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Cell Signaling in Cancer Microenvironment

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Abstract: Cancer microenvironment appears to include a lot of factors leading to tumor growth and angiogenesis. Understanding such factors and how they participate in the signaling pathways inside the cancer microenvironment guide to the best targeting strategy for the cancer treatment. This work focuses on three of these factors, which are: Platelets Derived Growth Factor (PDGF), Hepatocyte Growth Factor (HGF) and Matrix Metalloproteinase-9 (MMP-9). It emphasizes the role of each in cancer progression and metastasis, and elucidates the extent to which these factors can be used as drug targets or inhibited for stopping the spread of cancer.

Keywords: Cell Signaling, Cancer microenvironment, Platelet-derived growth factor, Hepatocyte growth factor, Matrix Metalloproteinase.

Abbreviations:

ADAM	a disintegrin and metalloproteinase		
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs	**MMPs**	Matrix Metalloproteinase
		MPPIs	Matrix Metalloproteinase Inhibitors
CAFs	Cancer associated fibroblasts	**PDGF**	Platelet-derived growth factor
FSP1	Fibroblast-specific protein 1	**PI3K**	Phosphoinositide 3-Kinase
HGF	Hepatocyte growth factor	**RTKs**	Receptor Tyrosine Kinase
JM	Juxtamembrane	**ROS**	Reactive Oxygen Species
MAPK	Mitogen-Activated Protein Kinase	**αSMA**	Alpha Smooth Muscle Actin
		shRNA	short hairpin RNA

Core Tip: Cancer is a complex disease that involves many cellular signalling pathways. Also, tumours showed wide range of signalling pathways' patterns according to many current therapeutic strategies. Consequently, it requires deep understanding for each signalling pathway inside the microenvironment to ease designing efficient targeting strategies.

1 Introduction

Cell signaling is one of the most complex systems in the biological system. Cells do not exist as individual cells but in form of tissues, organs and systems. Those complex tissues and organs regulate their function by communicating together in specific way. Cell signaling does not happen between the same type of cells only, but even between human epithelial cell and bacterial cell as for the gastrointestinal tract. Cell signaling mechanisms are varied but they could be classified based on the distance between signaling and receptor cells [1]; as: *Intracrine signals* (which are the signals produced from a cell then stay within this cell), *Autocrine signal* (which are the signals that are secreted by a cell and then affect the same cell but via receptors) and *Paracrine signals* (which are secreted by a cell then affect nearby cells). Moreover, any mistake in the signaling cascades will result in various diseases like Cancer, Autoimmune diseases and Diabetes.

Despite the known reasons for initiating cancer, the problem is not in how the cancer initiates. However, how cancer progresses and metastasizes are the real problems that cause death. There are plenty of mutated cells in our bodies every day, only when there is an accumulation of mutations, this will give rise to cancer [2].

Cancer cells are uncontrolled continuously divided cells which start to form bulk tumors and eventually metastasize leading to death. The tumor microenvironment is the key role of cancer progression and metastasis. The tumor

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microenvironment is a highly active system with many constituents such as immune cells, fibroblasts, lymphocytes and blood vessels [3].

Various cytokines and signaling molecules are involved in the tumor microenvironment. Recently some of them were discovered and studies still in progress to discover more. This work has focused on three signaling molecules which are Platelet-derived growth factor (PDGF), Hepatocyte growth factor (HGF) and Matrix Metalloproteinase (MMPs). They will be discussed elaborately.

2 Discussion

2.1 Firstly: Platelet-derived growth factor (PDGF)

Platelet-derived growth factor (PDGF) is one of the many growth factors that are responsible for the cell division and growth. It is also one of the major contributors of the tumor microenvironment growth. Platelet-derived growth factors (PDGF) constitute a family of growth factors; PDGF-AA, -AB, -BB, -CC and –DD [4]. These factors exert their cellular effects through two tyrosine kinase receptors.

PDGF has both autocrine and paracrine activity, each with specific function in Angiogenesis or recruitment of Cancer associated fibroblasts (CAFs). PDGF receptor signaling has been characterized by ligand binding to receptors induce receptor dimerization [5].

PDGF insight on structure and signal transduction mechanism

Most receptor tyrosine kinases (RTKs) are single subunit receptors but some exist as ligand binding to the extracellular domain *(Figure 1)* induces formation of receptor dimers [6].

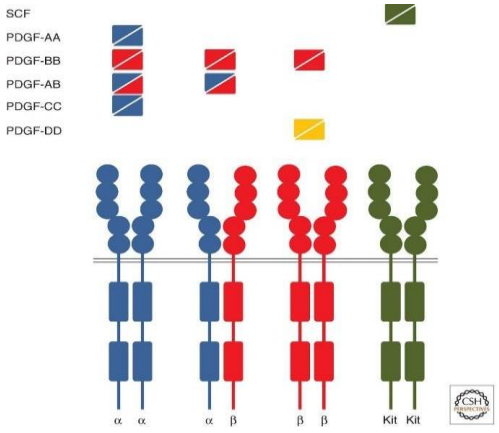

Figure 1: PDGF receptor types compared to SCF(green), Heldin, C. H., & Lennartsson, J. (2013). Structural and functional properties of platelet-derived growth factor and stem cell factor receptors. Cold Spring Harb Perspect Biol, 5(8), a009100. doi:10.1101/cshperspect.a009100

Paracrine activity of PDGF

PDGF was proved to be a significant driving force in the recruitment of CAFs in xenograft studies. CAFs are major contributors of the cancer microenvironment growth and metastasis. That is why the recruitment of such type of cells is one of the most studied areas in oncology research. Different types of PDGF have been identified for certain cancer types. PDGF-AA has been identified in breast and lung carcinoma, PDGF-CC in liver, kidney and heart carcinoma [7]. The most interesting fact is that the recruited fibroblasts were of different subtypes depending on their expression of PDGFR-alpha, fibroblast-specific protein 1 (FSP1), and α-smooth muscle actin (α-SMA).

Autocrine activity of PDGF

Autocrine activity of PDGF is involved in multiple tumor-associated processes including autocrine stimulation of tumor cells growth, stimulation of tumor angiogenesis and recruitment of fibroblasts. The role of PDGF in regulation of tumor angiogenesis and tumor fibroblasts are more general, and probably occur in most common solid tumors [8]. Most studies focus on the importance of PDGF receptor signaling for tumor pericyte recruitment and its relation of Angiogenesis [9].

PDGF signaling and tumor pericytes

Other studies suggested the role of PDGF signaling in recruitment of tumor pericytes. Forced PDGF over production by tumor cells was associated with increased pericyte abundance which lead to an increased tumor growth rate [10].

2.2 Secondly: Hepatocyte growth factor (HGF):

HGF-MET pathway has a major role in cellular growth, cell mobility and transition of tumor cells from the origin position. HGF is a growth factor for hepatocytes and has an important role in fibroblast derived cell mobility. Moreover, HGF is a scatter factor of epithelial cells [10]. The tyrosine kinase MET is known to be the receptor for HGF.

Moreover, MET transfers intracellular signals via the mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)-Akt pathways. That plays an important role in invasion, wound healing and survival of cells due to the suppression of the apoptosis [11].

Mesenchymal cells are the site for secretion of HGF, however epithelial cells are the site of HGF action. HGF stimulates the cell growth, mobility and induces the multicellular architecture [12]. HGF plays a major role in the liver and plasma of the rats that suffer from liver injuries. HGF also acts as a renotropic factor in renal regeneration' [12]. HGF RNA is also found in non-injured organs such as: the lung and spleen following hepatic or renal injury. That is because injurin (a protenous factor which induces HGF

mRNA expression) was identified in the plasma of rats that suffered from organ injuries. HGF also contributes to the regeneration of the liver or kidney through an endocrine mechanism. HGF plays an important role in construction normal tissue structure during the different phases like: embryogenesis, organogenesis, and organ regeneration [12]. HGF plays an important role in building the normal tissue structure during the different phases of the growth like: embryogenesis, organogenesis and organ regeneration [12].

Despite the major role of HGF factor that was mentioned earlier, the overexpression of Met/HGF receptor is strongly associated with the development of cancer especially mesenchymal human tumors as: bone cancer. The expression of c-MET oncogene encodes the HGF receptor [13].

Hepatocyte Growth Factor-Scatter Factor (HGF) has a vital role in regulation of cell motility and growth due to its activity as a tyrosine kinase [14]. Moreover, HGF is a cytokine which stimulates the growth of both normal and neoplastic cells (cells that grow abnormally). The Met/HGF receptor was found to be phosphorylated in the tumor cell line. The Met/HGF receptor phosphorylation is suppressed by blocking autocrine loops (a known blocker is suramin). As the activation of the Met/HGF receptor by a paracrine or an autocrine mechanism is related to its role in developing cancer [13]. The phosphorylation of the HGF receptor results in linking the receptor with the Shc adaptor, via the SH2 domain. Mutation of the HGF receptor occurs due to the phosphorylation and therefore phosphotyrosines Y1349VHV and Y1356VNV sites of the receptor. The phosphorylation of Y1349VHV and Y1356VNV makes these sites acts as docking sites for Shc. This increases the affinity of the binding site for Grb2 present on the HGF receptor (Y1356VNV) [14]. Shc binds to the HGF receptor and acts as an amplifier of the phosphorylation of the receptor. HGF affects MACC1 mRNA expression. MACC1 mRNA expression in primary tumors is related to metastasis development and the survival of metastasis. The MACC1expression increases 13-fold in the tumors in comparison to control cells containing MACC1 short hairpin RNA (shRNA), MET shRNA or control MACC1 shRNA. Control cells showed reduced cell migration over five months. MACC1 induces the transcription of MET [14]. MACC1 plays an important role in cell motility and division [14].

Due to the correlation between HGF and MACC1, siRNAs target MACC1 or MET block in vitro and in vivo metastasis that depend on HGF scattering activity [14]. MACC1 overexpression is related to the development of tumor cells, because the expression of MACC1 may induce changes in cells and myriad of the cell domains that are involved in "protein-protein interactions and phosphorylation sites for tyrosine and serine-threonine kinases" [14]. One of the domains that are strongly affected by the overexpression of MACC1 is SH3 domains. That plays important role in association of signaling complexes. SH3 domain acts either as tyrosine kinases or substrates of protein kinases that affects Grb2 receptor [14]. The kinases trigger Ras-Rho-Rac G protein family members that are involved in cell signaling. As the HGF-MET pathway also includes the Grb2–Ras-signaling pathway, MACC1 is strongly involved in signal transduction. [14] MACC1 binds to the endogenous promoter of MET at 60–base pair [15].

The MACC1 domain structure is critical to MET activation. MET has also been identified as a transcriptional target of b-catenin and hypoxia-inducible factor-1 [15]. MACC1 is known to have a therapeutic importance due to MACC1 role in HGF-MET signaling. That is why the HGF-MET pathway is currently targeted with specific antibodies and small molecules in order to neutralize the receptor and block the MET gene that encodes HGF receptor or inhibit the receptor kinase [15]. As HGFMET signaling is found to be important in several tumor types, MACC1 can be used more in clinical aspects [15]. Another important role of Hepatocyte growth factor (HGF) that involves an extra pathway besides that of MACC1 and also plays an important role in the development of the cancer is the three-phase response leading to the formation of branched tubular structures in epithelial cell. The initial phase of the response that is induced from the cytoskeleton reconstruction, loss of intercellular junctions and cell mobility. That is dependent on phosphatidylinositol-

3-OH kinase and Rac activation' [16]. Ras-MAP kinase cascade activation initiates the second phase (growth) [16]. The third phase (tubulogenesis) that depends on the STAT mechanism. HGF induces the recruitment of Stat-3 to the receptor, tyrosine phosphorylation, nuclear translocation and binding to the specific promoter element SIE [16].

Tumor development is a multi-step process that involves a progressive selection of specific tumor phenotypes. Met initiation may induce different phenotypes depending on tumor stage: inducing division in primary tumors, stimulating motility to form micro-metastasis, and regaining the proliferation phenotype to form over-metastasis' [17]. SHIP-1, Src, and Stat3 directly bind to a Met docking site and induce different cellular responses. SHIP-1 is essential for the stimulation of HGF/SF-mediated branching morphogenesis. Src may stimulate HGF/SF-induced cell transformation [17]. The stimulation of Stat3 results in expression of genes required for HGF/SF that plays an important role in branching morphogenesis [17]. Moreover, Met mutations in the juxtamembrane (JM) domain is involved in gastric cancer and lung cancer. The JM domain is involved in the interaction between Met and c-Cbl that plays an important role in regulating the body functions. Mutations at the different domains of Met prevent the regulation of Met. Finally, the inability for the regulation results in Met overexpression and therefore extended Met signaling [17].

2.3 Thirdly: Matrix Metalloproteinases (MMPs)

As it has been mentioned, the progress in the field of cancer biology has revealed that there are wide interactions between

tumor cells and the microenvironment (tumor stroma), such that these interactions lead to the invasion of cancer throughout the whole tissue or even to its metastasis to a new tissue [18].

The extracellular matrix is the major component of the extra cellular environment; it is highly dynamic and constantly undergoing remodeling processes [19]. One of the important components of the extracellular matrix that help in cell migration, invasion, proliferation and metastasis is the Matrix Metalloproteinases (MMP) family [20].

It is a family of "zinc-containing endopeptidases" [21] that have a structural function. They help hold the cells together as well as keep the threedimensional structure of the body. This is because the MMPs degrade the components of the extra cellular matrix, thus help in shaping the tissues and help in metastasis through degrading the physical barriers for the cell migration [22]. Also, MMPs help in regulating signaling pathways, so they can be considered as key players in the molecular mechanisms that control the communication between the tumor and stroma, and actually one of the biggest challenges for researchers nowadays is to understand the molecular mechanisms between the malignant cancer cells and the dynamic non-malignant cells in the stroma surrounding it [23].

So many trials have been done throughout the past 20 years for developing MPPIs (Matrix metalloproteinase inhibitors), however the initial clinical trials for this kind of inhibitors were disappointing" [21].

About 23 MMPs have been identified and categorized according to their architectural features. Their structure consists of three main domains [23]. When the MMPs are firstly expressed, they are inactive due to the interaction between a cysteine residue in the pro-domain with the zinc ions in the catalytic domain. But when this interaction is disrupted through a mechanism known as "cysteine switch", where the pro-domain is proteolytically removed or some modification is done on the cysteine residue, the enzyme becomes active. The pro-domain contains a consensus sequence that needs to be proteolytically cleaved by convetrases. Such a cleavage is done through two ways: intracellularly by furin or extracellularly by other MMPs or serine proteinases as plasmin, depending on the sequence of the pro-domain [23]. Once the proteinases are activated, MMP-2, -3, -7, -9 and -12 can generate a negative feedback signal to degrade the plasminogen, which is to be converted into plasmin.

MMPs have also the ability to degrade (plasminsuppressing serpin proteinase inhibitors), thus they enhance the conversion of proMMPs [22] and they increase the duration of action for these proteinases. It is known that cells adhere to one another or to the extracellular matrix surrounding them, and this (cell-to-cell) and (cell-to-matrix) interaction is tightly controlled in normal cells. In the majority of human cancers, this cell adhesion has some defects; as the tumor cell can easily detach from the origin site and move to another location and disrupts the normal tissue [24]. Such a property is acquired through the high expression of proteolytic enzymes as MMPs, ADAM (a disintegrin and metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs), or through changes in the levels of adhesion molecules as integrins. Thus, the extracellular matrix will be degraded which pave the path for tumor cells to migrate and metastasize.

The function of MMPs in vivo is regulated by some physiological inhibitors, where a certain local balance is to be maintained between them (MMPs and inhibitors), so there are energy stores that inhibit the proteolysis in the extracellular matrix [23].

For instance, the inhibitors that are released by the liver into the plasma as: α1proteinase inhibitor (α1-PI), α2-macroglobulin (α2-MG), and α1-chymotrypsin (α1-CT), Such that, when these inhibitors are present in high concentrations, they efficiently bind to the proteinases forming a proteinase-inhibitors complex that is recognized by a scavenger receptor and then engulfed by macrophages [23]. Tissue inhibitors of metalloproteinases (TIMPs) have been reported to be the most important physiological inhibitors for MMPs [25], [26].

Basically, the activity of MMPs can be regulated at different levels: the gene level, compartmentalization, conversion from zymogen to active enzyme, and through specific inhibitors. That leads to when the expression of proteinases is high in the tumor tissue, it is important to check whether the activators (convetrases enzymes) or the inhibitors of proteinases are present in the microenvironment or not; so as to judge the pathophysiological condition [23].

In addition, MMPs are affected by the reactive oxygen species (ROS) that are secreted at the tumor site by the activated macrophages and neutrophils during the inflammatory response. Such that, the large amounts of ROS initially activate the MMPs through oxidizing the cysteine of the pro-domain, but finally the "myeloperoxidase enzyme in the inflammatory cells inactivate MMPs by modification of amino acids of the catalytic domain by hypochlorous acid" [23].

3 Conclusion and Recommendation

Cancer arises due to the integration of many genetic and environmental factors. That is why it is considered as complex disease, and finding a cure to treat cancer represents a big challenge nowadays. In such a review, Platelets Derived Growth

Factor (PDGF), Hepatocyte Growth Factor (HGF) and Matrix Metalloproteinase-9 (MMP-9) are discussed. However, it is highly recommended to study the other essential factors in tumor angiogenesis and progression as: Transforming growth factor-beta (TGF-β) and Fibroblast Growth Factors (FGFs); to understand the cancer

microenvironment more comprehensively and find the best targeting strategy for cancer therapy.

Conflict of interest

The authors affirm no conflict of interest

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