Review Article

A Review on Matrix Metallo Proteinases-9 (MMP-9) and Cancer Progression: Focusing Molecular Insights of MMP-9 Activation Signaling Pathways and Role in Breast Cancer and Esophageal Cancer

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ABSTRACT

Matrix metallo proteinases (MMPs) are a large family of proteolytic enzymes which are calcium-dependent zinc-containing endopeptidases. Matrix metalloproteinase-9 (MMP-9) also known as Gelatinase B from gelatinase class represents the largest and most complex member of MMP family. Gelatinase B is directly involved in extracellular matrix remodeling, tumor metastasis, and more recently MMP-9 activity has been linked with the process of tumor cell Intravasation leads to cancer progression. Cancer is a complex network of diseases which is charectarised by abnormal growth of cell involved in metastasis or invading the cells from site of origin to other sites in the body, which causes significant morbidity and mortality. MMP-9 has found to be involved in almost all cancer initiation, invasion and metastasis. Consistent with their role in breast cancer progression and esophageal cancer, high levels of MMP-9 have been found in patients with breast cancer and esophageal cancer, correlated with poor prognosis in patients. Many of the signaling induction pathways leads to activation of MMP-9 in various cancers. In this review article a brief on MMP-9, its role in cancer progression and molecular insights of MMP-9 activation signaling pathways leads to cancer progression has been given in different cancers mainly focused in breast cancer and esophageal cancer. By understanding activation pathways of MMP-9, various targets to inhibit MMP-9 can be traced in future to inhibit cancer progression that is can be used to provide new strategies for inhibition of cancer metastasis and angiogenesis.

Keywords: Matrix metallo proteinase-9 (MMP-9), Breast cancer, Esophageal cancer, Activation pathways, Gelatinase B.

INTRODUCTION

Matrix metallo proteinases (MMPs) are a large family of proteolytic enzymes which are calcium-dependent zinc-containing endopeptidases, can also be called as matrixins**,** are secreted by connective tissue cells, inflammatory phagocytes, and various different transformed cells pro-inflammatory cells which includes fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes.[1,2] They can be also called metallo, because of presence of zinc atom at their active sites. The breakdown of extracellular matrix (ECM) timely is essential for various process like embryonic development, morphogenesis, reproduction, tissue resorption and remodeling. MMPs are thought to play a central role in these processes which are expressed in normal physiological conditions. MMPs are involved in various physiological process such as Angiogenesis, Apoptosis, Blastocyst implantation, Bone remodeling, Cervical dilation, Embryonic development, Endometrial cycling, Hair follicle cycling, Immune response Inflammation, Nerve growth, Learning and memory, Cell migration and Wound healing also MMPs though to plays role in various pathological disorders such as Arthritis, Multiple sclerosis, Alzheimer's disease, Nephritis, Atherosclerosis, Neurological Disease, Osteoarthritis(OA), Cancer, Cardiovascular Disease, Rheumatoid, Central nervous system disorders, Skin Ulceration, Emphysema, Vascular Disease, Fibrotic lung disease, Gastric Ulcer, Organ morphogenesis, Guillian-Barre Disease, Liver Cirrhosis, Metastasis.[1,2] To date at least 28 human MMPs are known as shown in Table 1. On the basis of their specificity, these MMPs are classified into four classes as collagenases, gelatinases, stromelysins, matrilysins and membrane type MMPs.[3] Gelatinase B (MMP-9) from gelatinase class represents the largest and most complex member of MMP family.[4] MMP-9 can also be called as 92 kDa gelatinase, gelatinase B, macrophage gelatinase, matrix metalloproteinase 9, neutrophil gelatinase, type IV collagenase and type V collagenase. MMP-9 plays an important role in local proteolysis of the extracellular matrix and in leukocyte migration. MMP-9 is primarily produced by mesenchymal, epithelial, and hematopoietic cells and also by distinct tumor cell types.[5] Gelatinase B could also play role in various physiological and pathological process, we will discuss that later in chapter. MMP-9 has a complex structure as compared to other MMPs, MMP-9 is a synthesized as pre proenzyme which consists of 707 amino acid residues, which secreted as inactive pro MMP. MMP-9 structure contains various domain motifs as shown in Figure 1. The amino-terminal propeptide domain (80 amino acids), the zinc-binding catalytic domain (170 amino acids) and the carboxyl-terminal hemopexin-like domain (210 amino acids) are conserved. amino-terminal propeptide has conserved sequence of PRCGVPD. Cysteine switch within this sequence ligates the catalytic zinc which maintains the pro MMPs in inactive state.[7] Zinc binding catalytic domain consists of two zinc atoms and three calcium atoms which maintains the stability of enzyme. It also contains a zinc binding motif as H*EXXHXXGXX*H and a conserved methionine, which then forms a unique "Met-turn" structure. MMP-9 can be also termed as 'metzincin'

because of similarity of structure with other four metalloproteinase families which are serralysins, astacins, adamalysins, and matrixins which gives evidence that they can be grouped together to form a common family which can be called 'metzincins' because of the common zinc-binding region and methionine turn (metzincins).[7] Fibronectin type-II domains are required to interact the enzyme with collagens and gelatins, so that MMP-9 have three repeats of Fibronectin type-II domains which are inserted in zinc catalytic domain which facilitate the degradation of (large) gelatinous substrates. The carboxyl terminal hemopexin like domain exhibited shape of an ellipsoidal disk which has four bladed B-propeller structure. Each propeller blade is made up of four antiparallel B-strands and an a- helix connected in a W-like strand topology.

Table 1: Classification of matrix metallo proteinases (MMPs)

Sr. no.	Protein	MMP
1	Collagenase 1	MMP1
2	Gelatinase A	MMP2
3	Stromelysin 1	MMP3
4	Matrilysin	MMP7
5	Collagenase 2	MMP8
6	Gelatinase B	MMP9
7	Stromelysin 2	MMP10
8	Stromelysin 3	MMP11
9	Macrophage elastase	MMP12
10	Collagenase 3	MMP13
11	MT1-MMP	MMP14
12	MT2-MMP	MMP15
13	MT3-MMP	MMP16
14	MT4-MMP	MMP17
15	Collagenase 4 (Xenopus)	MMP18
16	(No trivial name)	MMP19
17	Enamelysin	MMP20
18	XMMP (Xenopus)	MMP21
19	CMMP (chicken	MMP22
20	From human ovary cDNA	MMP23
21	MT-5-MMP	MMP24
22	MT6-MMP	MMP25
23	Matrilysin-2	MMP26
24	CMMP	MMP27
25	Epilysin	MMP28

The function of carboxyl terminal hemopexin like domain is to cleave triple helical interstitial collagens also it functions as interaction with substrates such as gelatin, collagen type I, collagen type IV, elastin and fibrinogen, binding to inhibitors, binding to cell surface receptors and induction of auto-activation.[8] Uniquely, MMP-9 consists of a central O-glycosylated (OG) domain, previously called the collagen V-like domain which is a flexible 64 AA linker between the catalytic domain and the PEX domain. Gelatinase A (MMP-2) and gelatinase B (MMP-9) differ from other MMPs in that they have three tandem fibronectins type II repeats within the amino terminus of the catalytic module that mediates gelatin binding.[9]

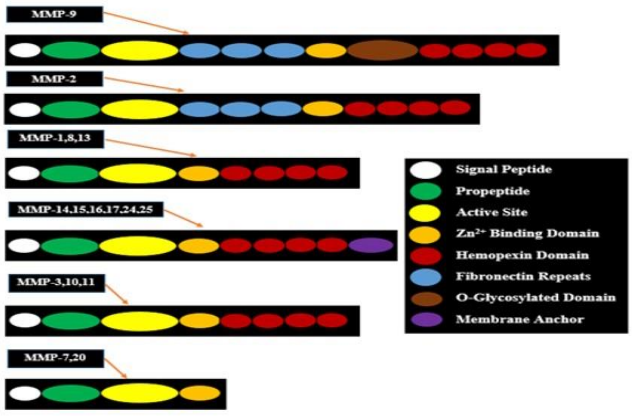

Figure 1: Domains of matrix metalloproteinase (MMPs)

These enzymes are expressed as zymogens, which are processed and activated by other proteolytic enzymes (such as serine proteases, furin, plasmin, and others) to generate the active forms.[1] Matrix metalloproteinase-9 (MMP-9) may play a critical catalytic role in tissue remodeling *in vivo*, but it is secreted by cells as a stable, inactive zymogen, pro- MMP-9, and requires activation for its catalytic function**,** Activation is achieved through an interacting protease cascade involving, other enzymes as trypsin, activation by plasmin, activation by MMP-2 (Gelatinase B), activation by MMP-3 (stromelysin-1), activation by urokinase type plasminogen activator (uPA), Activation by substrate binding or allosteric interactions, activation by kallikrein-related peptidase 7 (KLK7), activation by human neutrophil elastase, Priming of activation by meprins .[10]

MMP-9 is found to be involved in various physiological processes as discussed above mainly such as Reproduction, Growth and development, Angiogenesis and vascular remodeling, Bone development and remodeling, wound healing, Epithelial regeneration, Cell migration and tissue maintenance, Stem and progenitor cell migration, Tissue maintenance, Learning, memory and maintenance of the neuronal network, Synaptic plasticity, learning and memory, Regulation and maintenance of compartments in Schwann cells. Increased expression of Gelatinase B has been associated various inflammatory, autoimmune, degenerative and neoplastic diseases. MMP-9 involved in pathological process such as in lung conditions like chronic obstructive pulmonary disease (COPD), acute respiratory distress syndrome (ARDS) and asthma. in inflammatory diseases such as rheumatoid arthritis and multiple sclerosis. In autoimmune diseases such as type I diabetes, Systemic lupus erythematosus, Organ-specific autoimmune inflammation like glomerulonephritis, autoimmune carditis, bullous pemphigoid and multiple sclerosis, Allergy, Dentistry, in

several muscle diseases, in skin condition, in cardiovascular diseases such as Atherosclerosis and restenosis, Abdominal aortic aneurysm, Left ventricular hypertrophy, Stroke, Ischemia and reperfusion, Thrombosis., in Transplantation biology, in bone pathologies and mainly in various types of cancer.[10] We will here discuss briefly activity of gelatinase B in various cancer.

Matrix metallo proteinase-9 (MMP-9) expression related in various cancers

Cancer is a complex network of diseases which is charectarised by abnormal growth of cell involved in metastasis or invading the cells from site of origin to other sites in the body, which causes significant morbidity and mortality.[11] Over hundred types of cancers have been classified till date, in which the tissue of origin defines the unique charecterstics of the cancer.[12] The high proportion of premature deaths and mortality will promote and place the cancer as second prominent cause of death in the United States of America. The global burden of cancer data reported by American cancer society reveled that 1,688,780 new cancer cases and 600,920 cancer deaths were appeared to occure in United States in 2017.[13] Primarily cancer research has mainly focused on mutations happened in cancer cells that results in either gain-of-function in oncogenes or loss-of-function in tumour-suppressor genes. However, the extracellular matrix (ECM) of tumours and also non-cancerous, stromal cells of tumours also have a considerably important impact on tumour progression, invasion and metastasis.[14] ECM and dissolution of epithelial and endothelial basement membrane are remodeling processes that occur during tumor invasion and metastasis. A family of proteolytic enzymes that have been functionally linked to these remodeling processes are the matrix metallo proteinases (MMPs).[5] Gelatinase B (MMP-9) is directly involved in extracellular matrix remodeling, tumor metastasis, and more recently MMP-9 activity has been linked with the process of tumor cell Intravasation.[15] Metastasis is the spread of cancer from a primary tumor to distant sites of the body and is a defining feature of cancer.[16] Also mmp9 found to regulate apoptosis during development of tumor and also mmp9 directly regulates angiogenesis.[17] thus are a significant player in such processes as angiogenesis, cellular migration and formation of metastases. Metalloproteinases also participate in the activation of multiple proteins and thus take part in regulation of cellular proliferation and apoptosis.[17] Gene expression of MMP-9 (Average FPKM value of RNA in various cancers) is shown in Figure 2(a). Role of MMPs in cancer is depicted in Figure 2(b). Increased expression of MMP-9 found in various cancers such as.

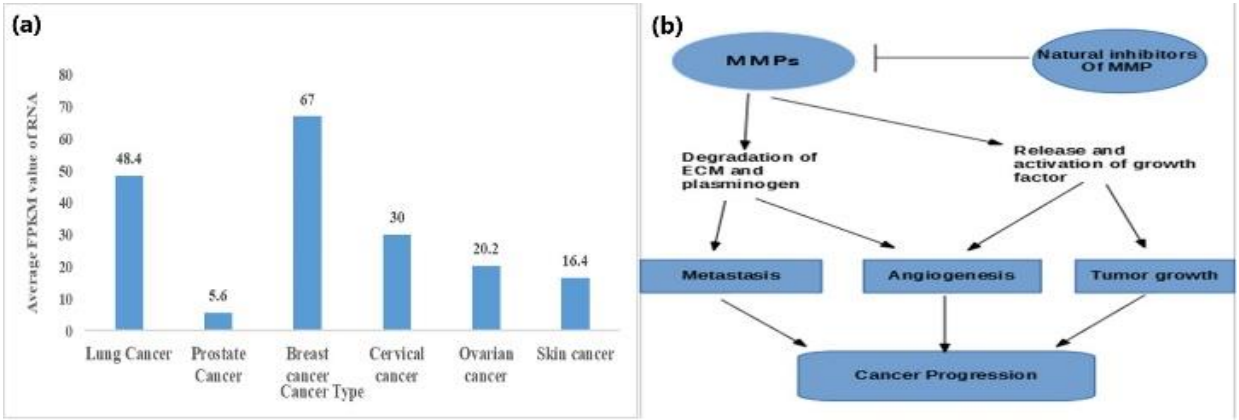

Figure 2: (a) Gene expression of MMP-9 in various cancers. (b) Role of MMPs in cancer progression

Bladder cancer

Bladder carcinoma is the fifth most common occurring cancer in men and one of the most important considerations in the management of patients with the bladder is the need to recognize which tumours are likely to progress to invasive disease. Bladder cancer can be classified into superficial (Cis, Ta and T1) and muscle invasive (T2, T3 and T4) stages; 50–70% of superficial tumours recur but 20% of these will invade the bladder wall. At present there is no reliable prognostic factor to identify which superficial tumours will become invasive. The MMPs are now being widely examined in a variety of tumour systems, including the urinary bladder, to ascertain their role in tumour progression and also as potential therapeutic targets. MMP-2 and MMP-9 (Gelatinase) have been reported to be expressed at a higher level in invasive bladder tumour than in superficial bladder tumours.[18,19] Epidermal growth factor (EGF) increases MMP-9 levels in bladder cells. MMP-9 (rather than MMP-2) may be one of the important enzymes involved in the progression of bladder cancer. The detection of high contents of MMP-9 enzyme in urine is related to tumour stage, and the induction of MMP-9 by EGF in one of the tumour cell lines, indicates that MMP-9 expression and activity should be investigated further for their significance in the invasive progression of bladder carcinoma.[19] Migration and invasion of bladder cancer cells is related to p38 MAPK activity and in production of increased levels of MMP-2 and MMP-9. The regulation of MMP-2 and MMP-9 was carried out by p38MAPK-driven MAPKAPK2 pathway.[20]

Cancer

Cervical cancer is the fifth most occurring malignant neoplasm throughout the world and the second most occurring in several developing countries. Death rate for cervical carcinoma, which was most prominent causes of cancer death for women in United States, had been declined by 74% between year 1955 and 1992, because of increased use of the screening test (Pap test). The American Cancer Society estimated record that in year 2018, ~13,240 cases of invasive cervical cancer will be diagnosed in the United States of America and probably~4,170 women will die from cervical cancer.[21] Cervical cancer is a multistep process in which, is a consequence of continuous infection with high-risk human papillomaviruses. Cervical cancer develops slowly, which take around 10-15 years to develop slowly into cancer, which starts with a pre-cancerous condition called as dysplasia that can be easily detected by pap smears and which can be treated fully. However, As the cervical carcinoma has metastasized, then it will be life threatening.[22] The ability of any cancer cells to migrate from the tissue of origin and metastasize to surrounding or distant organs is an essential feature of tumor progression. MMP-9 (gelatinase B) degrade components of extracellular matrix also able to cleave collagen IV which is the major collagen of the basement membranes, also it degrades laminin-5.[23] Gelatinase-B has been found to play a central role in the breakdown of certain cytokine receptors such as interleukin 2R on tumor-infiltrating lymphocytes which derived from human cervical carcinoma. Also association of Gelatinase-B expression with lymph node metastasis and compromised activity of immunocytes in patients with cervical cancer, as well as, up-regulated Gelatinase-B expression can be able to activate downstream MMPs, which therefore additionally enhances the degradation of ECM and further tumour invasion by various cancer cells. In a clinical analysis, thus increased MMP-9 expression levels were correlated with poor prognosis for the patients.[24] Transcription factors NF-kB and activator protein-1 (AP-1) are considered as the mediators of MMP-9 induction in different cell types. MMP-9 plays a crucial role in tumor development, in cervical carcinoma-associated myeloid cells, a STAT3- dependent molecular pathway was identified which leads to MMP-9 induction and further tumor invasion in cervical cancer.[25]

Ovarian cancer

Epithelial ovarian carcinoma, is the prominent cause of death from gynecological malignancy and also fifth most occurring cancer in the United States, ovarian cancer occurs primarily in post-menopausal women, as the ovarian cancers often remains clinically silent, the majority of ovarian cancer patients have advanced intraperitoneal metastatic disease at diagnosis which results in a poor prognosis. Generally, 80% of ovarian carcinoma cases are diagnosed only at an advanced metastasis stage.[26] MMPs expression has been found to be related with tumor invasion and metastasis in many different tumors.[27] MMP-9, which degrades collagen IV, the major ECM component of the basement membranes, have been seen to be critical for the invasive and metastatic potential in ovarian carcinoma. Gonadotropin-releasing hormone (GnRH) receptor is also present in 80% of ovarian cancer, Ovarian cancer cells could be stimulated with gonadotropin-releasing hormone (GnRH) to produce MMP-2 and MMP-9 mediated through c-jun NH2-terminal kinase (JNK) pathway, this increased expression resulted in increased cell motility and cell invasiveness.[28] Fibronectin (FN) in the media of human peritoneal tissues that plays role of activation of various MMPs. In one clinical study it was shown that fibronectin found to activate MMP-9 secretion through the MEK1-MAPK and the PI3K-Akt pathways in ovarian cancer cells.[29]

Lung cancer

Lung cancer is the commonest cause of cancer death in developed countries such as Europe and the United States. Despite advances in surgery, chemotherapy, and radiotherapy in the last two decades, the death rate has remained little changed. (MMP-9) have been associated with important roles in tumour invasion. MMP-9 (gelatinase B) is produced by inflammatory cells as well as by stimulated connective tissue cells.[30] In one clinical study it was found that Increased levels of MMP-9 shows poor prognosis in lung cancer.[31] Skp2 is one of the components of the E3 ubiquitin ligase which is necessary for the degradation of tumor suppressor p27. It was also found that Skp2 overexpression leads to increase in the expression of Gelatinase-A and Gelatinase-B and invasion of lung cancer cells which leads to metastasis.[32] Cigarette smoke found to induce MMP-9 expressions as it contains several carcinogenic components including free radicals (superoxide radicals, hydroxyl radicals, hydrogen peroxide) and benzo[a]pyrene which can activate signal transduction pathways, which results in lung inflammation and malignancies. These toxic compounds are thought to activate NF-Kb signaling and MMP-9 induction.[33]

Skin cancer

Non-melanoma skin cancer (NMSC) is the commonest group of malignancies in certain developed countries (white population) with annual occurrence rates of approximately 40 000 and 1 million in the UK and USA respectively. In the first collaborative report given by the Northern Ireland Cancer Registry and the National Cancer Registry, NMSC accounted for 30% of all recorded cancers in Ireland in the period of 1994–1996 and was by far the most common type of cancer in both men and women, with approximately 7334 new cases.[34] One clinical study showed that MMP-2, MMP-9, TIMP-1 and TIMP-2 plays an important role in the athogenesis of non-melanoma skin cancer, but they differ significantly in their expression levels between the various tumour types examined. The immune expression of these proteins may be useful indicators of cutaneous cancer invasion and progression.[35] One of the study done on mouse model of multi- stage tumorigenesis elicited by HPV16 oncogenes, showed that MMP-9 which was supplied

by bone marrow–derived cells further contributes to skin carcinoma.[36]

Infrared (IR) radiation from natural sunlight found to induce MMPs levels, leads to heat shock response and resulting in the increased production of MMP-1 and MMP-9 in human keratinocytes by inducing ERK, JNK and p38 kinase signaling pathways.[37] An important process in the progression of skin cancer is the epithelial-tomesenchymal transition (EMT) of keratinocytes. This transition is believed to require TGF-b signaling. Interestingly, TGF-b has a negative influence in later stages of cancer cell development, but it plays a protective role in early stages.[38] But it was found that in immortalized keratinocytes, TGF-b can induce MMP-9 expression.[39] Transforming growth factor-b (TGFb) signaling pathways has found to regulate a number of keratinocyte functions during epidermal carcinogenesis and wound healing which includes proliferation, survival, and migration. TGFb also can induce expression of the Gelatinase B (MMP-9), which has critical roles in promoting ECM remodeling and angiogenesis during tumorigenesis and tissue repair. It was found that a3b1 activates TGFb-mediated induction of MMP-9 in immortalized or transformed keratinocytes during skin carcinogenesis, also indicating involvement of SFK pathway.[40] The migration of keratinocytes during tumor progression found to be regulated by TNF-a via an MMP-9- and avb6 integrin-dependent pathway.[41]

Prostate cancer

Prostate cancer (PC) is the second prominent cause of death in men worldwide. metastatic growth at distant organs is the primary cause of morbidity and mortality. Bone is the preferred organ site for metastasis of PC.[42] Most deaths from prostate cancer are due to metastases that are resistant to therapy.[43] As we know different steps include in metastasis are tumor cell proliferation, angiogenesis, detachment, invasion, intravasation, survival in the circulation, growth in distant organs, extravasation, and adhesion to endothelial cells.[44] and various MMPs specially type IV collagenases as MMP-2 and MMP-9 expression found to play important role in these above process and leads to metastasis.[43] one clinical study showed that NF-kB signaling blockade signifcantly inhibited in vitro and in vivo expression of three major proangiogenic molecules, VEGF, IL-8, and MMP-9, and hence decreased neoplastic angiogenesis and metastasis also further downregulation of MMP-9 mRNA and collagenase activity, resulting in decreased invasion through matri-gel.[43] also MEK5 (mitogen/extracellular-signal-regulated kinasekinase 5) overexpression is found to associate with metastatic prostate cancer, which stimulates proliferation, increases MMP-9 expression and invasion through MEK5/ERK5 pathway. Increased expression levels of MMP-9 Mrna through activation of AP-1 takes place.[45] In prostate carcinoma tissue, co-expression of matrilysin-2 and proMMP-9 were found to play an important role in tumor progression since matrilysin-2 is an activator of proMMP-9.[46] 3,3¶-Diindolylmethane (DIM) has been shown to repress neovascularization and inhibit cell proliferation, migration and invasion in prostate carcinoma. Inhibition of NF-KB DNA binding activity by B-DIM contributes to the regulated bioavailability of VEGF by MMP-9 and uPA and, in turn, inhibits invasion and angiogenesis in prostate cancer animal model. Targeted genes, including VEGF, IL-8, uPA, and MMP-9 are involved in angiogenesis, invasion, and metastasis.[47] Docetaxel has improved patient survival in prostate cancer but it has certain amount of toxicity and Genistein, derived from soybeans, has been found to inhibit cancer cell growth without toxicity. genistein down-regulated the expression and activity of MMP-9, which was induced by docetaxel treatment. So it is clear that antitumor and antimetastatic activities of docetaxel are enhanced by genistein in the SCID-human model of experimental bone metastasis could be mediated by regulation of OPG/RANK/RANKL/MMP-9 signaling, resulting in the inhibition of osteoclastic bone resorption and prostate cancer bone metastasis .[48]

Leukemia

B-cell chronic lymphocytic leukemia (B-CLL) is the accumulation of monoclonal, slow-dividing CD5_ B lymphocytes in the peripheral blood. Most of the time, these cells progressively infiltte the bone marrow and secondary lymphoid tissue, resulting in poor prognosis in BCLL.[49] It was found that adhesion of B-CLL cells to the fibronectin fragment FN-H89, VCAM-1, or TNF-α which further activated human umbilical vein endothelial cells (HUVECs) and up-regulated MMP-9 expression, this effect was mediated through α4β1 integrin and required PI3-K/Akt signaling pathway.[50] As well as, chemokine (CXCL12) also upregulated MMP-9, without α4β1 and involving ERK1/2 but not Akt pathway. α4β1 activated the PI3-K/Akt/NF-kB pathway and CXCL12/CXCR4 interaction activated ERK1/2/c-Fos signaling pathway. It was found that MMP-9 is physiologically regulated by α4β1 integrin and CXCL12 and plays a key role in cell invasion, thus contributing to B-CLL progression.[50] One of the study done on acute myeloid leukemia states that, Matrix metalloproteinase MMP-9 expression is linked with myeloid cell differentiation, as well as inflammation and angiogenesis processes related to cancer progression. It was found that the chemo preventive effects of green tea catechins, epigallocatechin-gallate, catechin-gallate, and epicatechingallate, inhibited in a time- and dose-dependent manner MMP-9 secretion.[51] Similar to matrix metalloproteinases (MMP-9/-2), IL-18 was also overexpressed in some hematologic malignancies such as acute myeloid leukemia (AML), Further, IL-18 could significantly increase MMP-9 but not MMP-2 production at both mRNA and/or protein level, IL-18 may play a role in the clinical aggressiveness of human myeloid leukemia by stimulating MMP-9 production.[52]The expression of MMP-9 is dependent on the activity of a p38 MAP kinase and blocking of this kinase and MMP-9 resulted in impaired survival of the B-CLL cells and thus reduced cancer cell progression. The p38 kinase becomes activated by stress signals such as LPS and inflammatory cytokines .[53]

Breast cancer

Breast cancer is the most common cancer occurred in women these days. It is the leading cause of death from cancer for women aged between 35 and 55 years in world. One in nine women has to suffer from breast cancer during her life span and also 1 lac 30 thousand women die from breast cancer each year.[54] Accounting the second leading cause of cancer death for women in the United States. In 2005, about 215,000 cases of invasive breast cancer (IBC) and 50,000 cases of ductal carcinoma *in situ* will be diagnosed and 40,000 women will die from IBC in the US.[55] Among various cancer types, breast cancer stands out for its increasing death incidence rates and high mortality in world. Like most solid tumors, metastatic disease rather than the primary tumor itself is responsible for death. The metastasis process is a multistep process, involving the organized breakdown of the extracellular matrix (ECM) by matrix metalloproteinase enzymes.[56]

Role of MMP-9 in breast cancer

MMPs are involved in the early stages of tumor development in cancer. They found to degrade the extracellular matrix (ECM) and basement- membrane, which contributes to the formation of an ideal environment for further growth of tumour and tumor development. MMPs further promote metastasis.[13] Also Gelatinase B is also found as a secretory product of recruited inflammatory cells such as neutrophils and macrophages in tumour environment.[57] As well as various tumor promoting and carcinogenic agents, various oncogenic proteins, growth factors and hormones also contribute to the regulation of MMP-9 production by various cancer cells in tumour.[4] MMP-9 which is secreted in the tumor microenvironment by stromal cells which originates from the bone marrow, found to potentiates the release of growth factor (VEGF), resulting in increased levels of VEGF in serum and then further attraction of bone marrow-derived cells. It is estimated that when we remove MMP-9 from the tumor microenvironment, it breaks the VEGF/bone marrow/MMP-9 loop which results in reduced serum levels of VEGF and therefore it reduces angiogenesis and myelopoiesis.[59] In order for a small tumor to grow, it requires the formation of tumor-associated vascular structures. At that point, tumor cells starting producing proangiogenic factors to form these vascular structures, is often referred to as the angiogenic switch. In this switching process, MMP-9 was shown to act as a proangiogenic factor in several cancer models, thus promoting angiogenesis and tumour spread.[60]

Regulation and activation of MMP-9 in breast carcinoma

MMP-9 is regulated at various levels as; at first level is the regulation of MMP-9 under the control mechanisms of a multiple signaling pathways that either stimulate or reduce the expression of the MMP-9 gene. Second, MMP- 9 is regulated at the level of mRNA and at translation into the pre proenzyme.[13] many members of the MMP family are transcriptionally and tightly controlled by various cytokines, growth factors, hormones and cell interactions.[61] Primarily, MMPs are regulated by the mitogen-activated protein kinases (MAPKs). This family of kinases has three members mainly: 1) the extracellular signal-related kinases (ERKs) activated by various mitogens and phorbol esters, 2) the c-Jun N-terminal kinase (JNK)/stress-activated protein kinases and 3) p38 which are activated by cellular stress and inflammatory cytokines. MMP-9 is mainly regulated by ERK1/2 pathway.[13] Many members of the MMP family are transcriptionally controlled by various cytokines, growth factors, hormones and cell interactions.[3] and Gelatinase-B also follows this rule. In breast cancer MMP-9 gene expression is regulated by multiple factors. More recent evidence suggests that MMP-2 (Gelatinase A) and MMP-9 (Gelatinase B) may also be involved in breast cancer initiation and growth through complex interactions with the main oncogenes and tumour-suppressor genes which are involved in the early stage of tumorigenesis.[62] One study showed that transfection of MCF-7 cells with the ets gene PEA-3 leads to increased production of MMP-9.[63] High activity plasma levels of MMP-9 in breast cancer patients are associated with a worst overall survival rate.[64] Local invasion into surrounding tissues and then spread to distant organs are important processes in metastasis of metastatic breast tumors. Both those processes require several proteolytic enzymes which can degrades protein components of the extracellular matrix (ECM) such as MMP's.[65] MMP-9 is highly expressed in human breast carcinoma tissue and appears to be associated with lymph node metastasis.[66] Furthermore, one study provided evidence that MMP-9 expression in tumor stroma is dependent on direct cell-cell interactions with tumor epithelium; while MMP-2 production can be stimulated by soluble factors via paracrine interactions. A plenty of tumor cell-derived cytokines have been studied that are potential candidates of MMP induction in neighboring tumor stroma such as: PDGF, IL-1β, EGF, FGF, and TNFα are produced by breast cancer cells and have been shown to elicit fibroblastic MMP expression *in vitro*.[67] As we know from previous studies that metastasis is the primary cause of death in breast cancer patients. whereas metastatic dissemination depends on various factors such as tumor cell adhesion, migration, and invasion, some studies showed that alphavbeta3 activation strongly enhances breast cancer migration toward specific substrates and that this process is mediated by cooperation between the integrin and matrix metalloproteinase type 9 (MMP-9) in an activation-dependent pathway. Thus promoting metastasis.[68] circulating MMP-9 activity has used to predict the prognosis of breast cancer patients. Studies found that high levels of circulating MMP-9 activity at the time of diagnosis were significantly associated with worse overall survival rate.[69] CD44v3,8–10 is closely related with the active form of various MMPs, such as MMP-9, in a complex within ''invadopodia'' structures, several studies suggest that CD44v3,8–10 plays an important role in linking ankyrin to the membrane-associated actomyosin contractile system which is required for ''invadopodia'' formation (coupled with matrix degradation activities) and tumor cell

migration during breast cancer progress.[70] One study showed High expression of MMP-2 and MMP-9 in carcinoma cells, but not in stromal cells, was related to high AP-2 expression. Positive stromal MMP-2 expression was associated with HER2 overexpression in the whole patient group and in the node-negative patient subgroup. Positive stromal MMP-9 expression was related to HER2 overexpression in estrogen receptor (ER) .[71] MMP-9 expression can be induced by tumor cell-derived TNF-α and TGF-β, dependent on Smad-, Ras-, and PI3-kinase-signaling pathways, and likewise modulated by subsequent HGF- and EGF signaling. Together, studies indicate that MMP-9 levels in tumor fibroblasts are regulated by a complex tumor stroma cross-talk, involving multiple ligands and cellular signaling pathways.[73] Epidermal growth factor (EGF) and amphiregulin induces expression of matrix metalloproteinase-9 (MMP-9) in human breast cancer cells thus leads to invasion of metastatic breast cancer cells .[73] Heregulin-β1 found to activates various signaling pathways in breast carcinoma cells, which includes pathways such as Erk, p38 kinase, PKC, and PI3-K. One clinical study examined the pathways involved in heregulin-b1-mediated MMP-9 activation using specific chemical inhibitors which specifcally inhibit each of these above pathways. Study showed that PKC inhibitor RO318220 and p38 kinase inhibitor SB203580 completely blocked activation of MMP-9 mediated by heregulin-β1. MEK-1 inhibitor PD098059 partially blocked MMP-9 activation, but in case of PI3-K inhibitor wortmannin had no effect on heregulin-β1-mediated MMP-9 activation. It was found that three signaling pathways may be involved in the heregulin-b1 mediated activation of MMP- 9. As we know that MMP-9 is generally associated with invasion/metastasis and angiogenesis, clinical studies suggest that blocking activation of MMP-9 mediated by heregulin-β1 which is achieved by inhibiting the related signaling pathways can be used to provide new strategies for inhibition of cancer metastasis and angiogenesis. Heregulin-β1 binds to ErbB3 or/and ErbB4 receptors that induce heterodimerization of these receptors with the ErbB2 receptor. This leads to further activation of down-stream signaling pathways, which includes MEK to Erk, PLCγ to PKC, PAK1 to p38, and PI3-Kto Akt pathways. Pathways are shown in Figure 3(a) This study shows that Erk, PKC, and p38 pathways are involved in heregulin β1-induced upregulation of MMP-9 .[74] One study carried out on breast cancer cell lines showed that, a splice variant of CD99 found to increase motility and MMP-9 expression in MDA-MB-231 and MCF-7 human breast cancer cell lines which mediated through the AKT-, ERK-, and JNK-dependent AP-1 activation Signaling Pathways, which further carries out functions as elevated motility, fibronectin binding, invasiveness and MMP-9 expression. Activation of CD99 type II protein primarily induces the Src mediated phosphorylation and then activation of ERK1/2 and JNK. CD99 type II activation also found to induce the activation of Akt via PI3K, which leads to full activation of ERK1/2 and JNK through the pathway, then highly activated ERK1/2 and JNK further stimulate FOSB gene expression mediated by Elk1- and c-Jun-mediated JUND gene expression, respectively. Then resultant JunD-FosB complex induces the transcription of the AP-1-responsive genes, such as MMP-9 and cell motility-promoting genes[75].as pathways are shown in Figure 3(b).

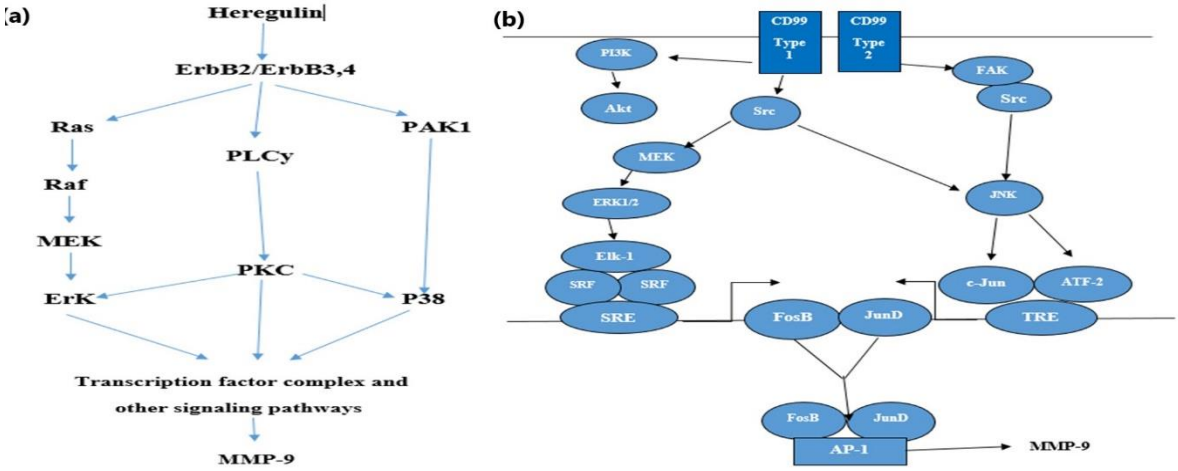

Figure 3: (a) Heregulin inducing downstream pathway to activate MMP-9 involving ErK, PKC and P38 pathways. (b) Activation of MMP-9 from CD-99, mediated through AKT-, ERK-, and JNK-dependent AP-1 activation Signaling Pathways.

These findings are really useful in designing therapeutic compounds which blocks AP-1 induction through Akt-dependent activation of ERK and JNK by the CD99 splice variant, resulting in further reduction of MMP-9 expression and then cell motility and then further blocking the invasion and metastasis of malignant breast carcinoma.[75] Amplified-in-breast cancer 1 (AIB1) is an overly expressed transcriptional coactivator in breast carcinoma, which functions as PEA3 coactivator that formed complexes with PEA3 on MMP-2 and MMP-9 promoters to increase their expression in mouse and human breast cancer cells. Clinical study done on 560 human breast tumors showed that AIB1 expression was found to be positively associated with PEA3, MMP-2, and MMP-9. These findings suggest that the

unwanted roles of these MMPs in breast cancer can be controlled by inhibiting their upstream coregulator AIB1. AIB1 plays a crucial role in the upregulation of MMP-2 and MMP-9 in mammary tumor cells.[78] Continuous activation of the MAPK cascade pathways has been mainly associate with the carcinogenesis and metastasis of human breast and renal cells by inducing expression of MMP-2 and MMP-9, because Mitogen-activated protein kinases (MAPKs) pathways found to play a major role in the mitogenic signal transduction pathway and these are essential components of both growth and differentiation The gelatinases B (MMP-9) and A (MMP-2) are 2 members of the matrix metalloproteinase (MMPs) thought to play a sever role in tumor cell invasion and metastasis. In a clinical study, it was shown that EGF and amphiregulin found to upregulate mainly MMP-9 in metastatic SKBR-3 cells but have no primarily effect on MMP-2 secretion.[79] It was found that TGF-b is thought to play a dual role in cancer progression. It may act as a tumor suppressor in the early stages of cancer, but promote cancer progression further in later stages of the disease. Also TGF-b is frequently overexpressed in breast cancer and its expression correlates with poor prognosis and metastasis.[80] As well as Smad3 and Smad4 pathways are found to play an important role in TGF-b-induced invasion by inducing expressions of MMP-2 and MMP-9. This supports the idea that targeting these molecules might aid in the prevention of metastasis in patients. The TGF-b/Smad pathway further induces breast cancer cell invasion through the up-regulation of MMP-2 and MMP-9 in a spheroid invasion model system.[81] Generally, bcl-2 overexpression is mainly associated with poor prognosis in several types of carcinomas. In one clinical study it was found that over-expression of bcl-2 in a human breast-cancer cell line (MCF7ADR) increases its tumorigenicity and metastatic activity by inducing increased secretion of the MMP-9. Also its overexpression is associated with the activity of the transcription factor NF-kB, which is an important gene regulator involved in metastatic activities, tumor progression and invasion. Experiments indicate that over-expression of bcl-2 in the MCF7ADR cell line, enhances the activity of NF-kB-dependent transcription. Since MMP-9 is a NFkB- regulated gene.[82] TGF-b1 found to modulates the homeostasis between MMPs and MMP inhibitors through p38 MAPK and ERK1/2 in highly invasive breast cancer cells.[83] Protein arginine methyl transferase 7 (PRMT7) increases the expression of Gelatinase B (MMP-9), which is a well-known mediator of breast cancer metastasis. Importantly, results demonstrate that upregulation of PRMT7 in breast cancer may have a significant role in promoting cell invasion through the regulation of MMP-9.[84] MMP-9 is produced primarily by the tumor cells themselves. One clinical study done on a mouse orthotopic model of basal-like breast carcinoma has shown that human breast cancer cell-produced MMP- 9 is primarily required for invasion in cell culture and for pulmonary metastasis. Tumor cell-produced MMP-9 further promotes tumor vascularization with minimal impact on primary tumor growth. MMP-9 is mostly overexpressed in human basal-like and triple negative tumors, mainly it contributes to metastatic progression.[85] In one clinical study determines that wnt-5A shows inhibitory action on MMP-9. Wnt-5A triggers Cdc42 activation leading to an ERK1/2 dependent decrease in MMP-9 activity and invasive migration of breast cancer cells.[86] In some studies, an oncogene testes-specific protease 50 (TSP50) was found to overexpressed in breast cancer samples, also it was shown that TSP50 overexpression increased expression and secretion of MMP-9, which is a target gene of NF-κB signaling pathways. As well as, knockdown of MMP-9 resulted in inhibition of cell migration and invasion *in vitro*. The co expression of TSP50 and p65 and TSP50 and MMP-9 were correlated with increased metastasis and poor survival. As well as estrogen receptors and progesterone receptors levels, were found to associated well with TSP50/p65 and TSP50/MMP9 expression status.[87] As we know that cancerous tumors which are fast growing often have areas of low level of oxygen content and hypoxia condition is maintained in tumours. This low oxygen environment tends to stimulates the production of Hypoxia-inducible factor 1 (HIF1) that in turn induces SDF-1/CXCL12 pathways in tumor cells and which further recruits MMP-9 producing monocytic cells from the bone marrow. These cells are sufficient to generate an angiogenic switch in cancer by increasing expression of MMP-9 and leads to metastasis.[88]

In one clinical study Increased levels of MMP-9, NGAL and the MMP-9/NGAL complex were measured in the serum of patients with invasive ductal carcinoma. These parameters were correlated with disease severity and further invasion of tumor and metastasis.[89] The PN-1/protease complex when binds to LRP-1 it activates ERK signaling pathway which leads to increased expression of MMP-9 and which further contributes to tumor metastasis. It was found that the serine protease inhibitor protease Nexin-1 controls mammary cancer metastasis mainly through LRP-1–mediated MMP-9 expression.[90] MMP-2 and MMP-9 expressions in breast cancer is related to the expression of the transcription factor AP-2 and oncogene HER2. MMP-9 expressing stromal cells are related with poor prognosis in hormone-responsive small tumors. AP-2 has been shown to be critically responsible for MMP-2 transcription and also in inducing MMP-9 promoter activity.[91] Shp-2, an src homology (SH) two-containing phosphotyrosine phosphatase, has various important roles such as control of cell spreading, cell migration, and cytoskeletal architecture. SHP-2 found to promotes invasion and metastasis of MCF-7 breast cancer cell lines with the loss of E-cadherin, the FAK dephosphorylation and also through the secretion of MMP-9 which is induced by IL-1b. The invasive potential of human breast cancer cells can be enhanced by adding IL-1b which acts through a SHP- 2-dependent signaling pathway. Activation of this pathway also results in higher levels of secreted MMP-9 .[92] avb3 (Adhesion receptor integrin) promotes metastasis of human breast cancer cells in cooperation with MMP-9. Only cells with activated integrin avb3 had activated 82 kDa (MMP- 9) in the cell culture supernatant.[93]

Esophageal cancer

Esophageal cancer is one of the least studied and deadliest carcinoma worldwide. Cancers arising from the esophagus are relatively uncommon in the United States of America, almost 13,900 new cases and 13,000 deaths anticipated in 2003. The lifetime risk is higher in males as compare to females like 0.8 percent for males and 0.3 percent for females. The risk of esophageal carcinoma increases with age, with a mean age at diagnosis of cancer is 67 years. Statistics shows that esophageal cancer is the seventh leading cause of death from cancer among American males, mainly found in black males, who have a higher incidence of this disease (13 cases per 100,000 persons) than do males in other racial or ethnic groups. Worldwide, it is the sixth leading cause of death from cancer.[94] There are mainly two types of esophageal cancer are there as squamous-cell carcinomas (ESCC) and adenocarcinomas. More than 90 percent of esophageal cancers are either squamous-cell carcinomas or adenocarcinomas, SCC is more prevalent in the upper and mid-esophagus, whereas the adenocarcinoma predominates in the lower esophagus.[95] Data from studies in animals suggested that oxidative damage from factors such as smoking or gastroesophageal reflux, which then cause inflammation, esophagitis, and increased cell turnover, may initiate the carcinogenic process in esophageal carcinoma.[96] Various risk factors for esophageal cancer to happen are tobacco use, alcohol use, barrett's esophagus, weekly reflux symptoms, obesity, poverty, achalasia, caustic injury to the esophagus, nonepidermolytic palmoplantar keratoderma (tylosis), plummer–vinson syndrome, history of head and neck cancer, history of breast cancer treated with radiotherapy, frequent consumption of extremely hot beverages, prior use of beta-blockers, anticholinergic agents, or aminophyllines.[97]

Regulation and activation of MMP-9 in esophageal cancer.

The role of MMP-9 in esophageal cancer is relatively same as we were already discussed in various cancers. MMP-9 overexpression leads to metastasis, angiogenesis and tumor invasion. As we previously stated that spread of malignant tumors is a multistep process which is involved with rapid growth and invasion into the lymph node and blood vessels, Degradation or breakdown of the extracellular matrix are the main structural changes which are necessary for migration of tumor cells, the regulation of tissue remodelling is accomplished by complex control of the expression and activity of matrix metalloproteinases in esophageal cancer.[98] MMP-9 (Gelatinase B) and MMP-2 have been reported to be important in the process of tumour invasion and metastasis in esophageal cancer.[99] In esophageal squamous cell carcinoma, cytoplasmic expression of MMP-9 is predominantly observed in carcinoma cells at the invasion front as compared to prostate cancer. MMP-9 expression often correlated with the expression of other MMP such as matrilysin-2/MMP-26, which is an activator of proMMP-9. Concomitant expression of matrilysin-2 and MMP-9 at the cancer invasive front was correlated with poor prognosis in esophagus squamous cell carcinoma.[100] One study conducted on esophageal squamous cell carcinoma tissues concluded that increased levels of MMP-2 and MMP-9 proteins in ESCCs as compared to normal esophageal tissues suggest their association with esophageal cancer invasion. Increased levels of these MMPs are observed in majority of dysplasias analyzed, which indicates that these alterations may be early events in esophageal tumorigenesis.[101] Matrix metalloproteinases, especially Gelatinase A (MMP-2) and Gelatinase B (MMP-9), and also their inhibitors, TIMP-1 and TIMP-2 found to play a crucial role in tumor invasion and metastasis. Physiological balance between MMPs and their inhibitors has maintained in healthy condition. But in disease condition such as cancer the imbalance between MMPs and their inhibitors may facilitate tumor progression. Percentages of MMP-9 positive immunostaining (diagnostic sensitivity) in esophageal cancer tissue reached 78% in the study by Murray et al. and increased with tumor stage. The diagnostic sensitivity of MMP-9 in the study of El-Shahat et al. was 100% in stage IV EC.[102] Id-1 (inhibitor of differentiation or DNA binding) is a helix-loophelix protein overexpression of this protein has been observed in many types of carcinomas including esophageal squamous cell carcinoma (ESCC). Also it exhibited increased invasive potential, which was mediated by PI3K/AKT dependent pathways and activation of MMP-9 expression, as it promotes tumorigenicity and metastasis of human esophageal cancer cells by activation of MMP-9 mediated activation of PI3K/AKT signaling pathway.[103] Concerning Gelatinase B (MMP-9), It was found that 71% of esophageal carcinoma cases associated with intense MMP-9 immuno reactivity in tumor tissues and found almost no expression in normal tissues so it was significantly related with tumor stage; One clinical study demonstrates that ESCC produces MMP-9, those findings suggest that the ability of MMP-9 production by the tumor can play significant role in its malignant behavior and tumor in esophageal carcinoma. CD34 and MMP-9 are highly expressed in human ESCC and a significant relationship was noted between MVD count and expression of MMP-9.[104] One study done on human esophageal squamous cell carcinoma cell lines shown that esophageal squamous cell carcinoma can able to produces a variety of MMPs including proMMP-1, -2, -3, and -9 in vitro, which suggests that the ability of MMP production of the tumour may play an important role in its malignant behavior of esophageal carcinoma. The production of proMMP-9 can be regulated by EGF via overexpression of EGF receptors.[106]One clinical study carried out on human esophageal cancer cell line, (TE-1), detected significant overexpression of placental growth factor (PLGF) and matrix metalloproteinase 9 (MMP-9) in the esophageal cancers with metastasis. Placental growth factor (PLGF) levels of PLGF in TE-1 cells found to positively affected the MMP-9 expression but in contrast the levels of MMP-9 did not affected the levels of PLGF, which demonstrates that PLGF may activate MMP-9 in esophageal cancer cells.[107] MMP-9 production in ESSC is regulated by various multiple signaling transduction pathways. Primarily Ras/Raf

extracellular signal regulated kinase (ERK)1/2 cascade pathway is associated with induction of MMP-9 expression in esophageal carcinoma, but more strongly induction of MMP-9 expression requires a combination of multiple signaling pathways. The signaling molecules and the phosphorylation of cytoplasmic substrates activated by Axl in various cell types include phosphatidylinositol 3-kinase (PI3-K), Akt, S6K, Src kinase, ERK, p38 mitogen-activated protein kinase (MAPK) and nuclear factor-kB. Growth factor inducing MMP-9 by combination pathways are shown in Figure 4(a) .[108] In one clinical study it was found that Axl induces both the PI-3K/AKT and ERK pathways but not able to activate other mitogen-activated protein kinase (MAPK) pathways. Activation of the ERK signal transduction pathways by Axl further leads to the activation of two factors named as nuclear factor-kB (NF-kB) and Brg-1 chromatin-remodeling factor. Translocation of Axl-activated NF-kB and Brg-1 into the nucleus induces MMP-9 expression, which plays an important role in invasion and metastasis of tumors mediated through ERK–NF-kB and Brg-1 pathways. Thus producing tumor invasion. Signal transduction pathways are shown in Figure 4(b) .[109]One study demonstrates that osteopontin is able induces pro-Gelatinase A (MMP-2) and pro-Gelatinase B (MMP-9) activations through two distinct pathways. Nuclear factor inducing kinase (NIK) plays an important role in OPN-induced NFκB activation, uPA secretion, and activation of pro-MMP-9 through MAPK/IKKα/β- mediated signal transduction pathways, which further controls the cell motility, invasiveness, and tumor growth. When OPN binds to αvβ3 integrin receptor it induces phosphorylation and activation of NIK and its subsequent interaction with IKKα/β, which further activates NFκB through phosphorylation and degradation of IκBα. also in addition, NIK in the presence of OPN induces MEK-1/ERK1/2 phosphorylation, which further found to activate NFkB. OPN stimulates NIK-dependent IKK/MAPK-mediated uPA secretion, which regulates pro-MMP-9 activation. OPN inducing MMP-9 activation pathways are shown in Figure 5.[110]

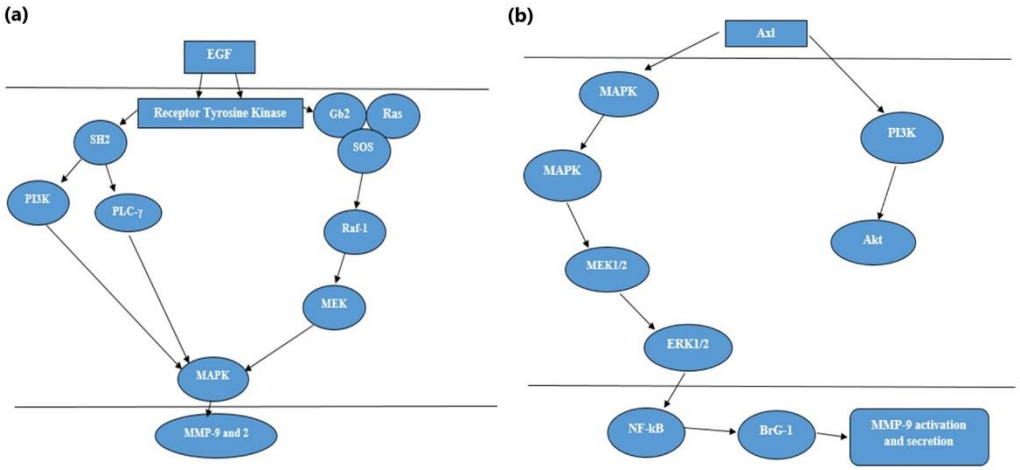

Figure 4: (a) Signal transduction pathways for growth factor inducing MMP-9 expression (b)Signal transduction pathways for Axl inducing MMP-9 activation and secretion

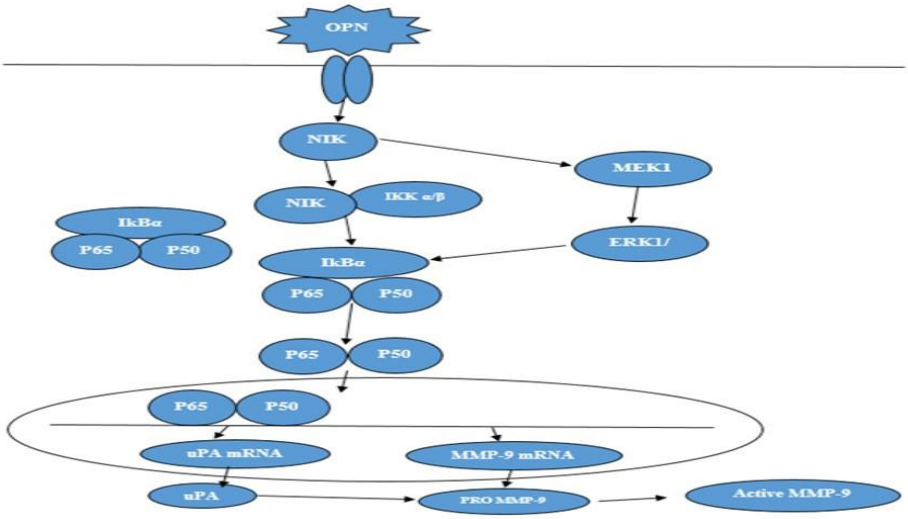

Figure 5: Osteopontin inducing NFκB activation, uPA secretion, and activation of pro-MMP-9 through MAPK/IKKα/β-mediated signal transduction pathways

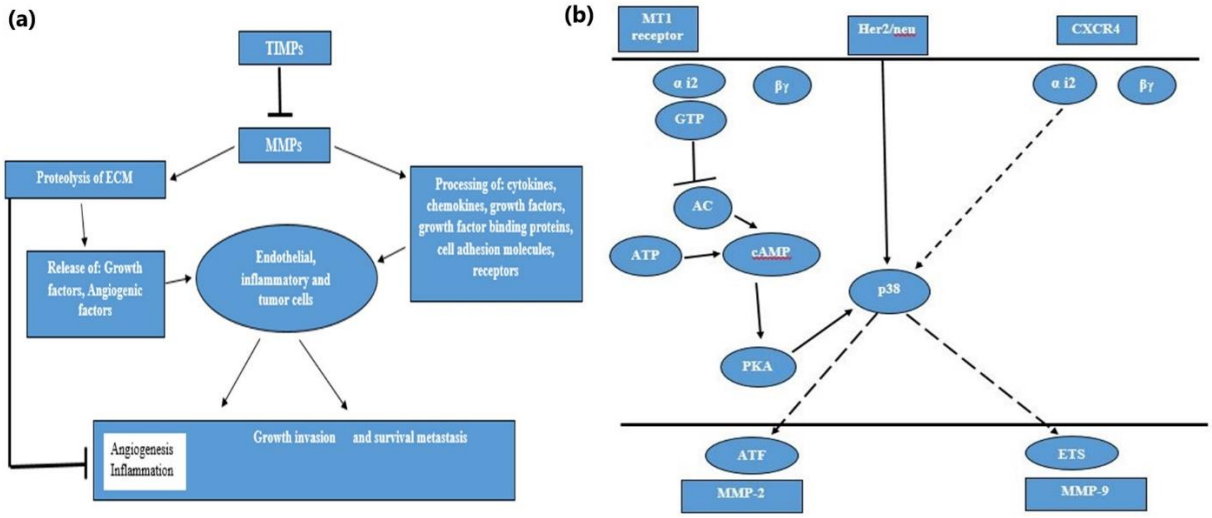

Figure 6: (a) Inhibition of MMP-9 by TIMP (b) Downregulation of MMP-9 and MMP-2 by melatonin through MT1 receptor mainly by downregulating the p38 MAPK signaling pathway

Table 2: Synthetic MMP inhibitory drugs in clinical trials

Sr. No.	Drugs	Targeted MMPs	Cancer type	Clinical trial status
1	Batimastat (BB-94) 5362422a	Broad spectrum including MMP-1-2-3-7-9-14	Malignant carcinomas including pancreatic, colorectal, gastric, ovarian, mesothelioma	Cancelled in phase 2 clinical trials, because of local toxicity and slow accural
2	Marimastat (BB-2516) 119031a	Broad spectrum including MMP-2-3-7-9	Breast, lung, colorectal, pancreatic, gastric, prostate, glioblastoma	Cancelled in phase 3 clinical trials because of prolongation of survival in gastric cancer
3	Tanomastat (BAY 12-9566) 6918336a	MMP-2-3-8-9-13	Pancreatic, ovarian and small cell lung	Cancelled in phase 3 clinical trials
4	Prinomastat (AG3340) 466151a	MMP-2-3-9-13-14	Non-small cell lung and esophageal carcinoma	Cancelled in phase 3 clinical trials
5	Rebimastat (BMS-275291) 9913881a	MMP-1-2-3-8-9-13-14	Non-small cell lung, breast and prostate	Cancelled in phase 3 clinical trials
6	Andecaliximab (GS-5745)	MMP-9	Gastric, breast, pancreatic, non-small cell lung, colorectal and esophageal	Ongoing phase 1,2 and 3 clinical trials
7	AB0041, AB0046, GS-5745	MMP-9	Colorectal carcinoma	Active in preclinical studies
8	DX-2400 Monoclonal Antibody	MMP-14-1-2-3	Breast, melanoma, fibrosarcoma	Both are active in preclinical studies
9	AG3340	MMP-2 -3	-	In phase 2/3
10	BAY 12-9566	MMP-2 -3	-	Development halted
11	BMS-275291	MMP-2 -9	-	Phase 1
12	CGS 27023A	Broadspectrum	-	Phase 1
13	Col-3 (metastat)	MMP-2 -9	-	Phase 1

Inhibition of MMP-9

MMP9 activity found to be tightly controlled by multiple agents to maintain the physiological balance in the host. Inhibition of Gelatinase B (MMP-9) has been done by various inhibiting agents such as various proteins as RECK, alpha macroglobulin, TIMP, by fatty acids and by certain chemicals such as various tetracyclines. There are four specific inhibitors of MMP are found as TIMP-1, TIMP-2, TIMP-3 AND TIMP-4. Primarily MMP-9 is inhibited by TIMP-1. And it binds to the hemopexin domain of pro-MMP-9.[111] The hemopexin domain of MMP-9 interacts with the carboxyterminus of TIMP-1.[112] Free TIMPs play other functional roles such as cell growth control, blocking angiogenesis and induction of oligodendrocyte differentiation, in normal conditions a physiological balance exists between TIMPs and MMPs, disease states are mainly associated with an imbalance. TIMPs are able to inhibit the active forms of MMP-9. Nevertheless, TIMP-1 (and TIMP-3) complexes preferentially interact with the C-terminal hemopexin domain of proMMP-9[113] schematic representation shown in Figure 6(a).

Targeting MMPs for anticancer treatment

MMPs may represent ideal pharmacologic targets for treatment of cancer. From the 1990s to early 2000s, synthetic inhibitors of MMPs (MMPI) were studied in various cancer types. Unexpectedly, due to lack of strongly promising preclinical data, all trials were gone unsuccessful in reducing tumor metastasis and burden or improving overall patient's survival in addition, unforeseen, severe side effects were also occurred because of MMPI. Two main reasons can be explained for the failure of MMPIs in clinical trials. Firstly, some MMPs have antitumor effects; therefore, the broad-spectrum MMPIs used in the initial trials might block these MMPs and which results in tumor progression. Secondly, in addition, although MMPs are involved in the early stages of tumor progression, MMPIs were tested in patients with advanced disease, beyond the stage when these compounds could be effective. As more specific MMPIs are now available, MMP targeting could be reconsidered for cancer therapy; however, new trials should be designed to test their anti-metastatic properties in early-stage tumors, Given the robust experimental and clinical evidence associating MMPs with tumor progression and poor prognosis, several MMPIs were synthesized and trialed from the late 1980s into the early 2000s for various cancer types.[116,117,118] As shown in **Table. 2.**

Some studies were carried out to target MMP-9 to decrease the tumor invasion and metastasis in breast cancer and esophageal cancer as follows,

Berberine is an isoquinoline derivative alkaloid compound. A clinical study carried out on MDAMB- 231 human breast cancer cells, has shown the inhibitory effects of berberine on MMP-9. TNF-α-induced AP-1 DNA binding activity was inhibited by berberine. TNF-α-induced cell invasion was significantly decreased by berberine treatment. Taken together, it was suggested that TNF-α-induced MMP-9 expression and cell invasion are mainly decreased by berberine through the suppression of AP-1 DNA binding activity in MDA-MB-231 human breast cancer cells.[114] One clinical study had shown inhibitory effects of blueberry on MMP-9 in breast MDA-MB-231 breast cancer cell lines. Blueberry Phytochemicals found to inhibit growth and metastatic potential of MDA-MB-231 Breast Cancer Cells. That action was mediated by modulation of the phosphatidylinositol 3-Kinase Pathway. Blueberry treatment decreased the activity of MMP-9 and the secretion of uPA while it increases expression of TIMP-1 and plasminogen activator inhibitor-1 secretion. It was found that treatment with blueberry decreased (PI3K)/AKT and NFκB activation in MDA-MB-231 cells, but protein kinase C and extracellular signal-regulated kinase (ERK) were not affected.[115] One inhibitory study of MMP-9 states that, Melatonin can be used in the regulation of breast cancer cell invasion. Melatonin, through MT1 receptor, plays an inhibitory role in breast cancer cell invasion, mainly by downregulating the p38 MAPK signaling pathway, further downstream activity of MMP-2 and MMP-9. G protein coupled receptor MT1 leads to coupling of Gi2 protein to MT1 receptor. As a result, the Gi2 subunit dissociates from the Gbg subunits and inhibits the activity of adenylcyclase (AC), which leads to a decrease in the levels of cAMP and protein kinase A (PKA) activity is inhibited. Further cAMP/PKA pathway tends to cross-talks with the p38 pathway through PKA. Activity of p38 is suppressed, and this causes further downregulation of Gelatinase B (MMP-9) expression through repression of ETS1 transcriptional activity and, specifically, downregulation of Gelatinase A (MMP-2). Schematic pathways are shown in **Figure 6(b).**[76] As we know matrix metalloproteinase-9 (MMP-9) plays an important role in the invasion and metastasis of cancer cells. One clinical study demonstrated the inhibitory effect of Silibinin, a flavonoid antioxidant from milk thistle (Silybum marianum L.) on PMA-induced MMP-9 expression carried out on MCF-7 human breast carcinoma cells. Silibinin significantly and specifically suppressed PMA-induced MMP-9 expression. This has been mediated through blocking the activation of AP-1 mediated by MAPK signaling pathways thus it inhibits PMA-induced MMP-9 gene transcriptional activity.[77] Propofol has shown inhibitory action on MMP-9. Propofol inhibited proliferation, invasion and angiogenesis of human Eca-109 carcinoma cells *in vitro* mediated through ERK-VEGF /MMP-9 signaling pathway. Propofol not only can be used as an anesthesia agent which reduces pain but plays an important role of inhibiting the migration and angiogenesis of ESCC cells in the therapy of ESCC patients. By reducing MMP-9 expression.[105]

CONCLUSION

By understanding various signaling pathways which leads to activation of MMP-9, and thus progression of cancer and poor prognosis of patients. In future various molecules in steps future can be found as profound targets for MMP-9 inhibition and can be used to provide new strategies for inhibition of cancer metastasis and angiogenesis.

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