CHAPTER

3

Role of proteases and cytokines in cancer growth

Renganathan Senthil[1], *Thirunavukarasou Anand*[2], *and Konda Mani Saravanan*[3]

[1]Department of Bioinformatics, Vels Institute of Science, Technology and Advanced Studies, Chennai, Tamil Nadu, India [2]Faculty of Clinical Research, Sri Ramachandra Institute for Higher Education & Research, Chennai, Tamil Nadu, India [3]Department of Biotechnology, Bharath Institute of Higher Education and Research, Chennai, Tamil Nadu, India

3.1 Introduction

Proteases are categorized into certain families based on shared characteristics in their main structure (Caminero et al., 2023; Koistinen et al., 2023; Polgár, 2005). Homologous families are further organized into clans (Di Cera, 2009). Proteases constitute a clan with a common catalytic mechanism determined by the amino acids in the active site (Craik et al., 1987; Tyndall et al., 2005). These amino acids are aspartic, cysteine, glutamic, metallo, serine, or threonine (Herman et al., 2024). However, there are also proteases for which the specific amino acids in the active site are either unknown or a combination of several types (Lupas et al., 1997). Additionally, asparagine peptide lyases belong to this group of proteases (Rawlings et al., 2011).

To maintain homeostasis within the body under typical physiological circumstances, the activity of proteases is carefully regulated through various mechanisms (Singh et al., 2019). These mechanisms operate at multiple levels, including genetic and epigenetic factors that control the expression of genes and the synthesis of proteins (Rando & Verstrepen, 2007). Additionally, post-translational modifications play a role by influencing the trafficking and compartmentalization of proteins (Navarro-lérida et al., 2021). Another important aspect of regulation involves the activation of zymogens (Khan & James, 1998). The degradome refers to the intricate network and interplay of proteases, their inhibitors, and substrates. Its functionality is contingent upon the specific cellular environment and tissue physiology. In addition, proteases play a crucial role in a well-coordinated network of proteolytic processes and a hierarchical sequence of actions have been described in protease signaling (Turk et al., 2012).

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In the human genome, around 7% of the total genetic material has been identified as consisting of 1208 recognized proteases and 1857 probable protease inhibitor genes (Pennisi, 2001). When the state of homeostasis is interrupted, there is a potential imbalance in the protease-inhibitor-substrate interactions, leading to changes in protease signaling (Meyer & Jaspers, 2015). This phenomenon can be attributed to a pathological condition's etiology or outcome, as observed in numerous disorders (Moloi & Ngara, 2023). The phenomenon of modified protease signaling in cancer is commonly called the "cancer degradome" (Hanzl & Winter, 2020). The process of protease signaling is intricately interconnected with other forms of cellular signaling, collectively contributing to a wide range of physiologic and pathological phenomena. Common instances in cancer involve the interaction between kinases and proteases, as well as the interplay between proteases and cytokines that regulate angiogenesis, the composition and integrity of the extracellular matrix (ECM), invasion of cancer cells, and other signaling pathways inside the tumor micro environment (TME) (Martin & List, 2019). The dysregulation of protease homeostasis is a significant factor in the progression of tumors. Within the TME, cancer cells and stromal cells collaborate to form an intricate proteolytic network. This network has promotive and inhibitory effects on tumor growth (Vizovisek et al., 2021).

3.2 Proteases in cancer

3.2.1 Matrix metalloprotease

Matrix metalloproteases (MMPs) are a well-studied group of proteases in the context of cancer (Cabral-Pacheco et al., 2020). These proteases are zinc-dependent endopeptidases and can be classified into four main subclasses based on their protein-domain structure and sequence homology. The MMPs are responsible for the direct degradation of ECM proteins, but they exhibit variations (Levin et al., 2017). Furthermore, Endocytosis is possible through diverse mechanisms, leading to uptake into early endosomes. At the cellular membrane, MMPs have the ability to form associations with tissue inhibitors of metalloproteinases. The impact of MMP-dependent signaling on gene transcription is widely recognized. As an example, the upregulation of vascular endothelial growth factor-A was observed in response to the activation of MMPs (Eisenach et al., 2010). Conversely, in fibrosarcoma cells, membrane type 1-matrix metalloproteinase (MT1-MMP) was shown to modulate the expression of genes associated with the inflammasome. However, MMPs themselves can traffic to the nucleus and there act as transcription factors (Hey et al., 2022). In this connection, the relevant protease family is A disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) (Kelwick et al., 2015). These enzymes mostly function near cells, also known as the pericellular milieu. The secretion of proteases by cells can affect the TME through several pathways associated with oncogenic and tumor-protective functions (Quail & Joyce, 2013).

3.2.2 Serine protease

The serine proteases, which can be further categorized as trypsin-like and chymotrypsin-like serine endopeptidases based on their substrate selectivity, have been extensively

studied in relation to cancer progression (Patel, 2017). This system plays a crucial role in activating plasminogen to plasmin, which acts as a feedback activator for plasminogen activators (PAs) and various MMPs. Activating these enzymes leads to the degradation of ECM proteins (Saravanan et al., 2022; Sundaram et al., 2022). In addition to cysteine cathepsins, the urokinase-type plasminogen activator (uPA) and its receptor (uPAR) signaling pathways trigger the initiation of a proteolytic cascade, thereby promoting the invasive behavior of cancer cells (Adenis et al., 1995). In addition to its proteolytic activity, the uPA/uPAR system plays a significant role in cancer cell invasion through proteolysis-independent mechanisms (Johnsen et al., 1998). Specifically, the uPAR, attached to the plasma membrane, directly interacts with integrins and vitronectin, and subsequently facilitates cell adhesion and migration. The upregulation of uPA, uPAR, and PAI-1 has been observed in various cancer types, leading to their early identification as potential biomarkers (Duffy et al., 2014).

3.2.3 Kallikreins

Human tissue kallikreins (hKs) are released and exhibit trypsin or chymotrypsin-like activity (Yousef & Diamandis, 2001). One well-recognized biomarker in the field is hK3, also known as prostate-specific antigen, which is commonly employed for identifying and assessing prostate cancer. However, its significance is scrutinized due to its substantial elevation in non-neoplastic inflammatory conditions (Diamandis, 2012). Although there is still a limited understanding of the specific roles of different hKs in cancer, several research suggest that pericellular proteolysis mediated by hKs may play a significant role in cancer cell invasion, angiogenesis, and growth. This is primarily attributed to the modulation of cytokines and proteases. As previously indicated, it is important to note that not all proteases exhibit tumor-promoting properties (López-Otín & Bond, 2008).

3.2.4 Cysteine protease

Cysteine proteases, including papain-like cathepsins and caspases, were initially identified for their activity within lysosomes (Löser & Pietzsch, 2015). However, it is now understood that these proteases are also active in other subcellular compartments and even beyond the cell. Cathepsins were initially linked to heightened metabolism and the degradation of lysosomal proteins in cancer cells (Turk et al., 2001). However, subsequent research has shown a wide range of other diverse and different roles (Yadati et al., 2020). One notable example is their role in developing resistance to therapeutic interventions. Strojnik et al. (2009) provided evidence indicating that cathepsin L has anti-proliferative properties as a tumor suppressor in the Rip1-Tag2 mouse model (Strojnik et al., 2009). However, in APCmin and K14-HPV16 mice, cathepsin L was found to promote carcinogenesis. The study conducted by (Kenig et al., 2011) revealed that the elevation of cathepsin L in glioblastoma leads to an antiapoptotic impact by enhancing caspase 3 production (Kenig et al., 2011). This finding aligns with previous research showing that cathepsin L promotes drug resistance (Lankelma et al., 2010).

3.2.5 Caspase

Caspases play a significant role as proteases in both the initiation and execution stages of apoptosis. It is worth noting that disrupted apoptotic signaling pathways are considered a characteristic feature of cancer (Hanahan & Weinberg, 2011). The initiation of the caspase cascade involves the activation of caspases-2, -8, -9, and -10, which then activate effector caspases-3, -6, and -7 through direct or indirect mechanisms, ultimately leading to the induction of apoptosis (Zuzarte-Luis et al., 2007). The downregulation of caspases has the potential to result in compromised apoptotic processes, increased survival of cancer cells, and resistance to therapeutic interventions. As an illustrative instance, the lack of caspase-8 expression has been linked to the progression of certain types of cancer, such as glioblastoma. In contrast, it has been observed that caspase-8 is upregulated in various forms of cancer and contributes to the progression of malignancy, potentially through its non-apoptotic mechanisms. These mechanisms include the induction of nuclear factor-kappa B (NF-κB) signaling, modulation of endosomal trafficking, and regulation of autophagy (Viswanathan et al., 2019).

3.3 Role of proteases in stromal cells

Stromal cells play a crucial role in facilitating cancer cell proliferation, invasion, metastasis, and drug resistance within the context of TME (Bougnaud et al., 2016). Nevertheless, it is worth noting that the stromal cell expression may exhibit variations compared to cancer cells, potentially leading to distinct implications for the advancement of tumors. On the contrary, the expression of cathepsin L by cancer cells plays a significant role in developing pancreatic neuroendocrine tumors. This observation suggests that the impact of proteases produced by cancer and stromal cells varies and can even be contradictory, contingent upon the specific form of cancer being studied. The diverse and adaptable nature of the tumor concerning the evolution of cancer cells may have varying effects. Consequently, cellular cross-talks exert selection pressure on cancer cells, compelling them to compensate for the absence or inhibition of a specific protease by activating alternative proteases. The mechanism of molecular crosstalk between proteases and cytokines of tumor cells and stromal cells is depicted in Fig. 3.1. The TAMs in the RT2 PanNET mouse model exhibit elevated levels of cathepsin X, which serves as a functional substitute for the depleted cathepsin B and cathepsin S (Jakoš et al., 2019). Notably, this compensation takes place in a manner that depends on the stage of the tumor, highlighting the adaptability of the cancer microenvironment.

These phenomena could explain the lack of success of protease inhibitors, namely MMP inhibitors, in clinical trials as medications for treating cancer. This failure may be attributed to the inhibitor development, which could be compensated for by non-targeted protease types. An instance of increased invasion in tumors lacking MMP-9 is linked to the migration of leukocytes expressing cathepsin B towards the invasive regions of the tumor, hence promoting the invasion process (Shchors et al., 2013). An instance of the pro-form of cathepsin X, produced from both TAM and cancer cells, has been observed to facilitate the

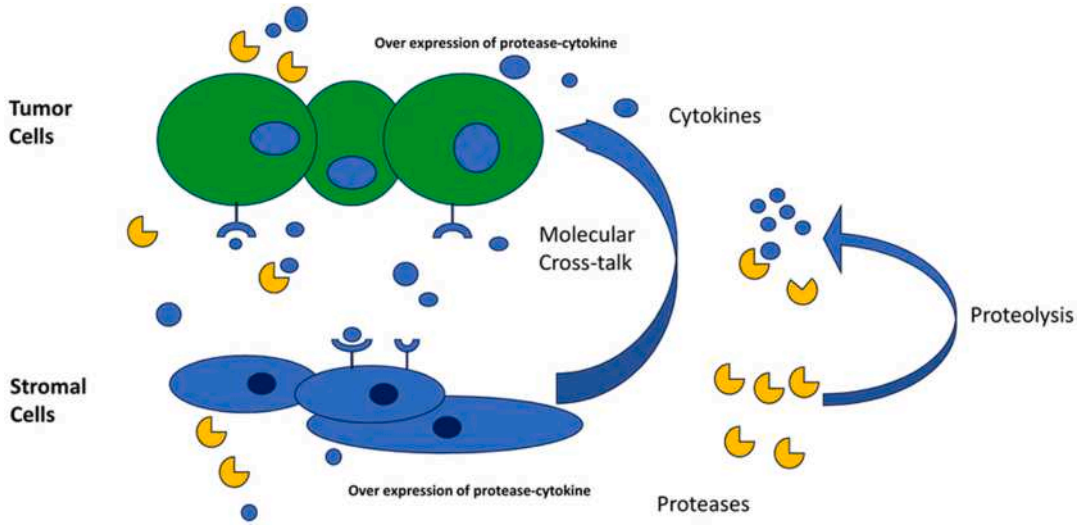

FIGURE 3.1 The mechanism of molecular crosstalk between proteases and cytokines of tumor and stromal cells is presented.

adhesion and migration of cancer cells (Kos et al., 2015). In addition, it has been observed that the inhibition of the hemopexin domain (HPX) in MMPs effectively impedes the progression of tumors (Kessenbrock et al., 2015). Collectively, these findings indicate that the intricate cellular environment, as well as the presence of several proteases with overlapping functions and specificities, pose significant challenges in cancer drug discovery.

3.4 Protease-cytokines crosstalk

Cytokines can perform their roles, such as controlling cell proliferation in the TME, through autocrine or paracrine mechanisms. Moreover, it has been observed that cytokines play a role in promoting cancer progression (Kenig et al., 2011; Wolf et al., 2007). The protease network plays a role in cancer progression by disrupting chemokine signaling, which subsequently impacts the recruitment and proliferation of immune cells within tumors. These immune cells can exhibit a range of activities, including pro-inflammatory, anti-inflammatory, and even immunosuppressive effects. Nevertheless, it is worth noting that there exists a significant overlap in target cell selectivity among chemokines, leading to a need for clearer differentiation in the specific functions of individual chemokines (Wolf & Albrecht, 2008).

Proteases exert an influence on the production of cytokines through several signaling pathways and modulate their activity after translation through proteolytic processing (Hu et al., 2014; Wilkinson et al., 2015) provided evidence to support the idea that activating the uPA-uPAR axis in cancer cells leads to an upregulation of interleukin-4 (IL-4) gene expression and protein secretion (Hu et al., 2014). Both cytokines have a crucial role in

the enhanced recruitment and polarization of macrophages towards the M2 phenotype, hence facilitating the advancement of tumors. The function of cytokines is exerted through their interaction with receptors connected to the cell membrane. The various groups of cytokines form bonds with specific receptors, and cytokines can control cell proliferation in the TME through autocrine or paracrine mechanisms. Furthermore, it has been observed that cytokines play a role in promoting cancer cell invasion and the development of new blood vessels in tumors by activating certain enzymes involved in invasion processes (Kenig et al., 2011). Chemotactic cytokines, or chemokines, are pivotal in regulating immune cell recruitment to tumors. The chemokine subfamily is categorized into four groups based on the location of the initial cysteines. Chemokine receptors are a class of G-protein-coupled receptors that can bind to multiple types of chemokines. As an example, we illustrate the cytokine binding with viral macrophage inflammatory protein 2 (a human chemokine homolog) to provide an immune response in Fig. 3.2. Nevertheless, it is worth noting that there exists a significant overlap in target cell selectivity among chemokines, leading to challenges in attributing a specific function to an individual chemokine (Wolf & Albrecht, 2008).

Proteases can selectively cleave cytokines, resulting in either the activation or inactivation of cytokine function and the alteration of receptor specificity. Certain cytokines necessitate proteolytic activation to perform their biological effect. As an illustration, MMP-8, MMP-9, and cathepsin L have been observed to facilitate the conversion of IL-8 into a significantly more active conformation, up to 30 times, through N-terminal cleavage (Van Den Steen et al., 2003). The activation of Tumor necrosis factor-alpha (TNF-α) subsequently leads to an augmentation in the infiltration of macrophages that promote tumorigenesis into the tumor. TNF-α has been found to enhance the proliferation of cancer cells through the activation of the transcription factor NF-κB (Kessenbrock et al., 2010).

However, it is worth noting that proteases have the potential to deactivate cytokines and modify their ability to connect to receptors, thus hindering the advancement of cancer. As an illustration, MMPs are responsible for the cleavage of CXCL12/SDF-1α, leading to the impairment of its capacity to connect with its corresponding receptor, CXCR4 (Manicone & Mcguire, 2008). The particular activity of MMP discussed in this study suppresses the chemotaxis of endothelial cells in nearby blood arteries, hence impeding the process of

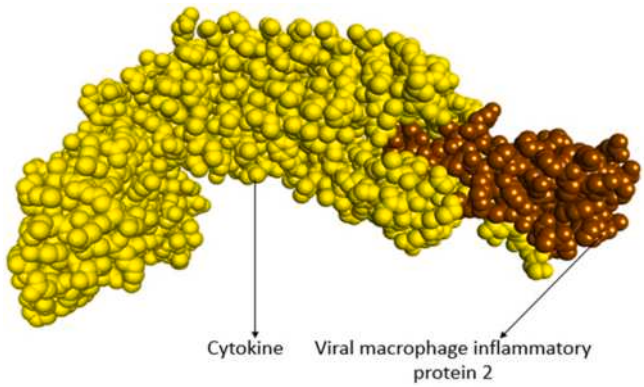

FIGURE 3.2 The cytokine binding with viral macrophage inflammatory protein 2.

tumor angiogenesis. Mortier et al. discovered that the diminished chemotactic response of tumors towards lymphocytes, which express CXCR4 receptors, can be attributed (Mortier et al., 2016).

Proteases play a crucial role in regulating cytokine bioavailability through the proteolytic cleavage of ECM proteins, which are the necessary binding partners for cytokines. Through a process of restricted proteolysis of the ECM, active cytokines are released. Consequently, this activation process induces the invasion of cancer cells (Yin et al., 2012). Furthermore, proteases play a crucial role in the recruitment of leukocytes and the promotion of tumor-associated inflammation through the modulation of cell surface proteoglycans. Proteases play a crucial role in developing metastatic and cancer stem cell microenvironments. MMPs and cathepsin K are proteases identified as contributors to the cancer stem cell niche establishment. The latter phenomenon has been linked to periarteriolar stem cell niches, where it is seen close to CXCL12/SDF-1α (Hira et al., 2015). This chemokine has chemotactic properties, facilitating the recruitment of glioblastoma cells toward certain niches, where they undergo a phenotypic transformation into glioblastoma stem-like cells.

3.5 Cytokine signaling affects protease activity

Cytokines play a significant role in paracrine cell signaling and serve as powerful regulators of protease activity, exerting their influence on many cell types inside the TME. The secretion of cytokines by stromal cells has been shown to trigger the expression of MMPs in cancer cells, hence facilitating their invasion. This phenomenon was demonstrated in an in vitro co-culture of glioma and endothelial cells (Kenig et al., 2011; Wang et al., 2013) conducted a study wherein they co-injected endothelium and hepatocellular carcinoma cells into nude mice and observed similar findings about the enhanced tumorigenicity of the carcinoma cells (Wang et al., 2013).

These cells have been found to release a diverse range of pro-inflammatory cytokines, which induce MMP activity inside the TME. In contrast, cancer cells enhance the expression of MMPs in stromal cells by producing soluble cytokines. As an illustration, IL-6 is a prominent inflammatory cytokine in advancing malignancy. Fibroblasts play a significant role within the TME by serving as a notable source of cytokines. An instance of dynamic interaction occurs between colon cancer cells and fibroblasts through the activation of transforming growth factor type beta 1 (TGB-β) signaling (Fig. 3.3). Consequently, these fibroblasts transform into cancer-associated fibroblasts, which play a significant role in multiple forms of cancer (Hawinkels et al., 2014).

Cathepsins inside the TME may arise from several cellular sources. Additionally, IL-4 promotes the growth of pancreatic cancer and stimulates angiogenesis in a mouse model of pancreatic neuroendocrine tumors (PanNET). According to Xie et al. (2011), the presence of elevated cathepsin K levels in fibroblasts leads to an augmentation of invasiveness in squamous cancer cells within fibroblast/cancer cell co-cultures in vitro (Xie et al., 2011). The significance of cytokine-mediated regulation of cathepsin L and cathepsin B has been documented with the emergence of chemoresistance (Yadati et al., 2020). According to a study conducted by Tuomela et al. (2013), it has been demonstrated that cancer cells can

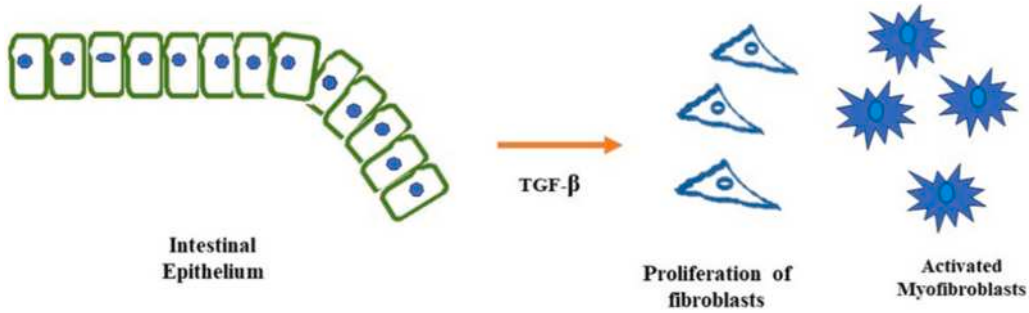

FIGURE 3.3 The dynamic interaction between cancer cells and fibroblasts through the activation of TGB-β signaling.

acquire DNA from deceased cancer cells that have been eliminated through chemotherapy (Tuomela et al., 2013). The aforementioned work illustrates that self-DNA originating from cancer cells that have undergone chemotherapy treatment is efficiently internalized by viable cancer cells. Subsequently, this self-DNA acts as a Toll-like receptor 9 (TLR9)-ligand, promoting invasion. Moreover, the invasion of surviving cancer cells is induced by DNA fragments derived from deceased cancer cells through the activation of TLR9 and the involvement of cathepsin. The cytokines released by endothelial cells, immune cells, and fibroblasts inside the TME impact the production of various proteases. This influence occurs through autocrine and paracrine signaling mechanisms, typically aimed at facilitating tumor advancement.

3.6 Conclusion and future perspectives

Proteases play a vital role in the regulation of cancer progression and metastasis. These proteases are present not only in cancer cells but also in stromal cells, including fibroblasts, Mesenchymal stem cells (MSCs), immunological cells, and endothelial cells, collectively called the TME. A protease and cytokine production by non-neoplastic cells promote the proliferation and invasion of cancer cells. One of the primary characteristics of cytokines and proteases, given their role as regulators of the immune response, is their pleiotropic effect. Various cytokines and proteases play a regulatory role in multiple immune cell populations, which can contribute to both anti-tumor and pro-tumor immune responses. Hence, the future prospects of cancer therapy utilizing cytokines and proteases will rely on the development of integrated strategies that seek to augment the anti-tumor immune response while concurrently inhibiting immune cells that facilitate tumor proliferation. Additionally, there are other notable challenges that arise, such as the limited duration of effectiveness and the potential for systemic toxicity, including pro-inflammatory and autoimmune reactions, when administering high doses of compounds that are required to achieve a substantial therapeutic outcome in individuals with cancer. Novel methodologies aimed at enhancing the precision of cytokine and protease targeting, as well as modifying their pharmacokinetic properties, could potentially offer valuable solutions to

address the constraints associated with various therapeutic interventions. The present direction of cancer immunotherapy research suggests that the optimal utilization of cytokines and proteases in therapy might be located in their combined administration with other medicines, such as immune checkpoint inhibitors.

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