eqolisins, a identify have arrived from the glutamic acid, active-site residues and glutamine. Members of this freshly diagnosed household of peptidases have a precedent undescribed ß-sandwich as a tertiary fold and a novel catalytic dyad incorporating glutamate and glutamine residues. The solely in the past remoted examples of glutamic proteases are from Scytalidium lignicolum, Sclerotina sclerotiorum, Cryphonectria parasitica Talaromyces emersonii and Aspergillus niger, all filamentous fungal species of the Ascomycota phylum.[21]

Figure 6. *scytalidocarboxyl peptidase B* [15]

Occurrence of Proteases

Proteases are a large group which are found in every organism from prokaryotes to eukaryotes and even viruses. These proteolytic enzymes that is the proteases are involved in large no. of organic reactions, which goes from synthesizing of food to highly regulated and maintained pathways.[18] A very common example is Blood Clotting Cascade. Let's see different roles of proteases in different organisms: -

- **PLANTS:** Plant proteases are associated with formative guideline and guideline of regulation of biological process like recognition of pathogens, and activating other proteases in photosynthesis.[9]
- **ANIMALS:** Pepsin (corrosive protease) emitted into the stomach and trypsin and chymotrypsin (serine proteases) present in the duodenum aids processing of proteins present in nourishment. Plasmin, Thrombin and so on. Proteases present in the circulation system, helps in the blood coagulating and the lysis of clumps. Cathepsin-G and elastase are the proteases which are available in the leucocytes and aides in controlling the digestion. Pit snake haem poison (snake venom) is additionally a protease, which hampers the casualty's blood coagulating course.[18]
- **BACTERIA:** Proteases emitted by the microorganisms, helps in hydrolyzing the peptide bonds present in the protein structure which further breaks the proteins to their separate monomers. Worldwide nitrogen and carbon cycles reuse the proteins which are helped by the bacterial and parasitic proteases. Peptidases are available in the microscopic organisms, which considers answerable for controlling the protein quality; by the corruption of misfolded proteins or the proteins left unfurled.[18]
- **VIRUSES:** One large polyprotein is expressed by viruses as their complete genome and they use a protease for cleaving the polyprotein into its constituent functional units like norovirus, polio and TEV proteases. TEV proteases are highly specific.[18]

Some examples of Proteases

- **PEPSIN:** it is an aspartic protease which is found in the stomach. It is said to be one of the first enzymes which was discovered and characterized.[18]
- **TRYPSIN AND CHYMOTRYPSIN:** they are serine proteases which was discovered from the pancreatic secretions.[18]
- **PAPAIN:** it is a cysteine protease which was discovered from papaya in the late 1800s[18]
- **THERMOLYSIN:** it is a metalloprotease which was discovered from thermophilic bacteria and it is said to be the first metalloendoproteinase to be crystallized and have its pure structure.[18]

CHAPTER 1

1.1 ROLE AND FUNCTION OF PROTEASES IN CANCER

In normal cells the proteases functions and show their activity in biological process. In the living systems there is an equilibrium between the proteases and anti-proteases, but disturbance in this equilibrium can make up too many diseases such as cancer- initiating from tumor progression, metastasis and lastly takeover into some other active site.[29]

All the steps that lead to cancer the role of proteases has been found aggressively in many recent studies. They have been involved tumor growth and cancer progression present both at primary and metastatic sites. The release of proteases has been monitored in many tumor cells that cause heavy devastation. The recent discoveries of the genome sequence seen in different organism has aid assistance in the recognition of the entire protease collection, which is also called as degradome. This degradome consists a minimum of 569 proteases and homologs. [8]

The process leading to cancer is a complex process which involves certain alterations in the normal cell. Tumor growth drives continuous rounds of mutation and survival**.** Neoplastic cells are formed slowly and gradually from normal cells. Epigenetic changes that is observes in normal epithelial cells causes tumor production and growth. These changes in the epithelial cells leads to disruption of the intracellular contacts and thus leading to the discharge of cells from the epithelial tissue. From here tumor invasion takes place followed by metastasis which occurs due to interconnection in endothelial cell, fibroblasts, tumor cell and invading inflammatory cells.[37]

Throughout all the steps leading to tumor growth and then cancer all the types of proteases are involved. Recent studies and discoveries have proved that these proteases target wide range of substrates and govern many processes that are important for life and death of cell altogether. The

organism which promotes this tumor evolution, an incontrovertible fact that significantly changes the normal view of the function of proteases performed in different stages of cancer.[19]

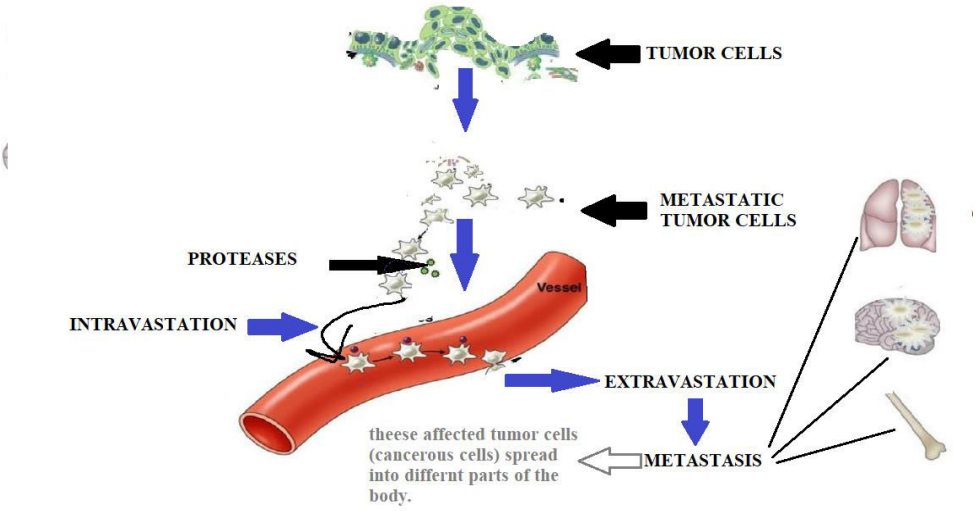


Figure 1.1

Dissemination and colonization of tumor cells, showing the role of proteases activating other protease cascades

1.2 Proteolytic cascades: maintain the activation of protease

So basically, the activity of proteases is seen in the last stages of tumor growth, metastasis and invasion. The extracellular proteases simultaneously sway over matrix degradation and invasion in the tumor cell through proteolytic cascades. Each protease plays a definite role starting from tumor growth to progression and then metastasis. The proteases are manufactured as zymogens which is then converted into an active enzyme by the process of autocatalysis. These proteases then interact with each other that results in the invigoration of other proteases forming protease cascades and unitedly degrading all extracellular matrix components. Therefore, during cell invasion these proteases debase the extracellular matrix by degrading all the components of it. [19]

1.3 Tumor stromal interactivity: maintaining the expression of proteases

Indirect and direct relationships between stromal cells and tumor cells can stimulate the appearance of protease in vitro. In fibroblast expression of protease gelatinases, A and B is escalated due to unmediated contact with tumor cells. This indicates that when cancerous cells attack the basement membrane and then

travel to stroma, these cells construct a straight contact with fibroblast cells and thus elevate the protease expression in them. proteases are also regulated by diffusible factors like growth factor, e.g. Insulin shoot up the proclamation of cathepsin D in human breast carcinoma cells.[19]

1.4 Matrix Degradation both Intracellular and Extracellular

CATHEPSIN one of its type plays potential role in degrading the extracellular and intracellular matrix of proteins. This activity put forward the progression of cancer activity. [26] Cathepsins are able to guide the proteolytic cascade due to which they activate other proteases like MMP's urokinase etc. in the tumor microenvironment. They are useful and potential biomarkers and help in detecting the patients of those suffering from breast cancer, colorectal cancer, tongue carcinoma and pancreatic cancer.[25]

FAMILY OF CATHEPSINS	PROTEASE	SITE /LOCATION	TYPE OF CANCER
Cysteine cathepsins	General cathepsin K	Lysosome, bone	Breast cancer
Aspartic cathepsins	Cathepsin D	lysosome	Breast, ovarian, colorectal
Serine protease caspases	Upa	Membrane	Lung, ovarian, prostate, head and neck
ADAM	MMP-14	Extracellular membrane	Malignant gliomas, breast, colorectal

Table 1.[12]

Different types of protease and their location in cancer

1.5 Involvement of proteases in pre malignant lessions

MMP'S and its type produce certain alterations that lead to tumor production. Increased activity of MMP's has been observed in many types of tumor. These MMP'S are responsible for altering and disturbing the original model of ECM proteins.[12] Clearly this proves that MMP's participate in initial stages of tumor progression and caner development inside the body.[1]

1.6 overexpression of protease in tumor environment

Cytotoxic agents like Adriamycin can be provided inactive by the peptide chains for example in cysteine proteases. The overexpression of proteases is acknowledged by inventing novel drug targets which remain inactive until activated by a protease. This shows their overuse and role in the tumor environment and in developing stages of cancer. [19]

expression of Proteases is also regulated by parcine factors which are produced from tumor and stromal cells. these studies done in vitro tells us about the increase in the role and expression of proteases performed and observed in the tumor microenvironment. [19]

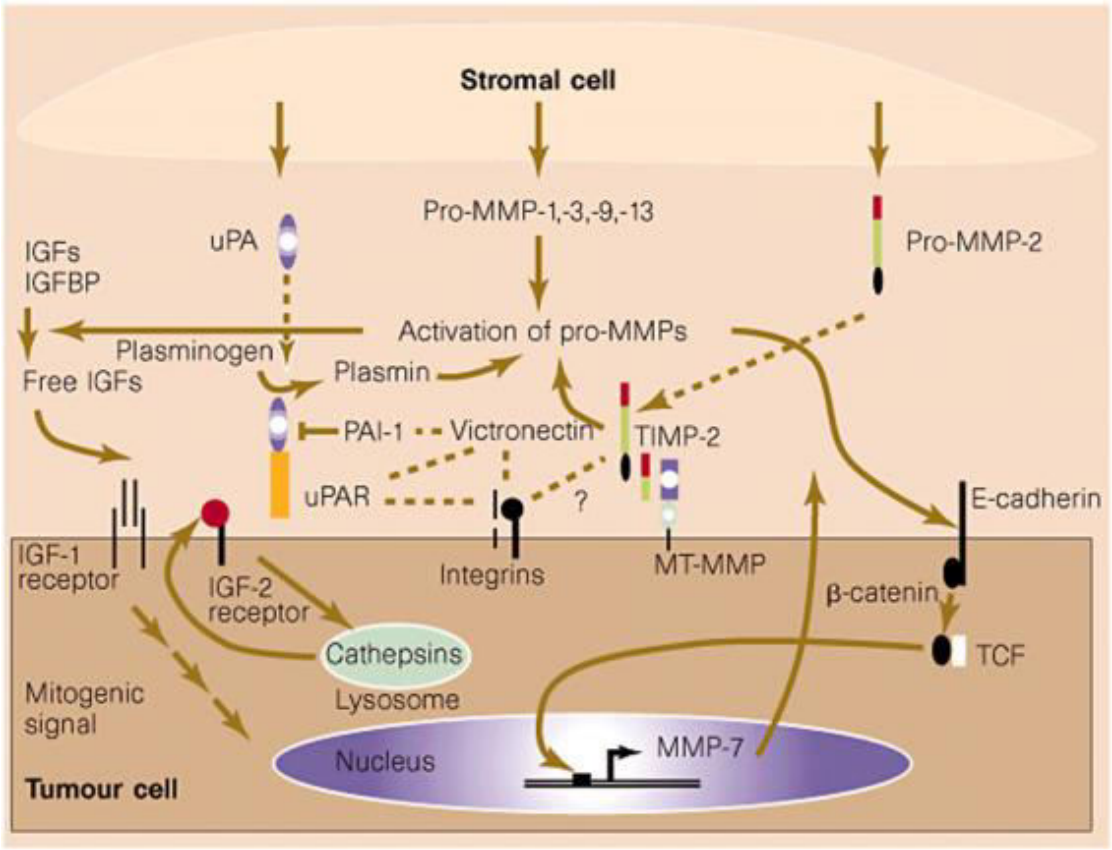


Figure 1.2[12]

the co relation between tumor cells and stromal cells as well as the role of proteolytic enzymes in cancer pathogenesis.

The participation of proteases in cancer advise the work of protease inhibitors as ANTI CANCER DRUGS in cancer therapy. The PI's are of several types depending upon their mechanism od of action or either the type of protease they hold back. These PI are therefore used as therapeutic targets to find against cancer for which we called as anti-cancer therapy.

CHAPTER 2

2.1 INTRODUCTION TO PROTEASE INHIBITORS

Protease inhibitors abbreviated as PI's are the family of drugs that is used to fight viral infections by preventing it from viral replication. Recent studies have shown that proteolysis have been very important aspect for the treatment of cancer. The viruses which encode proteases are used as therapeutic targets for the treatment of viral infections, the most harmful being cancer.[12]

There are various methods by which PI's perform their role and their effect depends upon certain arenas- its nature, structure and their collaboration with microenvironment. It differs even between the cancer that has arose on the same source. Through recent studies and discoveries many cases have been outlooked of PI's treatment in cancer, there were failures as well as success in some areas in therefore the PI's were took into strong considerations as pharmaceutical strategies to fight against CANCER.[12]

2.2 Protease Inhibitors: Role and Function

PI's are used as anti-cancer agents. In addition to that the large molecular network of proteins that interact with other proteins posses' different roles showing the importance of each and every molecule same as the different types of PI's would play favoring in the biological processes. These molecules play a potential role as therapeutic targets in the treatment of cancer. The design of the protease inhibitor is complex and vast because different categories of cancer use different types of proteases that participate at the changing stages of cancer development. Now let's discuss different types of protease inhibitors that take part in cancer treatment.[12]

1) Cysteine cancer protease inhibitors

cysteine proteases which is lysosomal known as Cathepsins can be monitored by a cysteine PI which is endogenous called as CYSTATIN. It is found in normal cells and tissues. Cystatin is a batch of interconvertible and hog-tied competitive inhibitors that impedes the proteolytic role of the cysteine peptidases. Cystatin plays an important activity in cancer treatment by doing certain alterations with the proteolytic management. The recent works have shown that they block the metastasis.[12]

The major role played by cystatin is to degrade the additional cysteine proteases activity from the lysosomes. As these cysteine process helps in the process of tumor growth, metastasis, these cystatins help to limit and control these processes. types of cystatins (type 1 and 2) has been most researched in cancer domain. These different types of cystatins have been used to fight against breast cancer. The main species of cystatin type 1 like Stefins A and B have been used in treatment of lung cancer by increasing their levels. Low levels of stefins A can contribute into neck and head cancer and therefore their levels are amplified. [12]

INHIBITORS	TYPE OF CANCER	ILLUSTRATION
Stefin A	Breast cancer	Limits the process of metastasis
Stefin B	meningiomas	Degrade protein and message levels
Cystatin SN	Colorectal cancer	Tumor marker
Cystatin D	Human colon cancer	Subdue tumor growth gene which is induced by Vitamin D

TABLE 2.[12]

selected cysteine cancer protease inhibitors which help in the treatment of cancer.

2) Serine cancer protease inhibitors

Matriptase which is a transmembrane of type II is a serine protease is intricated in the decay of angiogenesis and in the maturation of epithelial cancers. In unaffected tissues and cells matriptase is constituted by a hepatocyte growth factor activator inhibitor called as **HAI-1**. The loss of this

inhibitor factor results in the maturation of human prostate cancer (CaP) and development of matriptase. These both proteins have been seen as a commending biomarker for the progression of CaP. It is also a marker for demonstrating the success of chemo preventive and therapeutic mediation.

SERPINS are as set of proteins which act as serine protease inhibitors to degrade and stop the activity of serine proteases involved in progression of cancer. [12]

TYPE OF SERPIN	TYPE OF CANCER	ILLUSTRATION
SERPINA1	Lung cancer, colorectal cancer	Act as serum biomarkers for the detection of these cancers.
SERPINA3	Prostate cancer	Foresee the bone metastasis of prostate cancer. Act as an biomarker.
SERPINB3	Breast cancer, tongue cancer, cervical cancer	Prognostic tool
SERPINI2	Pancreatic cancer	Inhibit the metastasis of pancreatic cancer.
SERPINB13	Brain and ovarian cancer	Plays role in single nucleotide polymorphism and in the treatment of cancer

TABLE 3.[12]

some of the important inhibitors that is used in the treatment of cancer.

3) Aspartate cancer protease inhibitors

There are several inhibitors which has been found in the treatment of cancer. Cathepsin is an aspartic protease which has been seen as overly expressed in the breast cancer epithelial cells. These proteases regulate the cell lines of breast cancer and the quantity of it decided the level of metastasis. So basically, it has been seen as a functional factor playing many roles most importantly in carcinogenesis. In breast cancer cathepsin D is formed under the regulation of estrogen hormones. High levels of cathepsin D have been seen in neoplastic tissues and therefore antagonists are designed in the treatment of breast cancer as it doesn't allow the protein to interact

with its receptor by blocking it. Pepstatin an aspartic protease inhibitor is been currently studied to test it in the treatment of cancer.[12]

4) HIV Protease Inhibitors

HIV-1 protease inhibitors (HIV-PIs), that focus on the viral aspartyl protease which divides forerunner proteins into segments of viral center in the HIV-replication process. Taking into account that the premise to the action in the two illnesses is the control of explicit objective chemicals which are required for the cell to build up the threat or on account of infection, the pathogenicity. The counter tumor impact of HIV-PIs fluctuates between every sort of inhibitor. Looking at some of most powerful HIV-PIs, the most productive inhibitor was nelfinavir. [2]

This inhibitor demonstrated to have fascinating outcomes with respect to its instrument; it had the option to repress the initiation of Akt flagging pathway in human non–little cell lung malignant growth cell lines and in human umbilical vein endothelial cells, yet not in human melanoma cells, where nelfinavir enact the Akt pathway. Nelfinavir without anyone else advances cell demise in two unique manners: with caspase-subordinate apoptosis and caspase-autonomous apoptosis went with autophagy and acceptance of ESR. The hindrance of cell development by nelfinavir is given for a G0-G1 cycle capture that is identified with nelfinavir-interceded proteasome-corruption of Cdc25A phosphatase, without restraint of chymotrypsin-like action of the proteasome; the debasement of Cdc25A triggers the restraint of cyclin subordinate kinase 2 (CDK2) and associative dephosphorylation of retinoblastoma tumor silencer.[2]

5) ER Stressors

Turmoil to Endoplasmic Reticulum homeostasis that can't be extricated by the unfolded protein response (UPR) leads to autophagy and cell death. Neuroblastoma cells when treated with ER stressors considerably leads to the formation of autophagosomes, which were diagnosed at the ultrastructural level.[6] The green fluorescent protein (GFP)-LC3-labelled structures, thus formed represent autophagosomes. They are extensively prompted in cells exposed to ER stress, with the conversion of LC3-I to LC3-II by using ER stress inducers like Tunicamycin, DTT and MG132. This conversion is the marker of autophagy and also the downregulation of mechanistic target of rapamycin (mTOR).[27]

6) Matrix Metalloproteinase Inhibitors

The MMP's show significant role in cancer progression by degrading the extracellular matrix and invading the normal cells and tissues. Throughout the process MMP plays role in tumor growth and therefore it is clear that the activity of MMP in controlled in this whole process of cancer progression.[21] The proteolytic functions of MMP is lower down by inhibitors which are non-specific protease inhibitors for e.g. α2-macroglobulin and α1-antiprotenase and also specific PI's like tissue inhibitors (TIMP). The application of TIMP was revealed by their capacity to inhibit tumor progression in cancer cells.

On the other side, there were many small molecule synthetic inhibitors which are involved as anti-cancer agents. One of its types called as peptidomimetric inhibitors copies cleavage site of collagen and helps in chelating the zinc atom on the active site of MMP. On the contrast there are non-peptidomimetics which are constructed according to the active site of MMP's. a large group of sulfonamides MMP inhibitors are designed in tumor necrosis factor. MMP along with TACE inhibitors is developed as a potential target for drug design in anti-cancer therapy.[2]

7) Other PI's

SECRETORY LEUKOCYTE PROTEASE INHIBITOR

SLPI's are the family of proteins which is majorly present at the upper respiratory tract. These SLPI's degrades the function and activity of many proteases involved in cancer activity such as cathepsin B and G, trypsin, chymotrypsin, mast cell chymase and tryptase. Though SLPI has been seen in many development roles like tissue repairing anti-inflammatory, it has also been seen in increasing the malignancy of cancerous cells. Therefore, this brings down to the formation of its inhibitor as it is principally corelated.[2]

Recent studies have also shown that SLPI has been functioning in lung cancer, gastric cancer, ovarian and cervix cancer, pancreatic carcinomas. This brings down to the fact that all the above cancer types can be suppressed and degraded if the level of SLPI is leveled down or completely absent. So, SLPI can become an efficient biomarker in these types of cancer. One favorable function of SLPI can be seen in liver metastasis as it acts as a protective gene because it decreases the potential of metastasis in liver carcinoma cells.[2]

An interesting fact about SLPI is that even when it is able to act as an anti-angiogenesis factor by repressing the travelling of newly formed blood vessels and is able to impede the migration

function of the vascular endothelial cells , both in vitro and in vivo, SLPI can also persuade sinusoidal vasculature in principal tumors which is marked as the first pace of an invasion - independent pathway of cancer metastasis. [2]

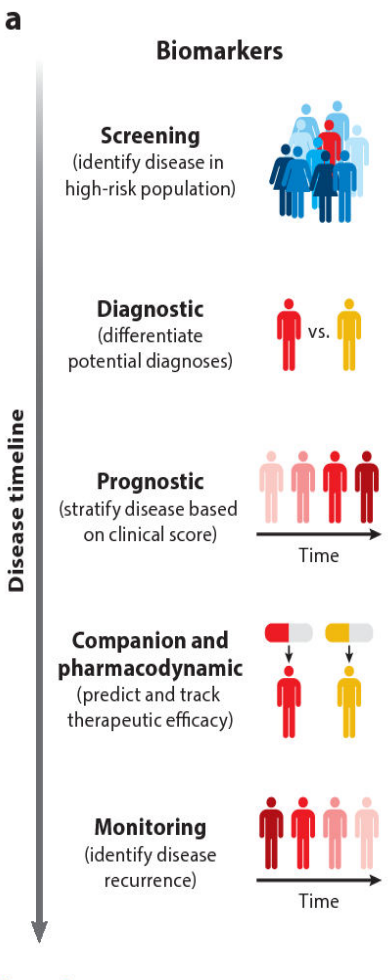
Governance of cancer will gain profit from the utilization of biomarkers. Observing and detecting tumors at early stages efficiently improves the result of both patients and healthcare system. target specific and sensitive biomarkers are highlighted through which therapeutics can be made and targeted during the early stages of cancer.

2.3 PROTEASE ACTIVITY AS A FUNCTIONAL BIOMARKER IN CANCER

Biomarkers are basically measured indicators of biological processes or they are the responses against the treatment. These biomarkers are very efficient and have the potential to impact on diseases. Since proteases portrays an essential role in the growth of cancer, its activity may bring us to make robust biomarkers. These protease-based biomarkers can be used for therapeutic response and targets and also to detect protease activity at an early stage. A good biomarker is decided upon which type of biomarker is needed at what time. In addition, that these biomarkers are needed according to the type of cancer. For say, prostate cancer type could be targeted by prognostic biomarkers which tells us about the effectiveness and aggressiveness of the disease at the time of speculation. Another one like in ovarian cancer biomarkers can be used to detect it at early stage because that is the only, we can cure it, as majority of patients are detected with ovarian cancer at late stages and then the cure seems to be quite impossible.[10]

2.3.1 Early diagnosis and detection: - the first method for therapeutic intervention is diagnostics and detection which should be precise. There have been several outcomes which approaches the function of protease activity seen in the early stage of the cancer growth. So, if the protease and its type is detected and diagnosed at early stage of cancer, chances of cure and treatment is increased to a very high level and through biotechnological methods it could be treated. There have been many tools used for e.g. substrate-based probes with fluorescence, near infrared spectrum etc. [17] these tools make a signal during a proteolytic cleavage in the tumor cell environment. For e.g. in mouse having lung cancer millimeter sized tumors were found using a three-dimensional fluorescence molecular tomography. [4]

2.3.2 Companion and pharmacodynamic: - the diagnostic and therapeutic strategies are crucial to make an accurate medicine. Many novel protease- activated therapeutics are combined with technology to examine proteolytic activity or predict the patient's movement for better therapeutic development.[10] There have been few technologies invented in which they can be specifically activated in the tumor cell area in-vivo so that they can detect the protease activity inside tumor microenvironment. Pharmacodynamic biomarkers can be effectively used to detect the protease activity as well as giving an evaluating and monitoring the efficacy of drug or antibody. [31]



b

	Technology	Methodology	Advantages and caveats
Imaging Cleavage-based	Quenched substrates (Hilderbrand & Weissleder 2010)	Peptide substrate cleavage generates image contrast	(+) Generalizable to any protease by changing peptide substrate (–) Lack of multiplexing of substrates due to limits of imaging and need for imaging equipment
	ACPP (Savariar et al. 2013)	Cleavage exposes cell-binding motif	
	Nanomaterial architectural changes (Harris et al. 2006)	Cleavage changes nanomaterial properties	
Imaging Binding-based	Antibodies (Thorek et al. 2016)	Antibody that only binds active protease	(+) Ability to identify protease target to validate specificity (–) Not generalizable to all proteases (e.g., MMPs for ABPs) and modification of proteases in vivo
	ABPs (Sanman & Bogyo 2014)	Covalent binding of probe after activation by protease	
Urine readout Cleavage-based	Synthetic biomarkers (Kwong et al. 2013)	Cleavage of peptide substrate releases urinary reporters for downstream analysis	(+) Generalizable to all extracellular endoproteases (+) Multiplexing of substrates and flexibility in urine analysis (–) Inability to localize proteolysis
Other ex vivo assays	Microfluidic or tissue biopsy imaging assays (Chen et al. 2013)	Measurement of protease activity in samples using fluorogenic peptides	(+) Measure changes in proteolysis without need for injected probes (–) Unclear how circulating changes of protease activity reflect tumor pathology
	Serum proteomics (Villanueva et al. 2006)	Tumor-specific cleavage patterns in serum	

Figure 2.1.[10]

Flow diagram showing how protease activity can be used as measurements to generate biomarkers and how they can be used in early detection of cancer.

2.3.3 Monitoring and Disease kinetics: - in this method monitoring of protease activity is done which should be actually simple and done at the time of care. Various types of assays are performed one of which is zymography. As these proteases have been seen in many biological samples like in blood and urine therefore, it is quite remarkable to make their biomarkers to measure its activity.[10] An alternate approach to monitor the therapeutic response is by using a radiolabeled antibody probe to measure and image the pathway of protease receptor in cancer which becomes an important therapeutic target. For e.g. in prostate cancer antibody probe is sent for active KLK2 to portray the androgen receptor (AR) pathway. KLK2 is managed by the antibody and AR pathway which is sent monitors the response of castration, this experiment showed us the specificity of KLK2, telling us the importance of selection of protease biomarkers.[33]

2.4 PROTEASES USED AS THERAPEUTIC TARGETS

Due to essential role of proteases in cancer they have been used as therapeutic targets. On one side where we focused on the main functions and signaling pathways of proteases, the other side they are also diverse in their roles in the proteasome where they can be therapeutically targeted.[23] Earlier studies showed that one of the proteases MMP's were targeted along with the inhibitor and came out with the result that broad inhibition would affect the cancer growth. Foer this to work efficiently there should be research on broad spectrum about therapeutic strategies and diseases. More advanced understanding of proteases and more approachable therapies have been taken in interest in proteases to be used as therapeutic targets. Another alternative approach which has been seen is using protease activity to activate therapeutics without directly balancing the enzyme on its own.[10]

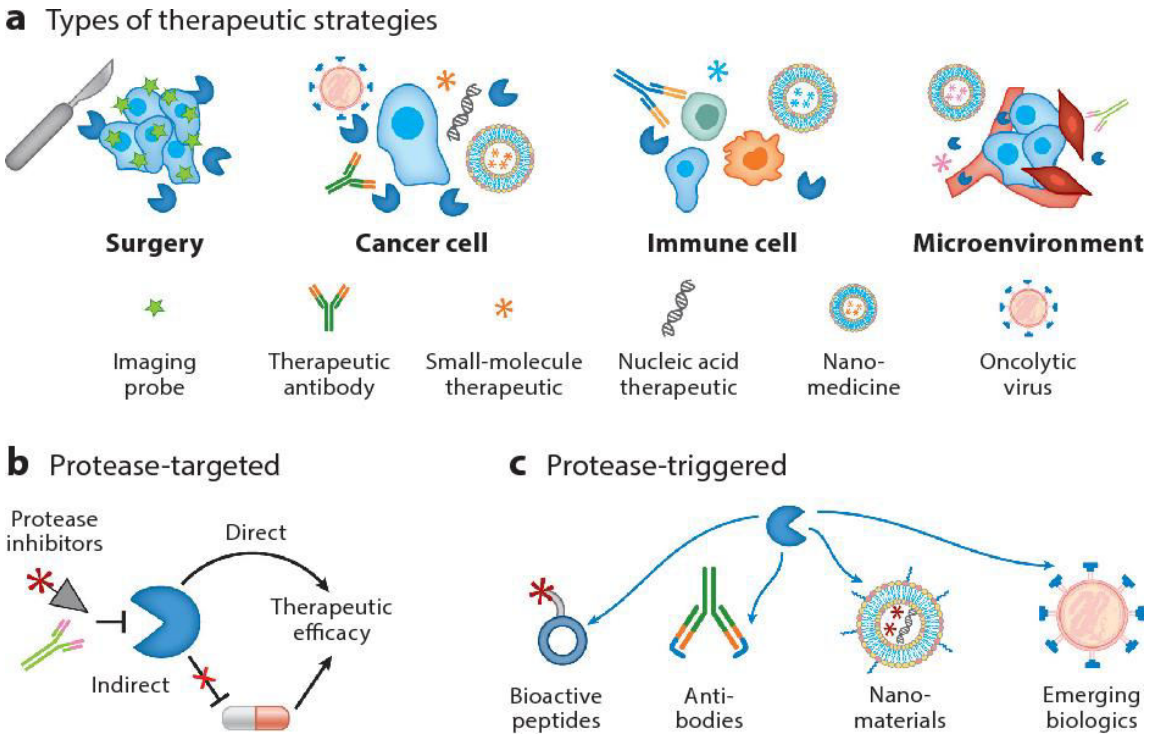


Figure 2.2.[10]

different types of therapeutic strategies. a- tells different methods to harness protease work like targeting cancerous cells with several immune cell targeting, therapeutics. b- inhibition of protease can be therapeutic straight away by obstructing the cancer growth activities or hindering with efficiency of other drugs. c- protease can pretend as targets to start off several kinds of therapies like antibodies and nanomaterials.

2.5 Importance of target selection of active proteases

There have been many failures in a wide spectrum of protease inhibitors which brings us to the fact that the selection of target is very important. It should specific and pharmaceutically suitable to in order to achieve successful protease inhibitors involved in the treatment of cancer treatment. Efficacy and specificity of target selection can be obtained by new screens that utilize ABP's as a display, permitting the simultaneous testing of a substance both happening off and on the enzyme target. Target sites of enzymes like hemopexin domain of MMP9, not highly protected around MMPs and therefore it has the potential for upgraded selectivity and its efficiency. [11]

Now there have been new targets enabled by biologics which is typically not authorized to small molecules approaches. MMP9 which is an allosteric binding antibody notably decrease the tumor growth and metastasis in orthotropic colorectal cancer mouse model. there were no toxic effects laid down. Antibodies like antibody variants or even antibody like molecules (e.g. knottins) has boosted up the therapeutic targeting of proteases.[10]

2.6 Active proteases as therapeutic targets

Active proteases are used as therapeutics since 1908 because protease cleavage moieties paired up to chemotherapies. There are many novel proteases which are triggered as therapeutic targeting incorporating more experienced activation strategies in cancer environment, which is advantageous for therapeutics with less toxicity.[5] The difficulty in the function of protease reveal the importance of target selection and therefore it is important to target the protease with the addition of more than one therapies. Therapies which are protease targeted specific can be useful to treat cancer by going into the activated tumor microenvironment. New techniques for e.g. protease-activated toxins, oncolytic viruses, cellular therapies are similarly advantageous and may benefit in the same way from selective activation in the tumor microenvironment. [10]

2.7 Mechanism of Protease Inhibitors in Anti-Cancer Therapy

The mechanism which are helping in the work of anti-cancer activity are Akt inhibition, endoplasmic reticulum stress unfolding protein response pathways. There are more effective pathways other than mentioned. They can be directly or indirectly dependent pathways. Some may have MMP-2 and MMP-9 obstruction, decrease in the regulation of androgen receptor, proteasome, FAS (Fatty Acid Synthase), survivin, autophagy, downregulation in cellular ATP concentration and increment of Bax, DR5 and

TRAIL receptor. These upshots result in reduction in invasion and upregulation in apoptosis of cancerous cells. Hence, the results satisfy in decrement in proliferation and therefore increment in cancer cells death. Special methods and trails are conducted at anti-cancer pharmaceuticals. In triple negative breast tumors, the major thing that came out is that, these cancers don't behave to ordinary anti-cancer therapies.[7]

- **NFV** is the right inhibitor in cancer treatment as it may work in different environments like in adipocytes, and hepatocytes and also in tumor cells. [20]
- **Phycocyanin** is a common, non-fatal, water dissoluble substance which has governing anti-cancer properties and therefore is regarded as the upcoming anti-neoplastic representative for triple negative breast tumor carcinoma.[20]
- **Epigallocatechin gallate (EGCG)** is a prime polyphenol which contains anti-cancer properties. To epitomize the arrangement of EGCG activity, restricting tests within the sight of EGCG-Sepharose and HSP90 freaks were performed. Scientists utilized, tumorigenic, nontumorigenic (NT) and metastatic tumor cells from a novel human prostate tumor little, to test that affectability is identified with HSP90 hindrance. From the investigations, it was apparent that EGCG – Sepharose crunched more HSP90 from metastatic cells when contrasted with nontumorigenic cells and restricting was through the HSP90 C terminal. Additionally, EGCG crunched HSP90 freaks that imitated both complex and uncomplex HSP90. EGCG initiated changes in HSP90 customer proteins which are available in nontumorigenic cells and huge contrasts were made in metastatic cells. This demonstrates EGCG might be compelling for the disease treatment and frustrates a chaperone; steady of the harmful phenotype.[20]

CHAPTER 3

FUTURE ASPECTS AND SCOPE

3.1 Potential Therapeutic Targets in Cancer

In the process of cancer, the cancerous cells gain a selective widening advantage to undergo the process of programmed cell death. Defect in genes present in the death of cell are actually related with different kinds of malignancies. The rapid death of cells is a hallmark of cancer cells. The cell death is an indication promoting carcinogenesis which is due to alterations in the gene and activation of oncogene in cancer cell and these cancerous cells have started to show resistance against chemotherapy by maintain a threshold against the death signals.[3]

The best described working of programmed cell death, implies targeted proteases by activated caspases, and this area of apoptosis is managed by the equilibrium of pro-apoptotic and anti-apoptotic signaling cascades. In the process of cancer increment in the anti-apoptotic survival pathways and decrement of pro-apoptotic pathway diminishes apoptosis.[3]

3.1.1 Conventional Targets: Conventional therapeutic targets are more reliable as these agents attain higher cytotoxicity in cancer cells. They reached this either by targeting the components of DNA synthesis, DNA damage or mitotic channel. Some of conventional targets are anthracyclines, anti-metabolites and topoisomerases. On the other hand, taxane, vinca alkaloids, and epothilone prey growth of cell cycle by hampering the cytoskeletal components which is acquired in cell division. [3]

3.1.2 Emerging targets: In the past few years, there have been many therapeutic agents discovered which is used as a combined therapy to produce a larger effect on cytotoxicity in cancer cells. This is achieved by increasing the specificity of targets that target into the biological pathways. These newly discovered agents comprise of small molecule inhibitors that actually targets the growth factors of the signaling pathways and survival pathways. Some of them are EGFR kinases, ErbB2, Ras. In continuation to this there are several other therapeutics like immunotherapy, cytokinin's, viral antigen-target therapy epigenetic therapies, metabolic pathways inhibitors are being researched and tested in laboratories in pre-clinical and clinical trials.[3]

CONCLUSION

Proteases and the concept of using them as therapeutic targets have become an interesting group of substances from the viewpoint in the modulation of research and finding out clinical remedies. As seen

the use of protease inhibitors have showed great efficiency against cancer, their mechanism of action has proved the extraordinary entangled process applied in the development of cancer. The anti-cancer consequences of PI's bring alterations at the genetic level as well as at protein level. Since proteases are functionally utilized in many stages of cancer, they are the most suitable to be targeted while being active in order to develop its treatment.[28]

There are two major ways where cancer can be prevented that is reduction of risk and early detection. Different techniques and methods may diagnose non-invasive cancer and provide immediate remedy before it becomes severe or on the other side is to detect the tumor/tumor cells at early stage which is treatable. This can be done by detecting the active proteases which function at the initial stage of tumor progression and thus used as drug targets for its remedy. The studies done on proteases as well as protease inhibitors describe well their role in cancer progression and therefore extend their therapeutic window for cancer treatment. Many studies and researches are still going on to find out different preceptive roles of proteases in cancer progression.[34]

Biotechnology once again proves here that natural and scientific methods can also treat a chronic disease like cancer. Biotechnological methods and research provide safe and natural remedy apart from chemotherapy in order to save patients' lives. There are many failures and success regarding protease used as therapeutic targets. Future studies observing in the interaction between proteases and its binding site are necessary to completely explore the efficiency of proteases for therapeutic intervention.

I hope that in this paper will throw light on this agenda and help in taking a fresh look at this topic on how proteases a simple group of proteolytic enzymes can provide medication and methods to treat a disease like cancer. This topic has diversified researchers the field of cancer and I hope that all the hard work and efforts will lead to greater techniques and methods as well as novel approaches to target cancer cells.

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