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Regulation of matrix metalloproteinase (MMP) gene expression by protein kinases

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TABLE OF CONTENTS

1. ABSTRACT

Matrix metalloproteinases (MMPs) are a family of structurally and functionally related zinc-containing endopeptidases that are capable of degrading almost all of the components of the extracellular matrix (ECM). Under physiological and pathological conditions, the MMPs play a significant role in the efficient tissue turnover and remodeling. Specific MMPs are responsible for the matrix degradation and remodeling. Maintenance of the equilibrium between deposition and degradation of the extracellular matrix is essential to the normal tissue development. Therefore, synthesis and breakdown of the MMPs are tightly controlled by protein kinases which mediate a host of other cellular processes. The MMPs are often induced by several agents and any uncontrolled expression of the MMPs can contribute to the pathogenesis of many human diseases. This review focuses on the regulation of the MMPs by the protein kinases at the level of gene expression and their signaling pathways.

2. INTRODUCTION

The matrix metalloproteinases (MMPs) are a family of structurally and functionally related zinc-containing endopeptidases that are capable of degrading almost all of the components of the extracellular matrix (ECM) (1). Most cells in the body express MMPs although some are often associated with a particular cell type (2). Based on their structures, sequence similarities and substrate specificities, they are often classified into subgroups of collagenases (MMP -1, -8, -13 and -18), gelatinases (MMP -2 and -9), stromelysins (MMP -3, -10 and -11), matrilysins (MMP -7 and -26), membrane-type (MT) MMPs (MMP-14 -17, 24-25) and other MMPs (1, 3-8) as shown in Table 1. The MMPs are known for their ability to cleave one or several extracellular matrix (ECM) constituents as well as non matrix proteins (3). The collagenases generally cleave the interstitial collagen isotypes I, II and III as well as non-ECM proteins (1). The gelatinases are particularly efficient in digesting elastin and

Table 1. Matrix Metalloproteinase (MMP) Family

MMP	Alternative names
MMP-1	Collagenase 1
MMP-2	Gelatinase A
MMP-3	Stromelysin -1, Transin-1, Procollagenase
MMP-4	(Obsolete)
MMP-5	(Obsolete)
MMP-6	(Obsolete)
MMP-7	Matrilysin; Pump-1, Matrin, Uterine Metalloproteinase
MMP-8	Collagenase 2
MMP-9	Gelatinase B
MMP-10	Stromelysin -2, transin-2
MMP-11	Stromelysin 3
MMP-12	Macrophage elastase
MMP-13	Collagenase 3
MMP-14	MT1-MMP (Membrane Type 1-MMP)
MMP-15	MT2-MMP (Membrane Type 2-MMP)
MMP-16	MT3-MMP (Membrane Type 3-MMP)
MMP-17	MT4-MMP (Membrane Type 4-MMP)
MMP-18	Collagenase 4 (Xenopus)
MMP-19	RASI (rheumatoid arthritis synovial inflammation)
MMP-20	Enamelysin
MMP-21	XMMP (Xenopus)
MMP-22	CMMP (Chicken)
MMP-23	CA-MMP (CA = cysteine array)
MMP-24	MT5-MMP (Membrane Type 5-MMP)
MMP-25	MT5-MMP (Membrane Type 5-MMP)
MMP-26	Matrilysin-2; Endometase
MMP-27	CMMP (Gallus); Human paralog of MMP-22
MMP-13	Epilysin

Comprised from Reference #1, 3-8

collagen IV, the major structural component of the mesengial ECM and glomerular basement membrane (GBM) (1). The stromelysins have broad substrate specificity for proteoglycans, fibronectin, laminin and GBM collagens. The matrilysins and MT-MMPs are known to digest a number of ECM components such as fibronectin, laminin and gelatin (9). The other MMPs form a broad heterogeneous subgroup because of their different substrate specificity, amino acid sequence and domain organization, and the fact that they cleave such substrates as aggrecan, casein, elastin and gelatin (10).

Maintenance of the equilibrium between deposition and degradation of the ECM is essential to normal tissue development wound repair or tissue damage due to inflammation. In different pathological states, degradation is disproportionately increased with the concurrent disequilibrium. Specific MMPs are responsible ECM degradation. MMP activity is demonstrated by the collagenases, the gelatinases and the stromelysins. Precisely how the production of these MMPs is regulated is important in understanding the physiologic and pathologic processes involving the ECM.

Under physiological and pathological conditions, the MMPs play a significant role in the efficiency of tissue turnover and remodeling. In addition to cleaving virtually all structural ECM molecules, the MMPs also cleave several, circulating, cell surface pericellular proteins (11). For this purpose, the MMPs are tightly regulated by different mechanisms during virtually every aspect of their life span, from induction to their ultimate destruction (3, 11). Therefore, the MMPs are variously regulated at three levels: (i) induction of gene expression; (ii) activation of the latent proenzymes; and (iii) inhibition of their enzymatic activities by inhibitors of metalloproteinases (TIMPS) (12). This review focuses on the regulation of MMPs at the level of gene expression and their signaling pathways.

3. MMP GENE EXPRESSION

Regulation of the MMPs occurs, at the level of gene expression, with precise spatial and temporal compartmentalization of both synthesis and secretion by resident cells as well as by those cells invading the tissue (13). The literature is replete with examples of normal MMP gene expression which may be cell type-specific, tissue-specific and even stage-specific as well as constitutive and inducible (14). There are also differences between *in vitro* and *in vivo* patterns of expression of the MMPs. Most of the MMPs are synthesized in latent forms in the cells in response to varying stimuli and immediately secreted into the ECM without being stored but require proteolytic cleavage for enzymatic activity (15). However, a few MMPs are activated intracellularly by a furin-like mechanism and, are therefore fully active when they reach the extracellular space (2,8). On the other hand, MMP-8 is synthesized by polymorphonuclear leukocytes, stored in their secretory granules and released in response to appropriate stimuli (16). Although MMP-8 was originally regarded as the exclusive product of neutrophils, where it was stored until needed (17, 18), recent studies have shown that it is also expressed by chondrocytes (19), synovial fibroblasts and endothelial cells (20) as well as by human foreskin fibroblasts(21). In cell type-specific expression, phorbol esters induce MMP-3 expression in fibroblasts, but induce MMP-10 expression in keratinoctyes (22). MMP-13 is mainly expressed by tumor cells in squamous cell carcinomas of the skin, oral cavity and larynx (23) but not in intact or re-epithelializing epidermis, healthy oral mucosa or normal kerotinocytes *in vitro* (24), demonstrating that MMP-13 is specifically expressed by malignantly transformed squamous epithelial cells (25). Also, while MMP-2 expression is generally restricted to connective tissue cells, it is rarely expressed in epithelial tissues, just as MMP-7 is expressed in glandular epithelial cells of normal small intestine but is not observed in stromal cells of the same tissues (22). In stage-specific expression, MMP-7 is expressed by promonocytes while MMP-1 and MMP-3 are expressed by activated macrophages (26). Expression of most MMPs is normally low in tissues but is usually induced by several extracellular stimuli when required for ECM remodeling (16). Sometimes, some of the external stimuli can have different effects in different cells or tissues. Signals that may coordinately regulate one MMP may differentially regulate other MMPs. Likewise, signals that up-regulate a

certain MMP in one setting may have no effect or even an opposite effect in another setting, depending on the absence or presence of specific signals (4). Glucocorticoids, for example, up-regulate MMP-1 gene expression in osteoblasts but inhibit it in nonskeletal fibroblasts (27).Transforming growth factor-beta (TGF-beta) suppresses the transcription of MMP-1 and MMP-3 genes but induces the expression of the MMP-13 gene. In addition, some MMPs are expressed in only a small repertoire of cell-types, like MMP-20 which appears to be confined to the enamel organ of developing teeth (11). In contrast, MMP-2 and MMP-14 (MT1-MMP) are expressed constitutively in several cell types with minimal regulation (16, 28). Additionally, MMP-9 is expressed by fibroblasts and epithelial cells *in vitro* but, *in vivo*, it is restricted to osteoclasts and bone marrow cells, suggesting that culture conditions may alter the normal expression patterns of MMPs (22, 29-31). Similarly, MMP-13 mRNA is abundantly expressed by tumor cells of vulva squamous cell carcinomas *in vivo* and by cervical carcinoma cells *in vitro* (25).

MMP-12 has been shown to play a critical role in atherogenesis. However, its expression is cell-stage-dependent. It is not expressed by undifferentiated U937 monocytic cells and human peripheral blood monocytes. By contrast, both U937-derived macrophages and human peripheral blood monocyte-derived macrophages showed spontaneous MMP-12 expression, which was significantly increased by addition of GM-CSF or anti-CD40 antibody, indicating that MMP-12 expression depends upon the state of cellular differentiation and which can be enhanced by cytokine and CD40 signaling (32). MMP-12 is also the most active MMP against elastin in human skin *in vivo* and its expression increased by heat treatment (33). MMP-19 is up-regulated in keratinocytes, smooth muscle cells, endothelial cells and macrophages (34). In addition, both leukemia inhibitory factor (LIF) and oncosstatin M (OSM) stimulate MMP-13 expression (35) while TGF-beta inhibits MMP-13 in osteoblasts (36).

MMPs have been reported to be the major factors responsible for aseptic loosening of artificial hip joints. Based on local expression pattern of the MMPs at the transcriptional level, aseptic loose artificial hip joint was analyzed for the expression of 16 MMPs. Five MMPs (MMP- 1, MMP-9, MMP-10 MMP-12 and MMP-13) were characterized by elevated expression, Nine MMPs (i.e. MMP-2, MMP-7, MMP-8, MMP-11, MMP-14, MMP-15, MMP-16, MMP-17 and MMP-19) were characterized by moderate expression. MMP-3 was characterized by reduced expression whereas MMP-20 expression was not expressed. These MMPs can potentially degrade almost all components of the periprosthetic extracellular matrix. Thus, many MMP type enzymes possibly contribute to prosthetic loosening and osteolysis through extracellular matrix degradation and connective tissue/ bone remodeling around prostheses (37). Since these MMPs have profound impact in their specific environment, their expression has to be carefully and strictly regulated by other enzymes in the system.

Under normal physiological conditions, the expression of MMPs is usually low to allow for healthy bone and connective tissue remodeling, embryonic development, angiogenesis, ovulation and wound healing (2, 8). In pathological conditions, however, the level of MMP expression increases considerably, resulting in aberrant connective tissue destruction (2).

Uncontrolled, excessive and inappropriate MMP expression contributes to the pathogenesis of many diseases such as atherosclerosis, inflammation, tumor invasion, metastasis, rheumatoid arthritis and periodontitis (2). To accomplish their normal functions, MMPs must, therefore, be present at the right time and in the right amount (11). Hence, MMP gene expression is primarily regulated at the transcriptional and post-transcriptional levels by numerous stimulatory and suppressor factors through multiple signaling pathways, and directed by the protein kinases (19).

4. DEFINING THE ROLE OF PROTEIN KINASES

4.1. Historical Perspective

Evidence for the pivotal role of PKC in the activation of many different cell types (38, 39) was largely obtained by showing the ability of exogenously supplied activators of PKC, such as phorbol esters and synthetic DAG analogues, to mimic the response of the physiologically relevant ligand. This evidence was, of course, supported by the ability of nonselective kinase inhibitors to inhibit responses to either physiological ligands or exogenously applied PKC activators in many systems. The evidence turned out to be circumstantial because a number of anomalies had arisen that cast doubt on many of the results generated following the use of those agents. The agents used, H7, staurosporine and K252a, were challenged for their poor selectivity (40). For example, H7 inhibited PKC by competing with ATP at the ATP binding site at such high concentrations that would actually prevent H7 binding to the enzyme. Staurosporine, an indolocarbazole with a broad spectrum inhibition, not only inhibited calcium-dependent but also the cAMP-dependent PKA and cGMP-dependent PKG as well as phosphorylase kinase, S6 kinase and *src* kinase with similar efficiency. K252a also showed some selectivity as an inhibitor of phosphorylase kinase.

4.2. Current Approaches

New agents and approaches have now been developed and used to define the precise role of PKC in signal transduction. Several potent and highly selective PKC inhibitors have now been developed based on the structure of staurosporine (Table 2). New techniques are also being used to clearly define the role of PKC in gene expression. Antisense oligodeoxynucleotides have been used to show the involvement of PKC in the synthesis and expression of several MMPs (41). Stable overexpression of dominant kinase-negative mutants of PKC isozymes has been used to confirm the participation of such isozymes in the gene expression of the MMPs. The discovery of RNA interference (RNAi) has opened up many new possibilities

Table 2. Protein kinase inhibitors

Protein Kinase Inhibitors	Action
H7	A broad spectrum serine-threonine kinase inhibitor of PKA, PKC and PKG
K-252a	A cell-permeable protein kinase inhibitor that inhibits CaM kinase II, PKA PKC PKG
Go6976	Selectively inhibits Calcium-dependent PKC isozymes and does not affect the kinase activity of calcium-independent isozymes
Ro318220	A competitive, selective inhibitor of PKC over PKA, CaM kinase and Phosphorylase kinase.
Ro318425	A potent and selective inhibitor of PKC
GF109203	A general Protein Kinase C inhibitor
Staurosporine	A broad spectrum inhibitor for PKA, PKC, PKG, phosphorylase kinase, S6 kinase and src kinase
Rottlerin	A specific inhibitor of PKC delta and theta
Calphostin C	A highly specific PKC inhibitor that interacts with the regulatory domain by competing at the binding site of diacylglycerol and phorbol esters.
Chelerythrine	A selective inhibitor of PKC that acts on the catalytic domain irrespective of the attachment of the regulatory domain.
PD98059	Specific inhibitor of ERK 1
SB203580	Specific inhibitor of p38MAPK.
SP600125	Specific inhibitor of JNK/SAPK
U0126	Specific inhibitor of ERK 1, 2

for suppressing gene expression to further define the role of PKC in MMP gene expression. Taken together, the new specific inhibitors and techniques have provided an understanding of the role of the specific PKC isozymes in physiological and pathophysiological process of MMP gene expression.

Mitogen-activated protein kinases (MAPKs) phosphorylate specific serines and threonines of target substrates and regulate many important cellular activities including gene expression, mitosis, movement, metabolism and programmed death. They have been studied extensively to define their roles in the regulation of MMP gene expression. In multicellular organisms, there are three well characterized subfamilies of MAPKs (42). These include the extracellular signal-related kinases, ERK 1 and ERK 2 ; the c-Jun amino-terminal kinases, JNK 1, JNK 2 and JNK 3 : and the four p38 enzymes, p38alpha, p38beta, p38gamma and p38delta. Specific inhibitors have also been used to evaluate the participation of these MAPKs in the regulation of MMP gene expression. (43).

5. REGULATION BY PROTEIN KINASES

Protein kinases are enzymes that covalently attach phosphate to the side chain of either serine, threonine or tyrosine amino acids of specific proteins inside cells. Protein phosphorylation can control protein kinase enzymatic activity, their interaction with other proteins and molecules, their location in the cell as well as their propensity for degradation by proteases. Protein kinases phosphorylate specific serine and threonine residues of target protein substrates and regulate cellular activities ranging from gene expression to apoptosis (43).

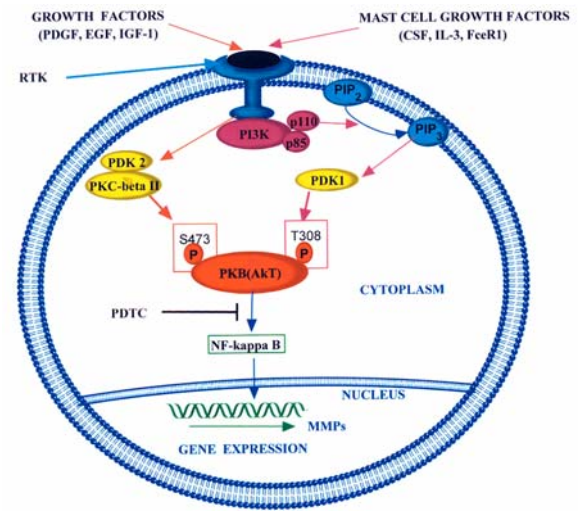
In multicellular organisms, cell growth must be tightly regulated. Individual cells must respond appropriately to a variety of signals, some of which have to reach the cell nucleus where gene transcription takes place (44). Since cells can both sense and produce signals, it is imperative that they communicate with each other for the efficient transduction of those signals through cellular receptors. Some of the chemical signals, which are membrane-permeable, pass through the plasma membrane of the cell, bind to specific receptors, which are localized in the cytoplasm or nucleus of the cell, and directly regulate gene expression (45). The membrane-impermeable signal molecules are recognized by specific receptors, which are localized on the plasma membrane. Upon binding of their ligands, the molecules transduce the signals by several mechanisms, with the binding resulting in the stimulation of intrinsic enzymatic activities of their receptors or the modulation of transducing proteins by covalent modifications, leading to the activation of effector proteins (45). One of the most important covalent modifications in this signal transmission is phosphorylation on serine/threonine or tyrosine residues mediated by the protein kinases. The different protein kinases involved in the MMP gene regulation are: Protein Kinase A (PKA), Protein Kinase B (PKB/Akt), Protein Kinase C and the mitogen-activated protein kinases (MAPKs).

5.1. Regulation by Protein Kinase A (PKA)

In the mechanism of PKA action, one of the downstream signaling targets of the PKA is the cyclic AMP response element-binding protein (CREB). This transcription factor is a CREB/ATF family member that is constitutively present in the nucleus. CREB binds to a DNA consensus called the camp response element (CRE) primarily as a homodimer, via a leucine zipper domain. CREB is activated by PKA-mediated phosporylation within its P-lot domain. CREB is activated by PKA-mediated phosporylation within its P-box domain, which permits its interaction with p300/CBP and other coactivators leading to gene transcription (46).

Cyclic AMP-dependent PKA has been implicated in both positive and negative regulation of several MMP genes. In human thyroid cells, the repressive action of parathyroid hormone (PTH) on MMP-1 mRNA was mimicked by the PKA agonists, forskolin and 8-bromo-cAMP, and was abrogated by the PKA inhibitor H-89 showing the TSH inhibitory action on MMP-1 mRNA to be PKA-mediated (47). This study also showed antagonistic cross-talk between PKA and tyrosine kinase pathway in terms of MMP/TIMP expression. This result was in agreement with another antagonistic cross-talk in which PKA repressed PKC-induced MMP-1 in human skin and synovial fibroblasts (48).

Recently, it was shown that MMP-14 was down-regulated in estrogen-deficient rat osteoblast in vivo (49). This finding was confirmed by parathyroid hormone (PTH) inhibition of MMP-14 in human osteoblast-like MG-63 cells. Inhibition was blocked by the PKA inhibitor, H-89, and also by the PKC inhibitor, staurosposine, showing that both the PKA and PKC pathways were cooperatively involved in MMP-14 down regulation by PTH (50), suggesting a role for MMP-14 in the process of bone loss during the pathogenesis of osteoporosis (49).



Figue 1. A schematic representation of the regulation of MMP gene regulation by protein kinase B (PKB/Akt).

One of the chondrocyte differentiation-related genes is the MMP-13 gene. Chondrocyte differentiation was inhibited by prostaglandin E2 (PGE2) through the inhibition of MMP-13, among other genes (51). This effect was abrogated by treatment with H-89 and GO 6976, inhibitors of PKA and PKC respectively. Complete and partial inhibitions were noted for PKA and PKC respectively, demonstrating cooperative cross-talk between the two pathways in their regulation of the MMP-13 and other genes during chondrocyte maturation. However, the action of PGE_2 is MMP-, tissue- and species-specific. In a recent report, PGE_2 induced the activity of the MMP-1 promoter in mouse osteoblasts, suggesting that PGE_2 controls MMP-1 gene expression at the translational level (52) through cAMP-PKA.

5.2. Regulation by Protein Kinase B (PKB;Akt)

Protein Kinase B (PKB), also known as Akt, belongs to the AGC family of proteins that possess a highly conserved activation loop phosphorylation site in the central kinase domain and a hydrophobic motif phosphorylation site in the C-terminus (53). PKB is a serine/threonine kinase and a critical mediator of diverse cellular processes including metabolism, cell migration, angiogenesis, cell proliferation, gene expression and apotosis. In mammals, three isoforms of PKB: alpha, beta and gamma, have been identified. They exibit a high degree of homology, but differ slightly in the localization of their regulatory phosphorylation sites. PKB-alpha is the predominant isoform in most tissues; whereas the highest expression of PKB-beta is observed in insulin-responsive tissues, and PKB-gamma is abundant in brain tissue. Each PKB isoform is composed of three functionally distinct regions: an N-terminal pleckstrin homology (PH) domain that provides a lipid-binding module to direct PKB to phosphatidylinositol 4, 5-bisphosphate (PIP_2) and phosphatidylinositol 3,4,5- triphosphate (PIP_3), a central catalytic domain and a C-terminal hydrophobic motif (54). The activity of PKB is markedly stimulated in a phosphatidylinositol 3 kinase (PI3K)-dependent manner. The mechanism underlying PKB activation has been defined. Extracellular signal molecules bind to a receptor tyrosine kinase (RTK) which possesses one or two tyrosine kinase regions with enzymatic activity. Upon ligand binding, the RTK is autophosphorylated and the resulting phosphotyrosine residues (PY) act as highly selective binding sites for other proteins which transduce the signals by changing their enzymatic activity or recruiting other proteins. Upon stimulation by ligands, PI3K is activated, which in turn generates the second messenger, PIP_3, to recruit PKB and 3-phosphoinositol-dependent kinase 1 (PDK1) to the membrane lipid rafts, where PKB is subsequently phosphorylated. The p85 subunit of PI3K is recruited to PY residues on RTK and is linked to the p110 subunit which is able to phosphorylate PIP_2. This generates PIP_3 which has several intracellular targets, such as PKB and PKC (45, 53). PKB-alpha is then activated by phosphorylation on threonine-308 (T-308) in the activation loop and on serine-473 (S-473) in the hydrophobic motif of the C-terminal tail. T-308 is phosphorylated by PDK 1 and s-473 is phosphorylated by a putative PDK 2, identified as PKC-beta II (Fig -stimulated mast cells. By contrast, PKC-beta II is not required for s-473 . 2). Hence, PKC-beta II can activate PKB by directly phosphorylating the critical residue s-473 *in vitro* and in FceR1phosphorylation in mast cells stimulated with stem cell factor (SCF) or IL-3. Therefore, PKC-beta II functions as a cell-type- and stimulus-specific PDK 2 (54). The putative PDK2 has also been identified as DNA-dependent Protein Kinase (DNA-PK) (43).

Dually phosphorylated PKB is fully active. In that regard, dually phosphorylated PKB phosphorylates and activates nuclear factor-kappa B (NF-*kappa* B) allowing its translocation to the nucleus to regulate NF-*kappa* B-dependent MMP transcription (Figure 1). MMP-9 gene transcription is regulated by NF-*kaapa*B because the MMP-9 gene has an NF-*kappa* B binding site in its promoter region and is, therefore, expressed in an NF-*kappa* B-dependent manner (55). The modulation of MMP-9 reporter activity by PKB was inhibited by the NF-*kappa* B inhibitor, pyrrolidine dithiocarbamate (PDTC), confirming that PKB modulates MMP-9 production by affecting the transcriptional activity of NF-*kappa* B in the highly metastatic cell line, HT1080 (56). MMP-9 is expressed in a large variety of malignant cells where it degrades collagen, a major component of the ECM and basement membrane. Increased MMP-9 expression correlates with the progression of various types of tumors and with the metastatic potential of the tumor cells (57). The involvement of PKB and PKC-beta II in MMP-9 gene expression also implies that PKB and PKC-beta II have important roles to play not only in cancer cell invasion but also in angiogenesis, wound healing, and autoimmune disorders, such as rheumatoid arthritis, in which MMP-9 also is a major factor. On the other hand, the Non-steroidal antiiflammatory drug, sulindac, caused a PKB-dependent down-regulation of MMP-2 and may be one of the mechanisms by which sulindac, with its metabolites, inhibits glioblastoma cell invasion via its chemopreventive and anti-tumorigenic properties (58).

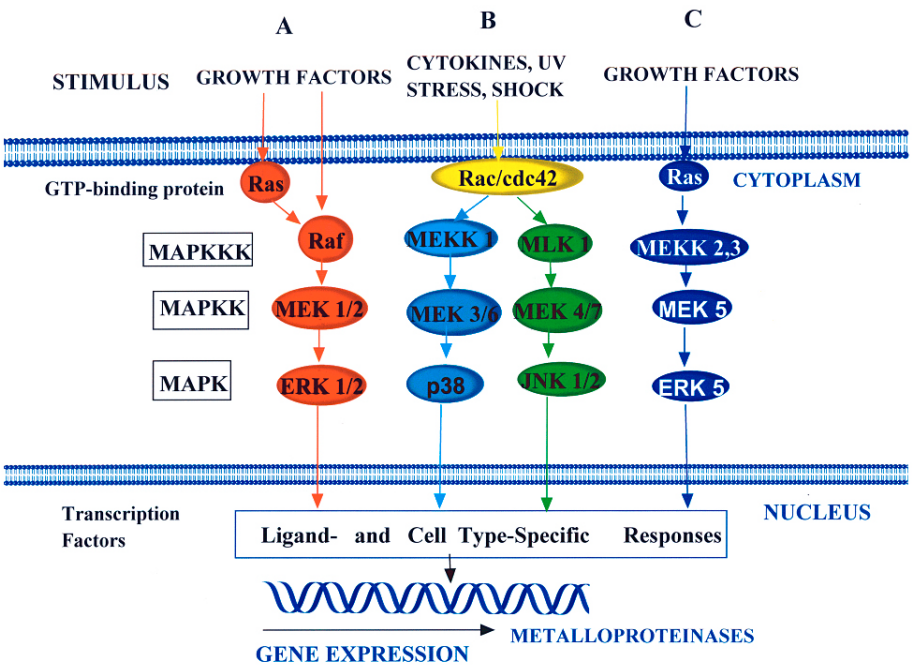


Figure 2. A schematic representation of the regulation of MMP gene expression by PKC isozymes.

Other studies have also been conducted to define the signal transduction pathway responsible for the increase in MMP-2 and MMP-14 that occur in response to mechanical stretching of trabecular meshwork cells because TM MMP and TIMP changes in response to mechanical stretching appear to be central to intraocular pressure homeostasis. The PI3K inhibitor, wortmannin, blocked the MMP-2 but not MMP-14 increase. PKB phosphorylation on T-308 and S-473 was significantly increased by stretching. The wortmannin sensitivity implicated PI3K as a modulator of the MMP-2 but not the MMP-14 increase and also implicated PDK 1 and PKB in the MMP-2 regulation, indicating that manipulation of these transduction pathways may provide new a approach to therapeutic intraocular regulation (59).

5.3. Regulation by Protein Kinase C (PKC)

PKC is a serine/threonine kinase that was first characterized by its dependence on calcium, phospholipids and diacylglycerol (DAG) for *in vitro* activation. PKC is also activated by phorbol esters and is considered the major phorbol ester receptor in the cell. Once activated, PKC plays a key role in a variety of cellular functions such as stimulation or repression of growth, changes in morphology and modulation of gene expression (60).

The PKC family of isozymes consists of three sub-families: the conventional, the novel and the atypical isozymes. While the conventional isozymes [alpha beta , beta II , and gamma] require calcium, phospholipid and diacylglycerol (DAG) for their activities, the novel isozymes [delta , epsilon , eta and theta] require only DAG and phorbol ester and the atypical isozymes [zeta , iota and lamda] require neither calcium nor DAG for their maximal activities. Human PKC mu and its murine analog, PKD, form a distinct class and are activated by DAG in the absence of calcium. Recently, this class has been named the PKD family which comprises three isoforms: protein kinase Cmu or its murine homolog, protein kinase D1 (PKCmu/PKD1), PKD2 and PKD3. In contrast to other PKC isozymes, PKCmu has a hydrophobic amino terminus, a transmembrane domain, a regulatory C1 domain with an extended spacing of two cysteine clusters and an additional 270 amino acid-sequence that separates the protein kinase domain from the regulatory C1 domain, resulting in an unusually large isozyme with an apparent molecular weight of 115kDa. In addition, PKCmu contains a PH domain that may exert an inhibitory effect on its kinase activity and also lacks the C2 domain that is responsible for the calcium sensitivity of the conventional PKC subgroup, thereby making it calcium unresponsive. Because of the lack of a pseudosubstrate sequence present in the other PKC family members, PKC-mu also displays a distinct inhibitor and substrate specificity (61).

Several studies have impicated PKC in the regulation of MMP gene expression with the use of PKC inhibitors. Production of MMP-2 and MMP-9, in a series of chondral, meniscal and synovial cultures of early osteoarthritis (OA), treated with or without catasbolic cytokines, IL-1alpha, TNF-alpha and lipopolysaccharide (LPS), was abrogated by treatment with the PKC-specific inhibitors, staurosporine, H7 and Go6976 (62) . A pan-PKC inhibitor, GF109206x, inhibited a PMA-induced an up-regulation of MMP-9 (63) while staurosporine, H7 and Go6983 inhibited MMP-2 and MMP-9 in cancer cells (63). Recent work showed that MMP-12 regulation in macrophages occured via PKC-activating G protein-coupled receptor PAR-1, which provided a distinct and focused regulation of this MMP (65). Several reports have also noted the involvement of some specific PKC isozymes in the MMP gene expressions as depicted in Figure 2.

5.3.1. Regulation by Protein Kinase C alpha

We previously showed (66) that basic calcium phosphate crystals induce MMP-1 and MMP-3 in human fibroblasts through the PKC-alpha-dependent pathway (Figure 2). In the mechanism, BCP crystals activate phospholipase C (PLC) leading to the hydrolysis of phosphatidyl inositol 4, 5-biphosphate (PIP_2) and the production of the intracellular messengers, IP_3 and DAG. IP_3 modulates the activities of the calcium-dependent enzymes such as, PKC-alpha, by releasing calcium from the endoplasmic reticulum, whereas DAG is a potent activator of protein kinase C. Further intracellular calcium was mobilized by transient opening of the calcium channel and by crystal endocytosis and dissolution (67). The mobilized calcium in the cytosol modifies the activation of PKC-alpha by DAG and induces its translocation to the plasma membrane where it becomes physiologically active (68). Some of the mobilized calcium diffuses through the nuclear pores into the nucleus (69, 70), where it enhances the BCP crystal induction of *c-fos* mRNA (68). PKC–alpha can also be redistributed from the cytosol to the nucleus (71).

It has also been shown that PKC isozymes can act in the cytoplasm and cause nuclear effects indirectly by triggering signaling pathways directed towards the cell nucleus or PKC itself can act in the cell nucleus (44). With a molecular weight of 80 kDa, PKC-alpha cannot enter the nucleus by diffusion because a protein with a molecular mass larger than 40-50 kDa cannot enter the cell nucleus by diffusion through the nuclear pores (72,73) and also because PKC does not have a nuclear localization signal (NLS) ((71). Therefore, the transport of PKC-alpha, with no NLS, into the nucleus differs from that of a protein with NLS. The simplest model of entry is that PKC-alpha has an unknown NLS which is hidden in its inactive form. Activation of PKC-alpha is accompanied by a conformational change which exposes the regions in the protein that are not accesable in the inactive form. An unknown nuclear import factor may then be able to bind to the NLS exposed after activation for translocation to the nucleus (71). This translocation may depend on the intergrity of the cytoskeleton (74). The PKC-alpha mediated up-regulation of MMP-1 and -3 was blocked by treatment with the PKC inhibitor, Go6976, thus confirming the involvement of this isozyme in gene regulation of the MMPs. It was also shown that the BCP crystal induced up-regulation of MMP-1 and MMP-3 was mediated by ERK 1, 2 (p44/42 MAPK) (66). This pathway was independent of the PKC alpha because the PKC inhibitors (staurosporine, bisindolylmaleimide I and Go6976) had no effect on BCP crystal induction of ERK 1, 2. Conversely, the ERK 1, 2 inhibitors (PD098058 and U0126) had no effect on the BCP crystal activation of PKC-alpha(61).

5.3.2. Regulation by Protein Kinase C beta

MMP-9 is the major MMP produced by human macrophages and PKC-beta is the most abundant PKC isozyme in these cells (75) which can be activated by protein tyrosine kinase. It was shown that the induction of MMP-9 gene expression during macrophage differentiation in HL-60 cells and human blood monocytes required PKC-beta to activate NF-*kappa* B to translocate into the nucleus to activate the transcription factors (Figure 2). PKC-beta also acts as an upstream signal to activate Raf in the Raf-MEK-ERK pathway (76). The above effects of PKC-beta were confirmed by the inhibition of PKC-beta activity with the PKC inhibitor, H-7, which also inhibited the MMP-9 gene expression. In addition, the use of PKC-beta-defficient HL-60 variant cells, (HL-525), failed to express MMP-9 (75).

5.3.3. Regulation by Protein Kinase C delta

A recent report showed that fibroblast growth factor-2 (FGF-2) and 12-O-tetradecanoylphorbol-13-acetate (TPA) induce MMP-9 secretion in MCF-7 cells through PKC of the Ras-Raf-MEK-ERK pathway (Figure 2). Using isoform-specific PKC inhibitors (Rotllerin and G06976), PKC-delta was identified as the PKC isozyme responsible for Ras activation (77). Other studies also found PKC-delta to mediate PMA-induced MMP-9 regulation (78) and fibronectin –induced MMP-13 gene regulation (79). In these studies, the PKC-delta activity was Ras-dependent. However, another study showed that the activity of PKC could be independent of Ras or dependent on Raf even though no MMP gene was expressed (80).

5.3.4. Regulation by Protein kinase C theta

PKC –theta is involved in the interleukin-1beta (IL-1beta)-stimulated regulation of MMP-2 and MMP-9. It activates the ERK and the JNK (Figure 3) pathways for the up-regulation of MMP-9. It also activates the NF-*kappa* B pathway for the up-regulation of both MMP-9 and MMP-2 (81; also see Figure 2).

5.3.5. Regulation by Protein Kinase C zeta

Activation of PKC-zeta has been shown in several studies to be essential in cytokine-induced MMP gene regulation. The inflammatory cytokines, TNF-alpha, IL-1 and LPS, can use ceramide as a lipid second messenger (see Figure 2). These cytokines activate a membrane associated sphingomyelinase which hydrolyzes the structural phospholipid, sphingomyelin, to phosphocholine and ceramide (82, 83). Ceramide enhances the MMP-1 expression via PKC-zeta which activates MEK. This in turn activates ERK and causes it to translocate to the nucleus where it interacts with the transcription factors. Other studies have shown transcriptional regulation of MMP-9 by PKC-zeta in glioma cells (84), hepatocellular carcinoma cells (63) and of MMP-1, -3, and -9 in rabbit smooth muscle (85). As seen with PKC-theta, PKC-zeta also activates JNK, ERK 1, 2 and NF-*kappa* B resulting in increased expression of both MMP-2 and MMP-9 induced by IL-1 beta (81).

5.3.6. Regulation by Protein kinase C mu

We had earlier reported that PKC-alpha was involved in basic calcium phosphate crystal induction of MMP-1and -3 through ERK 1, 2 and that the two pathways were independent of each other since the inhibitors of one did not affect the other (66). At that time, only the calcium dependent PKC isozymes were examined. Subsequently, all the isozymes were examined and PKC-mu was identified as one of the two PKC isozymes expressed in human

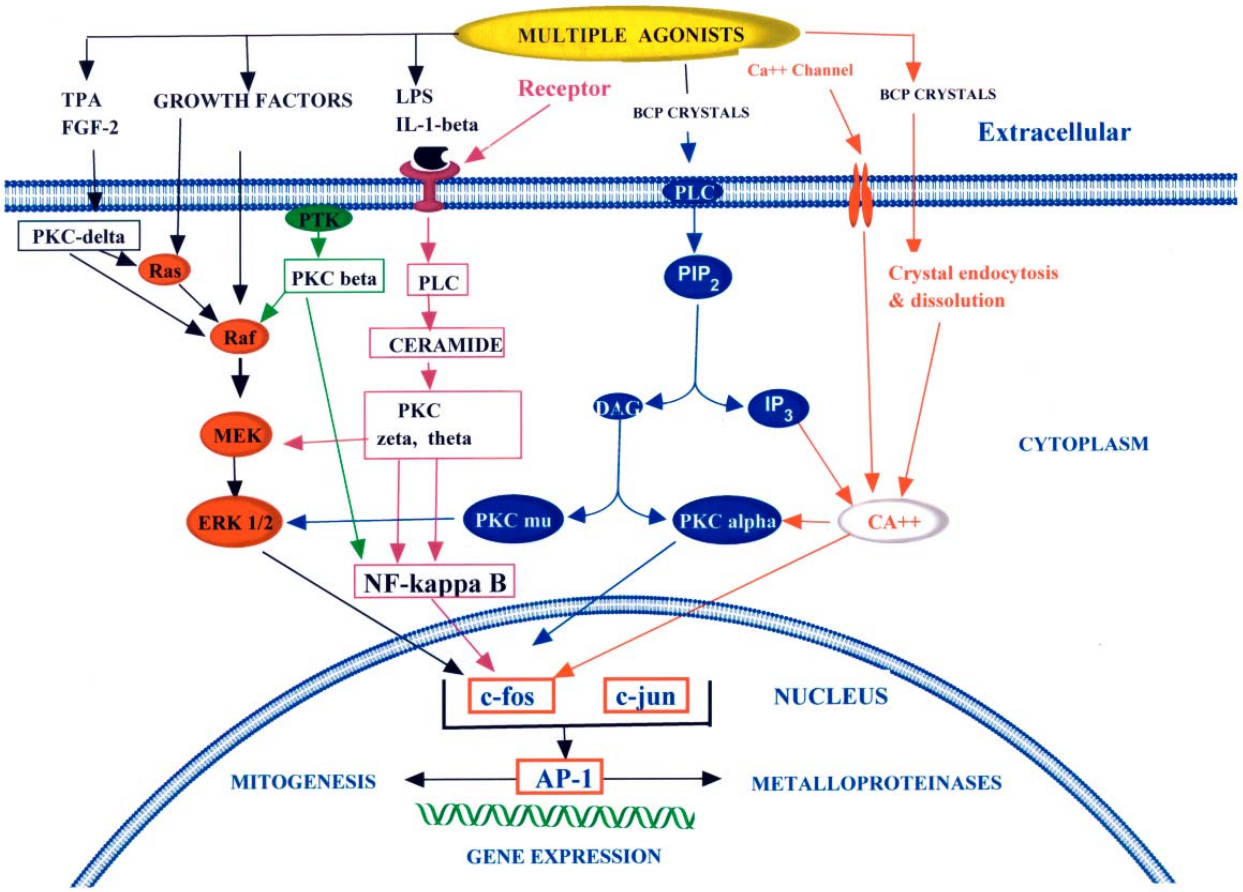


Figure 3. A schematic representation of the regulation of MMP gene regulation by the mitogen-activated protein kinases (MAPKs).

fibroblasts in response to BCP crystal activation (61). We also showed that BCP crystal activation of ERK 1, 2 was dependent upon PKC-mu because inhibition of PKC-mu, greatly attenuated the BCP crystal induction of ERK 1,2 and that inactivation of ERK 1,2 by PKC-mu inhibition, also inhibited MMP-1, -3 mRNA and protein expression (61). The fact that PKC-alpha is calcium- dependent and PKC-mu is calcium- independent and that each belongs to different PKC subfamilies, suggested differential roles for the two PKC isozymes in the BCP crystal activation of human fibroblasts. The identification of PKC-mu and the dependence of ERK 1,2 on PKC-mu helped to explain why the inhibitors of PKC-alpha did not affect ERK1, 2 and *vice versa* (66). In the subsequent work (661), it was established that BCP crystals modulated PKC-alpha and PKC-mu through the sequential hydrolysis of PLC and PIP_2, producing DAG, which independently activates PKC-alpha and PKC-mu (Figure 2) each with different substrate specificities (61). The PKC inhibitors used in the previous work (66) were specific for the calcium-dependent PKC-alpha which has a 10-fold higher sensitifity to bisindolylmaleimide and G06976 than PKC-mu. Whereas 2microM G06976 completely inhibited BCP crystal-induced PKC-alpha (66), it required 20microM G06976 and 300microM G06983 to suppress the phorbol ester-enhanced autophosphorylation of PKC-mu (61). Therefore, at the concentrations of inhibitors that inhibited PKC-alpha, PKC-mu was not inhibited and was still able to activate ERK 1,2 to facilitate translocation to the nucleus to transmit extracellular stimuli by phosphorylating several transcription factors, in agreement with a previous study which which showed that PKC-mu selectively activated p42 MAPK (ERK 2) (86).

Our studies have also provided evidence that activation of ERK1, 2 and its translocation to the nucleus were coordinated by PKC-mu. Confocal microscopy showed that the inhibition of PKC-mu activity also inhibited the activation of ERK 1, 2 and translocation to the nucleus (unpublished data). PKC-mu itself also remained in the cytoplasm. These results led us to the hypothesize that BCP crystal-induced activation of human fibroblasts and the up-regulation of MMP-1 and -3 follows two independent pathways. One pathway is the calcium-dependent PKC pathway characterized by calcium-dependent PKC-alpha and modulated by mobilized intracellular calcium as described before for PKC-alpha. The other pathway is the calcium-independent pathway which is mediated by the calcium-independent PKC-mu. Here, BCP crystals also activate the sequential hydrolysis

of PLC-PIP_2, producing DAG, which activates PKC-mu, which in turn, undergoes autophosphorylation , as a measure of activation (87) which activates ERK 1, 2 generated from the Ras-Raf-MEK-ERK 1, 2 signaling cascade (88). The activated and phosphorylated ERK 1, 2 then migrate to the nucleus to mediate BCP crystal-induced cellular responses. PKC-mu itself does not translocate into the nucleus because of its molecular weight of 115 kDa (61). ERK1, 2 can freely difuse into the nucleus because of their molecular mass of 44 kDa and 42 kDa respectively. By activating and causing the translocation of the ERKs to the nucleus, PKC-mu plays a unique role in this signal transduction pathway.

In gouty arthritis, monosodium urate (MSU) crystals induce MMP-3 expression in chondrocytes in a p38-dependent manner (89) and urate crystals also stimulate macrophages to produce MMP-9 through ERK 1, 2 (90). It is possible that their action is similarly mediated by PKC-mu. Of all the PKC isozymes identified in Figure 2, PKC-mu is the only one that appears to act direcly on the ERKs. The precise pathway of PKC-mu in relation to the ERKs is worth noting. One study, using TNF-alpha stimulating trabecular meshwork, says that PKC-mu does not appear to be upstream from ERK in the signalling cascade (91). Another study, using human adenosine A_1 in Chinese hamster ovary (CHO) cells, notes that it is likely that PKC-mu is acting downstream of ERK 1, 2 (92). In the BCP crystal -stimulated human fibroblasts, there appears to be a direct contact of PKC-mu with the ERKs. The precise pathway of PKC-mu, therefore, may be both stimuli- and/or cell type- specific.

5.4. Regulation by mitogen-activated protein kinases (MAPKs)

Mitogen-activated protein kinases (MAPKs) are part of a phosphorylation regulatory system composed of three sequentially activated kinases and, like their substrates, are regulated by phosphorylation (43). Among multicellular organisms, three sub-families of MAPKs have been characterized. These are the extracellular signal-related kinases 1 and 2 (ERK1/2), the p38 kinases and the c-Jun amino-terminal kinases (JNKs) (43). The ERKs are activated by phorbol esters, growth factors and cytokines while p38 and the JNKs are activated by inflammatory cytokines, osmotic stress and apoptotic signals (93). Activation of MAPKs in response to these stimuli controls gene expression, metabolism, cytoskeletal functions and other cellular regulatory events (94). The balance between the distinct MAPK pathways regulates cell growth, differentiation, survival and death (4). An examination of the individual pathways shows that cooperation between the multiple MAPK pathways results in the regulation of MMP transcription in response to different signals.

5.4.1. Regulation by Extracellular Signal-Regulated Protein Kinases 1 and 2 (ERK 1/2)

Extracellular signal-related Protein Kinases 1 and 2 (ERK 1 ,2) are also known as p44/42 Mitogen Activated protein kinase (p44/42 MAPK) because they are 44 kDa and 42 kDa enzymes, respectively. The signal transduction pathway leading to the activation of ERK is shown in Figure 3, Panel A. ERK activation begins when growth factors bind to cell surface receptors with intrinsic tyrosine kinase activity, resulting in autophosphorylation of the receptor which is followed by the recruitment of SH2 domain-containing adaptor proteins such as GRB2 or SHC to the activated receptor. The recruitment of the exchange factor Sos places it in the membrane next to its substrate, the small GTP binding Ha-Ras. Sos activates Ha-Ras by catalyzing GDP: GTP enchange. Once Ha-Ras is activated, it binds and recruits the serine/threonime kinase Raf-1 to the membrane. While membrane translocation of Raf-1 is essential, it is not sufficient. A second step leading to Raf-1 phosphorylation is also required. Raf-1 functions as the MAPKKK in the ERK cascade. Once activated, Raf-1 phosphorylates and activates the MAPKKs, MEK 1 or MEK 2. Once MEK 1 or MEK2 are activated, they can directly activate the ERKS (ERK 1 and ERK 2) by phosphorylating their conserved threonine and tyrosine activation sites. Once the ERKS are activated, they translocate to the nucleus where they phosphorylate transcription factors and, thereby, regulate gene expression (94).

In response to vascular injury, smooth muscle cells migrate from the media into the intima, where they contribute to the development of neointimal lesions. The MMPs are thought to be some of the principal regulators of matrix degradation in response to the vascular injury (95). The gelatinases, MMP-2 and MMP-9, have been implicated as mediators of lesion development in response to vascular injury and are expressed in human atherosclerotic lesions (96). Although MMP-2 and MMP-9 have similar specificities (97), there are differences in the regulation of their expression. MMP-2 is constitutively expressed by several cell types, including smooth muscle cells, and its expression is not induced by cytokines or growth factors (98). In contrast, the basal levels of MMP-9 are low, and its expression can be induced by treatment of cells with tumor necrosis factor-alpha (TNF-alpha) and interleukin-1 (IL-1) but not platelet-derived growth factor (PDGF) (99). Moreover, MMP-9 expression, both mRNA and protein, is induced early after the injury while MMP-2 expression is not changed following vascular injury (100). Previous studies had already shown that the activation of ERK1/2 is critical for the increased expression of MMP-9 in response to different agonists (101) and that nerve growth factor (NGF) is a potent activator of ERK1/2 in vascular smooth muscle cells expressing TrkA receptors, suggesting that NGF could also potentially regulate MMP-9 expression in smooth muscle cells (101). It was found in that study (101) that sustained NGF treatment of smooth muscle expressing TrkA induced MMP-9 expression, which was dependent on the activation of the MAP kinases, ERK1/2. This effect was abrogated by pretreatment of the cells with the MEK-1 inhibitor, U0126, confirming, the regulation of MMP-9 gene expression by ERK1/2 (102).

Protein kinases also regulate agonist-dependent reciprocal effects on MMP gene expression in the same cell. Thus, NGF activation of ERK 1, 2 induces MMP-9 gene transcription in smooth muscle cells (96). Evidence is also accumulating about the transcriptional down-

regulation of MMP-9 mediated by ERK 1, 2 in smooth muscle cells (102). In this regard, MMPs contribute to the destruction of the ECM at the shoulder regions of atherosclerotic plaques that lead to plaque destabilization and triggering clinical activation of cardiovascular disease. MMP-1, -3 and -9 are up-regulated by inflammatory cytokines and growth factors that are produced by plaque resident macrophages and smooth muscle cells through the transcription factor, NF-kappa B, which has a binding site in the promoter of MMP-9 gene and an NF-*kappa* B-like element in the promoter of MMP-1 gene. Inhibition of NF-*kappa* B by its inhibitor, Ikappa B alpha, also results in ERK inhibition and down-regulation of MMP-1 -3 and -9. This suggested that ERK participates in MMP down-regulation to promote plaque stabilization with a possible cardiovascular benefit.

The UV-induced production of MMP-1 mRNA by macrophage migration inhibitory factor (MIF) in human dermal fibroblasts was mediated by several pathways: PKC, PKA, Src family tyrosine kinase, MAPK, c-Jun and AP-1-dependent pathways. This was evidenced by the fact that the up-regulation of MMP-1 mRNA by MIF stimulation was found to be inhibited by a PKC inhibitor (GF109203X), a Src-family tyrosine kinase inhibitor (herbimycin A), a tyrosine kinase inhibitor (genistein), a PKA inhibitor (H-89) and a MEK inhibitor (PD98089) (103). However, the MIF-induced up-regulation of MMP-9 and MMP-13 in rat osteoblasts also depended on the Src-related tyrosine kinase- Ras-ERK1/2 and AP-1-dependent pathway (104). This intracellular signaling pathway may play an important role in the degradation of the collagen matrix and in bone remodeling. The biological significance of MIF in bone remodeling makes it a target molecule for therapeutic use in destructive bone diseases such as osteoporosis and rheumatoid arthritis (103).

The initial stages of diabetic nephropathy are characterized, in part, by expansion of the mesangeal ECM. Because of its significant role of insulin in the pathophysiology of diabetic glomerulosclerosis, insulin-mediated stimulation of MMP-1 gene transcription in mesangial cells is strictly regulated at the transcription level by ERK1/2 (105). It was also shown, in arsenic-induced skin carcinogenesis, that ERK MAP kinases were involved in arsenite-stimulated MMP-9 gene expression (106). Other reports of cigarette smoke-induced MMP-1 induction (107), osteopontin-induced activation of MMP-9 (3, 108) and over-expression of MMP-1 in invasing melanoma cells (109) have indicated the participation of ERK1/2 in the regulation of those MMP genes. These MAP kinases are also involved in reciprocal actions. Although the tumor metastasis supressor gene, LG11, mediated the suppression of MMP-1, -3 in glioma cells through the ERK1/2 pathway (110), work in our laboratory has shown that basic calcium phosphate (BCP) crystal induction of MMP-1, 3 in human fibroblasts is mediated by PKC and ERK1/2 signal pathways (111-113).

5.4.2. Regulation by p38 MAPK

The p38 MAPK was identified as the kinase that is activated by lipopolysaccharide (LPS) but it is activated by many other stimuli that activate the JNKs (Figure. 3 Panel B). Like the JNKs, p38 is activated by Rac and Cdc42 but it is not sufficiently activated by MEKK 1, 2 or 3. The serine/threonine kinase, ask 1, appears to be an effective MAPKKK in the p38 pathway, which can be directly activated by JNKK and also by MKK3 and MKK6 (95).

There are four well-characterized p38 kinases: alpha, beta, gamma and delta. P38 is activated by inflammatory cytokine and other stimuli such as stress, osmotic shock and heat shock (43). Mechanical stimuli induced MMP-13 in an osteoblastic cell line (114). In that regard, a unique feature of p38 is that it predominantly activates MMP-13 whose expression is inhibited by ERK1/2 (115). The involvement of p38 in expression of MMP-13 in a liver myofibroblast cell line (115) and transformed keratinocytes (116) was destroyed by the inhibition of its activity with the specific inhibitor of p38 MAPK (SB203580).

Another study showed that p38 alpha could inhibit ERK1/2 with a subsequent suppression of MMP-1 (117). Activation of p38 also inhibited PDGF-induced MMP-1 (118) and IL-1 induced MMP-1 and three-dimensional collagen-induced MMP-1 (119). These results point to p38 as a reciprocal negative regulator of MMP-1 via ERK. The bidirectional inhibition of three-dimensional collagen-induced MMP-1 pointed to p38, which provides the fine-tuned control of MMP-1 gene expression in response to biomechanical signals. Since MMP-1 and MMP-13 are implicated in arthritis and since p38 and ERK have reciprocal activities on these MMPs, these protein kinases should be evaluated for their potential role in the mechanism of controlling arthritic diseases.

5.4.3. Regulation by c-Jun Amino-Terminal kinase (JNK)

The c-Jun N-terminal Kinase (JNK) is also known as the Stress-Activated Protein Kinase (SAPK) or JNK/SAPK. There are three JNKs; JNK1, JNK2 and JNK3 (120). The JNK protein kinases are activated by Rac and Cdc42 and by phosphorylation on threonine and tyrosine by MKK4 (also known as SEK1) and MKK7 (Figure. 3, Panel B). The MKK7 protein kinase is primarily activated by cytokines (TNF-alpha and IL-1beta) and MKK4 is primarily activated by environmental stress (120).

The JNKs were discovered to bind and phosphorylate the DNA binding protein c-Jun to increase its transcriptional activity. c-Jun is a component of AP-1 transcription complex which is an important regulator of gene expression. AP-1 contributes to the control of many cytokine genes and is activated in response to environmental stress, radiation and growth factors. JNK is a critical regulator of mechanical stretch. Mechanical forces have profound effects on endothelial cells and TNF-alpha is a potent mediator of stretch-induced effects on MMP. Human umbilical vein endothelial cells (HUVEC), subjected to cyclical mechanical stretch in the presence of TNF-alpha, significantly increased mRNA expression and protein synthesis for MMP-2 and -14 (121). These increases were completely blocked after the addition of

TNF-alpha antibody or JNK inhibitor (SP600125), indicating that TNF-alpha mediated the stretch-induced MMP genes expression, at least in part, through the JNK pathway (121). SP600125 repressed the activity of PMA-stimulated MMP-9 promoter luciferase reporter, suggesting that the diminished secretion of MMP-9 reflected reduced transcription under the regulation of JNK (122).

JNK is also implicated in cancer development. Overexpression of JNK 1 in human squamous lines induced MMP-9 expression which correlated with increased tumor cell invasion of reconstituted basement membranes. Site-directed mutagenesis of the MMP-9 promoter revealed that JNK 1 cooperated with its transcriptional factor target c-Jun to increase MMP-9 expression at the transcriptional level via the proximal AP-1 site. These results suggested that elevated JNK 1 expression may contribute to increased MMP-9 activity and ECM invasion by tumor cells. (123). In a recent study, the expression MMP-1 in hepatocellular carcinoma cell line was suppressed by SP 60015, an inhibitor of JNK, but not PD98058 and SB820350, inhibitors of ERK and p38 respectively, suggesting JNK involvement in the MMP-1 expression and points to JNK as a target for cancer therapy (124).

JNK also plays an important role in rheumatoid arthritis (RA). The signaling pathways by which MMPs are induced vary depending upon the cell type and stimulus. While JNK appears to play a major role in MMP induction in rheumatoid arthritis synoviocytes (119), MMP-1 and MMP-3 gene expression was shown to be dependent on p38 MAPK (125).

In a few case, MMP gene regulation requires the coordination of two or more MAPK pathways. Thus, squamous cell carcinoma derived soluble factors activation of fibroblast MMP-1 expression and induction of MMP-13 by IL-1beta in articular chondrocytes was mediated by p38 and JNK (126, 127).

6. PERSPECTIVE

Much progress has been made in understanding the functions of the different matrix metalloproteinases (MMPs). A better understanding of how the MMP genes are regulated will also lead to a better understanding of how to regulate them in pathological situations. Identification of MMP- inducing agents will undoubtedly lead to the discovery of the mechanism of their induction and ultimately to the development of new strategies to counter MMP activity. The protein kinases play significant roles in the actions of the MMPs. Since their actions are significantly understood, effective drug development can be contemplated that will effectively bring hope to those afflicted by either the abnormal production of the MMPs or by the dysregulation of the protein kinases.

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