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Transcriptional regulation of matrix metalloprotease gene expression in health and disease

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1. ABSTRACT

The mammalian extracellular matrix (ECM) is a complex network of collagens, proteoglycans, glycoproteins, polysaccharides and other secreted proteins that plays fundamental structural and functional roles. In addition to its key function as an extracellular space-filling scaffold, the ECM is also implicated in the formation of important cell-cell and cell-ECM (i.e. juxtacrine) interactions that subsequently provide key regulatory signals that influence cellular proliferation and viability, differentiation, specialization and gene expression. Regulated turnover of the ECM, a process largely mediated by the tightly controlled matrix metalloprotease (MMP) enzyme family, is critical to a number of physiological processes involved in growth and development while aberrant turnover of matrix components is associated with congenital and metabolic diseases. The following review will focus on the transcriptional aspects of MMP gene expression, particularly in diseased states.

2. INTRODUCTION

Matrix metalloproteases (MMPs) are a large family of over twenty zinc-dependent endoproteases that are involved in the degradation of the extracellular matrix (ECM). Given the potent catabolic effects of MMPs on the ECM, MMP expression and activity must be tightly regulated *in vivo*. The importance of such fine regulation is highlighted by the various pathologies resulting from disrupted MMP regulation. The expression and activity of MMPs is controlled at multiple levels and by various mechanisms, including transcriptional activation and suppression, modulation of mRNA half-life, secretion, localization and enzymatic activation (via pro-enzyme cleavage) and inhibition (via their natural inhibitors, the tissue inhibitors of metalloproteases, TIMPs).

Positive and negative control of MMP gene expression occurs primarily at the transcriptional level (1) although post-transcriptional modulation of MMP mRNA

stability can also play a role (2, 3). Although certain MMPs are expressed in a constitutive manner, normally, MMPs are synthesized *de novo* upon the requirement for ECM remodeling via transcription from inducible promoters and then rapidly secreted. Transcriptional induction of MMP genes is regulated by a variety of physiologic and pharmacologic signals, including cytokines and growth factors, bacterial endotoxin, phorbol esters, hormones physical stress, oncogenic transformation, cell-cell and cell-ECM interactions. Each of these signals may result in the activation of specific signaling cascades, which ultimately leads to the targeting of positive or negative regulatory elements in the promoter regions of MMP genes by various transcription factors.

This review provides a detailed discussion of the transcription factors involved in the transcriptional regulation of different MMP genes within physiological and various pathological contexts.

3. TRANSCRIPTIONAL REGULATION OF MATRIX METALLOPROTEASE GENE EXPRESSION

3.1. Bone physiology and skeletal development

The complex process of bone formation (osteogenesis) involves essentially three main steps: 1) production and deposition of the extracellular organic matrix (osteoid), 2) matrix mineralization to form bone, and 3) bone remodeling through the highly regulated and interdependent processes of resorption and reformation(-) (see 4 for an extensive review). The combined cellular activities of osteoblasts, osteocytes, and osteoclasts are essential to the latter processes. Indeed, osteoblasts synthesize the collagenous precursors of bone matrix and also regulate its mineralization**.** As the process of bone formation progresses, the osteoblasts occupy small pits (lacunae) within the surrounding mineralized matrix and become osteocytes. The cell processes of osteocytes occupy minute canals called canaliculi that permit the circulation of tissue fluids. To meet the requirements of skeletal growth and mechanical function, bone undergoes dynamic remodeling by a coupled process of bone resorption by osteoclasts and reformation by osteoblasts.

Osteoblasts are mononuclear cells derived from mesenchymal stem cells of the bone marrow stroma. They synthesize and lay down precursors of collagen 1, which comprises 90-95% of the organic matrix of bone as well as proteins like osteocalcin, the most abundant non-collagenous protein of bone matrix and proteoglycans. The collagen 1 formed by osteoblasts is typically deposited in parallel or concentric layers to produce mature (lamellar) bone. The main mineral component of bone is crystalline hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] which comprises about 1/4 the volume and 1/2 the mass of normal adult bone. The mineral crystals, as shown by electron microscopy, are deposited along, and in close relation to, the bone collagen fibrils. Osteoblasts respond to extracellular stimuli and indeed express receptors for parathyroid hormone (PTH), growth factors, cytokines, steroid hormones, retinoids, bone morphogenic proteins and eicosanoids that act mainly on osteoblasts to bring about their effects on bone.

Osteoclasts mediate bone resorption and are derived from hematopoietic stem cells that also give rise to monocytes and macrophages. Typically multinucleated, osteoclasts adhere to the surface of bone undergoing resorption and lie in depressions termed Howship's lacunae or resorption bays. Increased remodeling and osteoclastic activity characterize several metabolic bone diseases such as hyperparathyroidism, Paget's disease, and others. Osteoclasts are apparently activated by "signals" from osteoblasts. For example, osteoblasts have receptors for PTH whereas osteoclasts do not, and PTH-induced osteoclastic bone resorption is said not to occur in the absence of osteoblasts.

At an early stage of human embryonic development, a cartilage model of much of the skeleton (of extremities, trunk, and base of the skull) is formed from the mesenchyme. In the further fetal development of long bones, a rim of primitive bone is first laid down in layers over the middle of the shaft by osteoblasts arising from the overlying periosteum, and subperiosteal bone formed in this way soon extends up and down the shaft (diaphysis). The process whereby bone tissue replaces membranous fibrous tissue is termed intramembranous ossification.

Skeletal formation and remodeling are strictly regulated both temporally and spatially by a variety of developmental factors and proteolytic remodeling of ECM is a *sine que non* in this regard (5, 6). A large body of evidence indicates that the MMPs play a central role in tissue-remodeling processes (1, 7). Among 26 members of this family of zinc-dependent enzymes, members of the collagenase subgroup (MMP-1, 8, and 13; collagenases 1-3, respectively) are the principal neutral proteases with the ability to degrade fibrillar collagens (among other macromolecules of the ECM). By far the best-studied collagenase in bone biology is collagenase-3, MMP-13. It generates fragments 3/4 and 1/4 the size of the original molecules, which denature rapidly and become susceptible to further degradation by other MMPs, such as the gelatinases, MMP-2 and MMP-9 (1).

It was reported that Cbfa1 (core binding factor alpha1), a transcription factor of the *runt* gene family involved in skeletal development (8), induced the expression of collagenase-3 (MMP-13) during bone formation (9, 10) subsequent to retinoid stimulation (7). Indeed, the MMP-13 promoter (approx. 1000 base pairs; bp) harbors a TATA box for enhancer complex assembly, a Cbfa1 site (-132 to -126; *note*: all nucleotide positions are relative to the transcription initiation site) that is also referred to as an osteoblast specific element (OSE-2), activator protein -1 (AP-1) (-50), polyoma enhancer A binding protein-3 (PEA3) (-78), and a TGF-beta inhibitory element (TIE) downstream at -523 (11) (Figure 1). In the rat osteoblastic cell line UMR 106-01, TGF-beta 1 stimulates collagenase-3 expression through Cfba1/Smad 2 transactivation of the Cbfa1 site (12). Cell permeable chemical inhibitors of the ERK1/2 and p38 MAPK pathways, but not the JNK pathway, reduced TGF-beta 1-stimulated collagenase-3 expression This indicated that the p38 MAPK and ERK1/2 pathways were required for TGF-

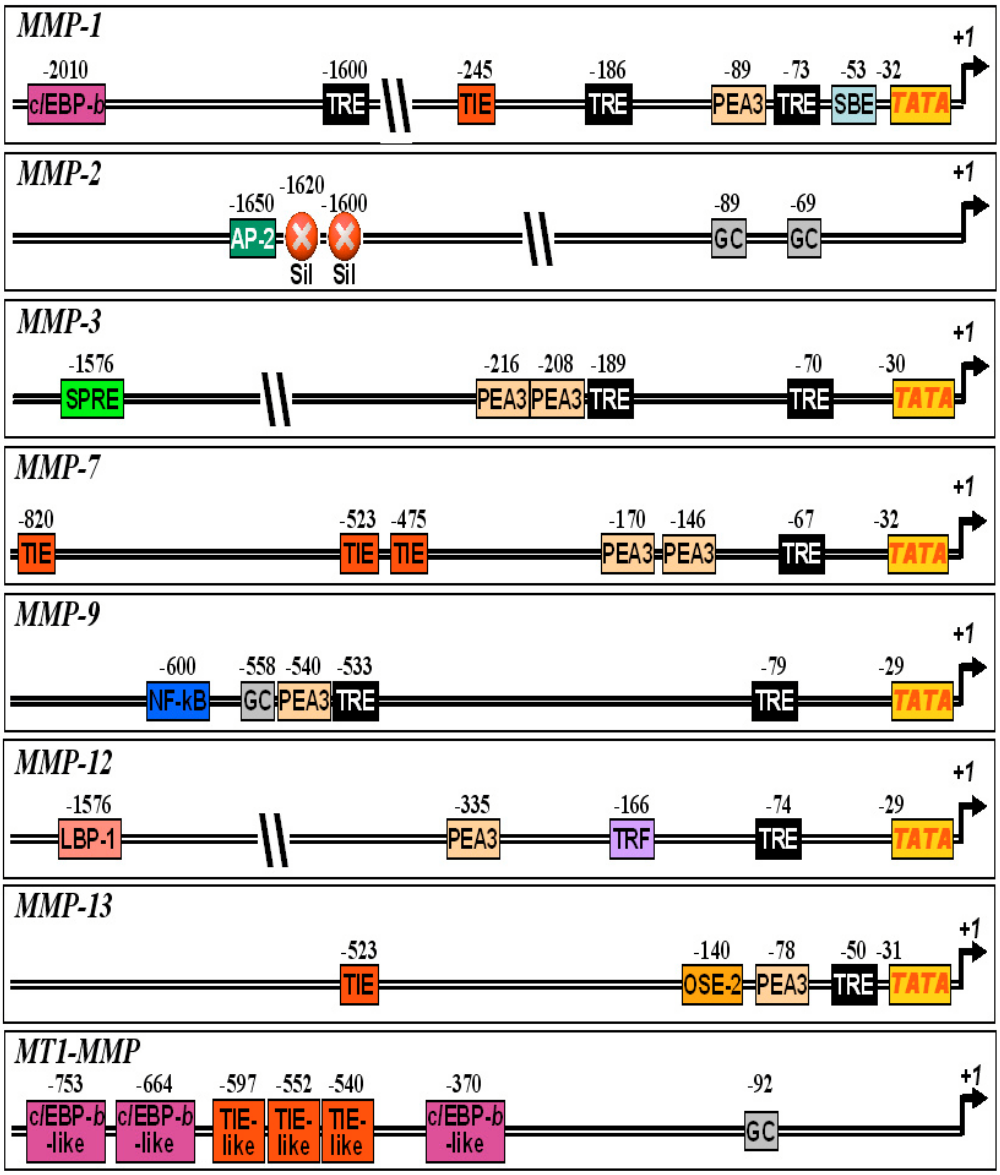

Figure 1. The regulatory elements in human MMP gene promoters. *Cis* elements are abbreviated as follows: AP-2- activator protein-2; c/EBP-*b*- CCATT/enhancer binding protein-beta; GC- GC-box (Sp1 and Egr-1 binding sequence); LBP-1- leader binding protein-1; NF-kB- nuclear factor-kappaB; OSE-2- osteoblast-specific element-2; PEA3- polyoma enhancer A binding protein-3 (Ets binding sequence); SBE- SIAT binding element; Sil- silencer; SPRE- stromelysin-1 PDGF response element; TIE- transforming growth factor beta inhibitory element; TRE- phorbol-ester response element; TRF- octamer binding protein. The number above each element represents its starting nucleotide position relative to the transcription start site (designated as +1).

eta 1-stimulated collagenase-3 expression (12). Cfba1/Runx2 is phosphorylated by p38 MAPK in response to TGF-beta 1 treatment in osteoblastic cells. Thus there seems to be an important role for cross-talk between the Smad and MAPK pathways in the expression of collagenase-3 following TGF-beta 1 treatment in UMR 106-01 cells.

With respect to steroid hormones, 1alpha, 25(OH)2D3 doesn't alter the decay of MMP-13 mRNA in transcriptionally arrested MC3T3-E1 cells; however, it increases the MMP-13 heterogeneous nuclear RNA (hnRNA) level and MMP-13 transcriptional rate (13). DNA binding activity in nuclear extracts of cells treated with hormone favored the AP-1 binding site as opposed to the Cbfa1 enhancer in the MMP-13 promoter region. Taken together, the data suggest that 1α, 25(OH) 2D3 directly or indirectly induces AP-1, which transactivated the MMP-13 promoter (13).

Basic fibroblast growth factor (bFGF) also stimulates collagenase-3 promoter activity in osteoblasts

through an AP-1 binding site (14). Mutational analysis revealed that the bFGF effect was mediated through an AP-1-binding site located at -48 to -42 nucleotides in the promoter. bFGF stimulated the binding of nuclear factors to the collagenase AP-1 site by 3- to 4-fold, as determined by electrophoretic mobility shift assays. Supershift analysis of nuclear extracts revealed that bFGF stimulates the occupancy of AP-1 site by c-Jun, JunB, JunD, c-Fos, FosB, and Fra2 (14).

Both AP-1 and Cbfa1-like factors are required for the induction of interstitial collagenase by PTH and indeed, PTH is a major regulator of calcium homeostasis as it mobilizes calcium through bone resorption (15). A *cis*-acting element in the collagenase-3 promoter was identified which, together with AP-1, is required for induction by PTH. This element contains CCACA motifs, which are required for binding of the 65 kDa osteoblast-specific splice variant of Cbfal (15). Introduction of mutations in this binding site that interfere with protein interaction also eliminates PTH inducibility and transactivation by Cbfa/ Runt proteins. While DNA binding activity of AP-1 is increased upon PTH treatment, high basal level of Cbfa/Runt binding activity is detectable in untreated cells, which is not further increased by PTH, suggesting that AP-1 and Cbfal contribute to transcriptional activation through different mechanisms in various cell phenotypes (15).

Recently, there were reports that Nmp4/CIZ regulation of MMP-13 in response to PTH could occur in osteoblasts (16). The PTH response region of the rat MMP-13 gene spans nucleotides (nt) -148 to -38 and supports binding of numerous transcription factors, including Runx2, necessary for osteoblast differentiation, c-Fos/c-Jun, and Ets-1. These trans-acting proteins mediate hormone induction via incompletely defined combinatorial interactions. Within this region, adjacent to the distal Runx2 site, is a homopolymeric(dA:dT) element (-119/-110 nt) that conforms to the consensus site for the novel transcription factor nuclear matrix protein-4/cas interacting zinc finger protein (Nmp4/CIZ). This protein regulates bone cell expression of type I collagen and suppresses BMP2-enhanced osteoblast differentiation. Electrophoretic mobility shift analysis confirmed Nmp4/CIZ binding within the MMP-13 PTH response region. Mutation of the Nmp4/CIZ element decreases basal activity of an MMP-13 promoter-reporter construct containing the first 1329 nt of the 5'-regulatory region, and overexpression of Nmp4/CIZ protein enhances the activity of the wild-type promoter. The same mutation of the homopolymeric(dA:dT) element enhances the MMP-13 response to PTH and PGE_2. Overexpression of Nmp4/CIZ diminishes hormone induction and mutation of both the homopolymeric(dA:dT) element and the adjacent Runx2 site further augments the PTH response (16).

It was found that all-trans retinoic acid (RA), which usually downregulates MMPs, strongly induced collagenase-3 expression in cultures of embryonic metatarsal cartilage rudiments and in chondrocytic cells (7). This effect was dose- dependent and time-dependent, required the *de novo* synthesis of proteins, and was mediated by RAR-RXR heterodimers. Analysis of the signal transduction mechanisms underlying the upregulating effect of RA on collagenase-3 expression demonstrated that this factor acted through a signaling pathway involving p38 mitogen-activated protein kinase. Treatment of chondrocytic cells with RA also induces the production of MT1-MMP, a membrane-bound metalloproteinase essential for skeletal formation, which participates in a proteolytic cascade with collagenase-3 (7). The production of these MMPs is concomitant with the development of an RA-induced differentiation program characterized by formation of a mineralized bone matrix, downregulation of chondrocyte markers like type II collagen, and up regulation of osteoblastic markers such as osteocalcin. These effects are attenuated in metatarsal rudiments in which RA induces the invasion of perichondrial osteogenic cells from the perichondrium into the cartilage rudiment. RA treatment also resulted in the up regulation of Cbfa1, a transcription factor responsible for collagenase-3 and osteocalcin induction in osteoblastic cells. The dynamics of Cbfa1, MMPs, and osteocalcin expression is consistent with the fact that these genes could be part of a regulatory cascade initiated by RA and leading to the induction of Cbfa1, which in turn would up regulate the expression of some of their target genes like collagenase-3 and osteocalcin (7).

Bone morphogenetic proteins (BMPs), members of the transforming growth factor-beta (TGFbeta) superfamily of growth factors, have multiple effects in osteoblasts including regulation of MMP-13. In osteoblast-enriched (Ob) cells from fetal rat calvariae, BMP-2 suppressed the activity of a -2 kb collagenase-3 promoter/luciferase recombinant in a time- and dose-dependent manner (17). The BMP-2 effect on the collagenase-3 promoter was confined to a -148 to -94 nucleotide segment of the promoter containing a runt domain factor 2 (Cfba-1/Runx2) site at nucleotide -132 to -126. The effect of BMP-2 was abrogated in a collagenase-3 promoter/luciferase construct containing a mutated Runx2 (mRunx2) sequence indicating that the Runx2 site mediates the BMP-2 response. Electrophoretic mobility shift assays, using nuclear extracts from control and BMP-2-treated Ob cells, indicated that the Runx2 protein is a component of the specific DNA-protein complex formed on the Runx2 site and that the BMP-2 effect may be associated with minor protein modifications rather than major changes in the composition of specific proteins interacting with the Runx2 site (17).

3.2. Arthritis

Osteoarthritis (OA) is the most common of rheumatic diseases and is idiopathic notwithstanding the compelling evidence that distinct forms of OA are inherited as dominant Mendelian traits (18, 19). While the disease was long believed to be a natural consequence of aging and/or the passive mechanical abrasion of cartilage, it is now obvious that metabolic alterations taking place within this tissue are at least partly responsible for the appearance of morphological and matrix degeneration (18). There is now wide agreement that proteolytic enzymes originating from either chondrocytes and/or the inflamed

synovium (e.g., interstitial collagenase (MMP-1), gelatinase A (72 kDa type IV collagenase, MMP-2), collagenase-3 (MMP-13), and stromelysin-1 (MMP-3), plasmin, elastase, cathepsin G, MMP-1, MMP-3 and gelatinase B (92 kDa type IV collagenase, MMP-9) from monocytes and differentiated macrophages, and finally MMP-8 (neutrophil collagenase) from neutrophils, all produced in abnormally high levels, are at least partially responsible for cartilage matrix degradation (20-24). In addition, the ADAMTS (a disintegrin and metalloproteinase with thrombospondin activity) metalloproteinases, comprising 19 ADAMTS genes, are capable of cleaving procollagen, aggrecan, versican and other minor proteoglycans of the cartilage matrix (25). They have been also linked to OA pathology particularly in terms of proteoglycan turnover.

The *in situ* activity of some of these enzymes is controlled at several functionally distinct levels. Firstly, most of the MMPs are inducible requiring extracellular signaling to up-regulate their expression and synthesis (22). Secondly, most proteolytic enzymes are synthesized and secreted in an inactive, zymogenic form with extracellular activation a prerequisite for full activity (22). Finally, a number of inhibitors found in plasma (ex. alpha–2 macroglobulin) or produced by connective tissues (tissue inhibitors of metalloproteases, ex., TIMP-1, TIMP-2, TIMP-3, TIMP-4) bind with high affinity and specificity to active and, in some cases, unactivated, zymogenic MMPs (ex. TIMP-2/proMMP-2 complex or TIMP-1/proMMP-9 complex) (22, 26, 27). Proteolytic activity in OA-affected tissues is apparently correlated directly with the severity of the disease and the appearance of lesions may be due to a quantitative imbalance in favour of proteolytic enzymes over TIMPs (27). The changes one sees in OA not only involve cartilage tissue but often the synovial membrane of the joint is inflamed. This inflammation is responsible for many of the clinical symptoms associated with OA (ex. joint tenderness and painful swelling) and the synovium is a fertile source of catabolic factors (reviewed in 28). The latter include matrix destructive MMPs, inflammatory cytokines (ex. IL-1, TNF-alpha) and possibly oxygen radicals (ex. superoxide anion) derived from infiltrating polymorphonuclear leukocytes during phagocytosis (28).

Rheumatoid arthritis (RA) is a chronic autoimmune inflammatory disorder, with systemic features and joint involvement, resulting in erosive synovitis, cartilage degradation and joint destruction (29). The typical histopathological changes include dense infiltration of the synovial membrane by mononuclear cells, lymphocytes, neutrophils, mast cells (degranulated), neoangiogenesis, cellular hypertrophy, and hyperplasia of the synovial lining (29). The underlying molecular basis for matrix degradation is thought to be dependent on the action of a variety of proteolytic enzymes that may be produced by both soft and hard tissue elements and by inflammatory cells (29). There are currently two major working hypotheses that attempt to explain the pathogenesis of RA; the T and B cell hypothesis and the macrophage-fibroblast theory (30-34). In both cases RA is initiated by the presentation of an unknown antigen (s) to CD4+T cells. In support of this hypothesis is the fact that more than 80% of RA patients express HLA-DR1 or DR4 subtypes alleles (35). It is now generally accepted that T cell activation is required in the early stages of the disease (31, 33). Histological analysis of the synovium demonstrates activated T cells in close association with antigen presenting cells (29, 30). Activated T cells release cytokines that clonally expand other T cells and activate macrophages and fibroblasts, triggering the immune-inflammatory cascade of synovitis. Detailed analysis of mediator production in the inflamed synovial tissue reveals the presence of T-cell-derived pro-inflammatory cytokines and anti-inflammatory Th2-derived IL-10 with some IL-4 (32, 34). There is also an abundance of cytokines and growth factors produced by macrophages and synovial fibroblasts. Macrophages play a central role in mediating joint damage and erosion (32) probably by producing locally large quantities of pro-inflammatory cytokines that have a general suppressive effect on tissue repair processes. It now seems likely that TNF-alpha and IL-1beta are pivotal mediators with a high ranking in the cytokine hierarchy, conclusions arrived at from data using anti-TNF-alpha antibodies both in vitro synovial culture systems and therapeutically with RA patients (32-34).

Tissue damage and/or structural alterations seen in the RA synovium may be related to extracellular matrix destruction caused by MMPs (29). Following stimulation with inflammatory cytokines, human macrophages synthesize and secrete a repertoire of MMPs (e.g., MMP-1, 2, 3, 8, 9) with specificity for matrix components such as collagens, elastins, proteoglycans, laminins, and denatured collagens (22, 26, 27). One quite typical macrophage metalloprotease is MMP-9 (92 kDa gelatinase), which plays a role in cellular diapedesis, augmentation of cellular invasion and tissue degeneration (26, 27).

A complex interaction exists between T cells and macrophages not only in terms of antigen presentation and clonal expansion but also in mutual control through chemical mediators. T cell factors control macrophage activation and macrophages may control the expression of many of the T cell cytokines (30-33, 36). T cells (CD45+RO+ helper cells) secreting IL-17 are among the first to be activated during the immune response suggesting that the latter cytokine plays an important role in the early stages of inflammation (36). Indeed, IL-17 strongly stimulated the secretion of the proinflammatory cytokines TNF-alpha and IL-1beta in addition to IL-12 and PGE_2 in human macrophages (37). The anti-inflammatory cytokine IL-10 was also upregulated as was the IL-1 receptor antagonist (IL-1Ra). It was reported that IL-17 also increased the release of the chemokine IL-8 in human vascular endothelial cells suggesting that IL-17 is also involved in leukocyte movement (chemokinesis) and directed movement (chemotaxis) (38, 39).

As previously alluded, the principal MMPs involved in connective tissue metabolism are those with specificity for collagens, denatured collagens, and proteoglycans. The most active MMPs in this regard are MMP-1, MMP-3, MMP-9, MMP-8, and MMP-13,

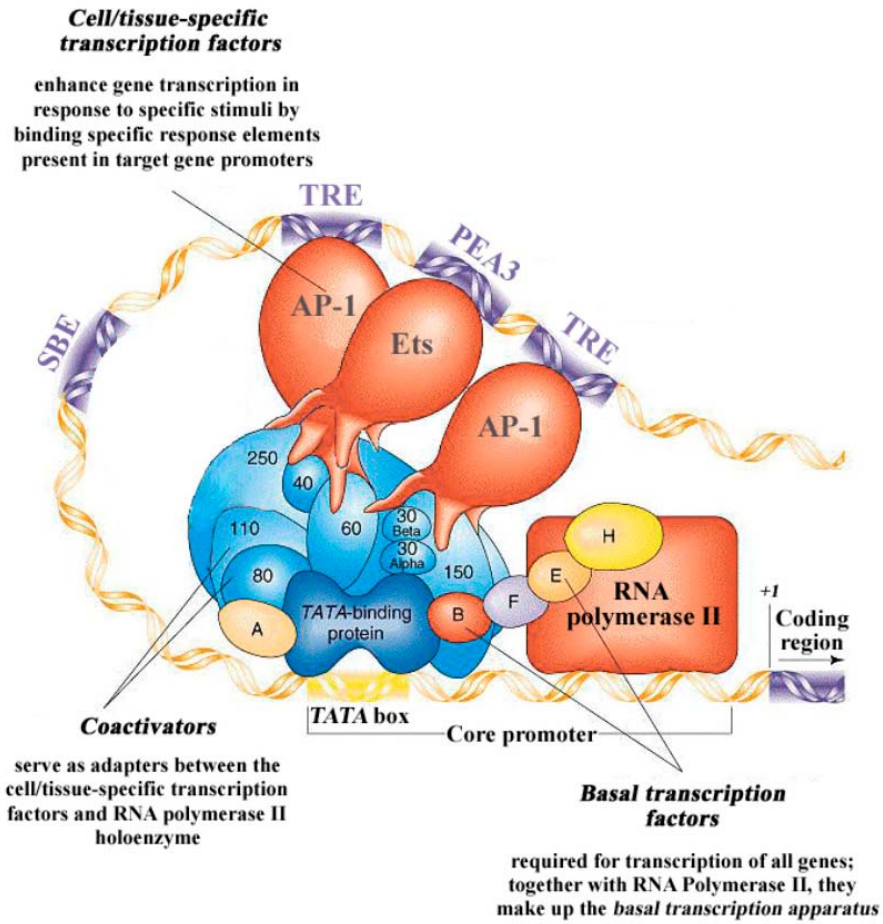

Figure 2. Prototypical transcriptional machinery involved in the induction of MMP promoters. Shown, is the transcriptional complex formed at the proximal promoter of the MMP-1 gene. Basal transcription factors, which assemble to form the TFII complex, are listed according to their designated letter names (i.e., TFIIA, TFIIB, etc.). The TFII complex interacts with RNA polymerase II and this interaction is the minimal requirement for RNA polymerase II to initiate basal (i.e., very weak) transcription. Numbered proteins, named according to their molecular masses in kilodaltons, represent RNA polymerase II subunits and coactivators. Abbreviations: AP-1- activator protein-1; Ets- erythroblastosis twenty-six; PEA3- polyoma enhancer A binding protein-3; SBE- SIAT binding element; TRE- phorbol-ester response element.

although membrane type-MMPs (e.g., MT-MMP1) play an important role in pro-enzyme activation (22, 26). The genes of MMP1, MMP-3, MMP-9, MMP-8, and MMP-13 are inducible by pro-inflammatory and pro-catabolic extracellular stimuli, growth factors and tumour promoters (11). Cellular mRNA levels are regulated primarily (but not exclusively) by transcriptional mechanisms with regulatory elements in the 5'-flanking promoter region (both silencers and enhancers) integrating intracellular signaling cascades. All in the inducible MMPs feature at least one phorbol-ester response element (TRE) located either just 5' to the TATA box or further upstream (e.g., -533 bp MMP-9) (Figure 1 and Figure 2). These elements bind AP-1 family members in their active form, which are composed of homodimers of c-JUN family members (i.e., c-*jun*, *jun*D, *jun*B) and heterodimers of c-*jun* isoforms and members of the c-*fos* group (40). They bind specifically to a consensus 5'TGAC/GTCA-3' palindromic sequence. Other responsive elements include a PEA3 site which binds the polyoma enhancer A binding protein-3, a TIE site, a GC-rich sequence which functions as a stimulatory protein 1 (Sp1) binding site, SBE or SIAT binding element, a c/EBP-beta site that binds the CCATT/enhancer binding protein-beta; OSE-2–osteoblast specific element-2 that prefers Cfba1/Runx2 family members, Sil – silencer sequence, a NF-kB-like site which binds nuclear factor-kappaB family members, a NF-1 site – nuclear factor-1, and a RARE sequence or retinoic acid responsive element, which binds homo/hetero dimers of RAR/RXR.

Responding to diverse extracellular stimuli, upstream intracellular signaling cascades regulate transcription factor activity and prominent among these are the mitogen-activated protein kinases (MAP kinases). The MAPK family of serine/threonine kinases consists of the c-Jun N-terminal kinases (JNKs), the extracellular signal-regulated kinases (ERKs) and the p38 kinases. The JNKs and p38 kinases are activated in response to inflammatory

cytokines, osmotic stress and apoptotic signals (reviewed in 41, 42), while the ERKs respond to cytokines, growth factors and phorbol esters (43). Upon activation by upstream MAPK kinases, MAPKs translocate to the nucleus to phosphorylate and activate appropriate transcription factors. Of particular relevance to MMP transcription, JNKs (synovial fibroblasts, chondrocytes) and ERKs (chondrocytes) phosphorylate and transactivate the AP-1 family members (e.g., c-*jun*: c-*fos*), which dimerize to drive transcription of inducible MMP genes (11, 40). The ERK pathway regulates the activity of erythroblastosis twenty-six (Ets) transcription factors that have been shown to bind specifically with PEA-3 sites, and which cooperate with AP-1 proteins in inducible MMP promoters (44). Though p38MAPK doesn't directly regulate MMP promoters in either chondrocytes or synovial fibroblasts, it can phosphorylate activating transcription factor-2 (ATF-2), which together with c-Jun stimulates both *c-jun* promoter transcription and the ternary complex factor Elk-1, which activates the *c-fos* promoter (40, 42). Thus, by promoting expression of AP-1 genes, p38 may indirectly contribute to positive and negative (see below) regulation of MMP transcription.

Another signaling pathway activated by cytokines that impacts on MMP- transcription is the NF-kB cascade (reviewed in 45), which is particularly prominent in OA/RA synovial fibroblasts following pro-inflammatory cytokine stimulation. After release from a molecular tether in the cytoplasm (inhibitor of NF-kB (IkB), p105, or p100, see 45 for a detailed description of the mechanism), NF-kB (p50), NF-kB (p52), and c-rel related factor A (RelA)/p65 NF-kB subunits form homo/heterodimers in the nucleus and bind to cognate enhancer sites in the MMP-1, MMP-3, MMP-9, and MMP-13 promoters (11, 41). Interestingly, p50 homodimers have been shown to repress NF-kB-dependent transcription by p50/p65 heterodimers (46). Transcriptional regulation by dimers of NF-kB containing p50 and/or p52 also seems to require an IkB-related protein, Bcl-3 (47, 48). Following degradation of p105 (precursor of p50), Bcl-3 promotes p50 homodimer formation by creating a stable p50/p50/Bcl-3 trimeric complex (47). Bcl-3 can then act as a coactivator molecule for p50 and directly contribute to transcriptional activation by p50 homodimers. Alternatively, Bcl-3 can inhibit the binding of p50 homodimers to certain promoter elements, and this liberates these sites for transactivation by p50/p65 heterodimers (48).

Though the proximal AP-1 site in inducible MMP promoters (e.g., IL-1, phorbol esters) contributes to basal and activated transcription, the more distal AP-1 like sites are functional upon the appropriate cell stimulation or they require interaction with an adjacent PEA-3 site (41). Furthermore, AP-1 and NF-kB cooperate under IL-1 (but not TNF-alpha) stimulation to up regulate the MMP-1 promoter in rabbit synovial fibroblasts (49, 50), presumably through simultaneous activation of the ERK/JNK and NF-kB pathways. The promoters of MMP-1 and MMP-13 are controlled by JNK and NF-kB signaling in human rheumatoid synovial fibroblasts while p38 MAPK, JNK, and NF-kB control promoter activity in human chondrocytes (51, 52).

The AP-1 site at –1602 bp of the human MMP-1 promoter cooperates with an Ets site that is created by a single nucleotide polymorphism (SNP) at –1607 bp. The SNP is the result of an insertional mutation involving an extra guanine base (G), which forms a core binding site for the Ets family of transcription factors (5'-GGA-3'). The '2G allele' has been identified in about 75% of the human population and its presence both in normal fibroblasts and some tumor cells is associated with enhanced MMP-1 transcription (53-55). Furthermore, multiple Ets sites occur in all inducible MMP promoters and the number of such sites and location within a given promoter vary among the MMP family members. These variations may influence the regulation of these target genes (53, 56).

The transcription factor Runt domain factor-2 (Runx-2)/Cfba1 is expressed almost exclusively in developing cartilage and bone (57-59). Among the MMPs, a Runx-2 binding site is unique to the MMP-13 promoter and chondrocytes and osteoblasts do express MMP-13 when activated. Runx-2 apparently cooperates with the AP-1 site to mediate MMP-13 transcription (60). Important future studies will define the role of MAPK in Runx-2 activation susbsequent to growth factor stimulation and in disease states (61), and if NF-kB interacts with the AP-1/Runx-2 complex.

In contrast to transcriptional mechanisms up regulating MMP expression, far less has been reported about MMP promoter suppression. Given the aneural and avascular nature of cartilage, joint destruction in arthritic diseases is less amenable to endogenous reparative mechanisms. The inhibition of the proteases responsible for cartilage degradation could be an important part of therapy and inhibition of MMP gene expression at the transcriptional level may be a viable alternative.

Synthetic glucocorticoids are prototypic anti-inflammatory compounds and have been used successfully in the treatment of OA/RA. Glucocorticoids bind to their hormone-inducible nuclear receptors (GRs) and interact with or transactivate glucocorticoid response elements (GRE) present in the promoters of many genes (62). Because the MMP promoters do not contain glucocorticoid response elements, inhibition of transcription probably occurs through an indirect mechanism. This 'transrepression' involves binding of the activated GR to Fos and Jun proteins present at the proximal AP-1 site of MMP-1/MMP-13 promoters, with a subsequent change in their conformation and a reduction in transcription (63). Activated GRs also abrogate NF-kB-dependent transcription by either enhancing IkB-alpha synthesis, resulting in sequestration of NF-kB in the cytoplasm or by specific protein-protein interactions with RelA/p65, resulting in inhibition of NF-kB-dependent transcription (64-66). This interaction is specific for p65, and is distinct from the domain involved in AP-1 transrepression.

The vitamin A analogues, retinoids, also block MMP transcription through the AP-1 site (67-70). Ligand-activated receptors (e.g. the retinoic acid receptors alpha, beta and gamma, and the retinoid X receptors alpha, beta

and gamma) reduce MMP transcription by binding to Fos and Jun proteins at the AP-1 site, sequestering these proteins away from the promoter and/or reducing the level of Fos and Jun mRNAs (71).

Prostaglandins, particularly of the E series, are also potent natural (feed-back) inhibitors of basal and induced MMP expression in human synovial fibroblasts and chondrocytes in vitro (72-76). In *ex vivo* experiments using cultured OA/RA membranes, it was demonstrated that proinflammatory and MMP genes are eicosanoid dependent, to the extent that their basal levels were 'superinduced' upon inhibition of endogenous prostanoid synthesis with NSAIDS (75). Physiological relevant levels of PGE_2 suppress MEKK1-induced MMP-1 promoter activation through a p38 MAPK-p53 dependent process (74, 76). In human synovial fibroblasts over expressing an active MEKK1 construct, induction of MMP-1 mRNA occurs through AP-1 and c/EBPalpha (CCAAT site -2013bp)-dependent promoter activation. Treatment with PGE_2 blocks the binding of c/EBPalpha (but not AP-1) to its cognate site through a transrepression process involving p53 (phosphorylated at Ser15 only) and through inhibition of c/EBP synthesis (74, 76). These data provide support for a previous study demonstrating that p53 suppresses MMP-1 mRNA through a promoter-based mechanism, although no mechanistic details were provided (77).

Activation of PPAR-gamma, either through the use of synthetic or natural ligands inhibits MMP-1 and MMP-13 gene expression, at least in part, by reducing IL-1-induced transcription (72, 78). Since PPAR-gamma can physically interact with c-Jun, it is tempting to speculate that PPAR-gamma ligand treatment induces an AP-1/PPAR-gamma association that is transcriptionally repressive.

3.3. Brain physiology

Much of the conceptualization concerning the physiological role of MMPs in the central nervous system was inspired by a rather voluminous literature describing the function of MMPs and their natural inhibitors (i.e., TIMPs) in embryonic development, tissue morphogenesis and remodeling, ECM destruction related to arthritic pathologies and in the immune and inflammatory responses pursuant to viral and pathogenic host invasion (79, 80).

Recently however, MMPs and the A Disintegrin and Metalloproteases (ADAMs) families of matrix destructive enzymes have been implicated in the formation of neural connections in the developing central nervous system (reviewed in 81). Indeed axons fail to extend and/or make guidance errors when MMP function is compromised. In this regard, cell culture studies revealed that the necessity for MMP activity resides in their ability to activate (cleave) ECM bound ligands (e.g., ephrinA2) and/or their cognate membrane bound receptors (82). In addition, a number of studies also implicate MMP activity in adult brain neuronal activity (reviewed in 83) particularly in terms of neuronal plasticity. The latter implies remodeling of synaptic connections, a process critical to learning and memory (84, 85). However, the preponderance of data regarding the MMP/TIMP system originated from studies involving neuroinflammation, seizures, viral infections, autoimmune diseases [e.g., multiple sclerosis (MS)] as well as studies on brain trauma, cerebral vascular ischemia, amyotrophic lateral sclerosis (ALS), Alzheimer's disease (AD), and gliomas (86-89).

It should be recognized that the mere presence of an MMP mRNA/protein doesn't imply physiological significance since MMP activity is regulated transcriptionally (promoter-based), post-transcriptionally (mRNA stability), post-translationally by cleavage activation, by S-nitrosylation (90), and spatially through intracellular and extracellular trafficking. In general however, MMPs are expressed at low levels in the adult mouse brain under quiescent conditions. Using highly sensitive procedures, such as RT-PCR and RNase protection assays, mRNAs for MMPs 2, 3, 8, 9, 11, 12, 13, 14, 15, and 24 were expressed primarily in the cerebellum (e.g., granule and Purkinje fibers) and hippocampus (91, 92). The latter reports were confirmed through analysis of proteins levels using, among other procedures, a gelatinase in-gel assay where MMP-2, 3, and 9 enzymic activities were detectable at low levels (93). The latter activities were enriched in Purkinje fibers while granular neurons express primarily MMP-3 and MMP-9 (93). Interestingly, MMP-3 and 9 levels peaked in the cerebellar structures in young rodent populations, presumably at periods of intense synaptogenesis. Subcellular distribution analysis revealed that MMP-24 was localized in the cell soma and dendrites (92) while MMP-2, 3, and 9 were apparently confined to the neuronal bodies (93). The distribution of MMPs in the hippocampal area of the brain (neurons of the dentate gyrus as well as CA1-3 subfields) is less well documented with MMP-2, 9, and 24 being most evident in the pyramidal neurons of the CA subfields (92, 94, 95). Szklarczyk *et al*. (96) observed that MMP-2 was confined to cells of a glial phenotype while MMP-9 was primarily neuronal.

The basal expression of MMPs reported in the hippocampal dentate gyrus responds to excitatory activity subsequent to glutamate-induced seizure. For example, MMP-9 protein, mRNA and enzymic activity along with TIMP-1 levels were transiently induced subsequent to glutamate treatment and coincided with intense neuronal activity (96-99). Interestingly, enhanced mRNA expression was observed in the neuronal cell bodies and the dendritic processes, suggesting activity-driven translocation of MMP-9 mRNA (96). The implications of these observations are relevant in terms of neural plasticity and its implications for learning and memory; proteins can be elaborated at the site of synaptic activity when the need arises (84). In support, Wright *et al.* (100) reported that MMP-9 activity was induced in rodent hippocampus during the course of spatial learning and Taishi *et al.* (101), showed that sleep and changes in ambient temperature can modulate MMP-9 mRNA expression in the cerebral cortex and hippocampus.

In order to invade and spread through surrounding normal tissue, tumor cells must degrade multiple elements of the ECM, including fibronectin,

laminin, noncollagenous glycoproteins (e.g., proteoglycans) and type IV collagen (reviewed in 89). Of the different families of ECM-degrading enzymes, the most extensively studied are the MMPs. The latter class is ideally suited to carry out these degradative processes, having all necessary inherent macromolecular degrading specificities. The gelatinases (A & B, MMP-2 and MMP-9) have received the widest attention, as their expression is substantially elevated in gliomas (CNS tumours of glial-cell origin, i.e., ependymal, oligodendrites, astrocytes and microglia cells). Furthermore, their levels correlate with the aggressiveness, extent of tumour invasion, and its overall mass (102). More recently, results have shown that active MMP-9 expression associated with the primary glioblastoma multiforme (GBM, grade IV) may be due to epidermal growth factor receptor (EGFR) signaling via MAPK/ERK activation (103). It has also been reported that endothelial cells (ECs) may also express elevated gelatinase levels which bind extracellularly to tumour cells by cognate MMP receptors on the cell surface (104). In addition, MMP-9 also has been shown to be expressed by other cell types that might form a significant proportion of the gliomas cell mass including infiltrating macrophages, microglia, and other infiltrating leukocytes (105).

Membrane-type MMPs (MT-MMPs, MT1-MMP to MT6-MMP, also designated MMP-14, 15, 16, 17, 24, and 25) are also believed to play a role in gliomal metastasis (106), ostensibly by trafficking MMP-2 via TIMP2 to the tumour cell membrane and site of matrix proteolysis. In addition, levels of MT1-MMP have been shown to correlate with high-grade gliomas.

Neuroinflammatory episodes in the absence of hypoxia also lead to elevated expression of MMPs, particularly MMP-9 and it is believed that leukocyte extravasation and tissue infiltration is aided by limited ECM degradation of the blood brain barrier (BBB) (rev. by Rosenberg, 88). Increased production of proinflammatory cytokines like IL-1beta and TNF-alpha by resident microglial cells and infiltrating neutrophils/macrophages is the likely stimulus for local brain cell production of MMP-9. Animal models of neuroinflammation include simple injection of inflammatory mediators like LPS/TNF-alpha into cerebral ventricles (107) or more elaborate murine models of autoimmune inflammatory demyelination diseases like experimental allergic encephalomyelitis (EAE). In EAE, mice are sensitized by immunization with basic myelin protein resulting in an autoimmune response characterized by symptoms resembling human multiple sclerosis and the associated pervasive demyelination. Elevated levels of MMP-9 are found around the time of onset of symptoms (loss of neuromuscular innervation associated with muscle weakness and loss of voluntary muscle control) and BBB leakage. Chemical inhibitors of MMP-9 and high dose anti-inflammatory steroids greatly attenuate the progression of the symptoms (108, 109).

Evidence from biochemical studies in permanent and temporary ischemia has shown that MMPs are also implicated in the disruption of the BBB that is followed by vasogenic cerebral ischemia and hemorrhage (110). Both MMP-2 and MMP-9 production are dramatically increased after middle cerebral artery temporary occlusion in hypertensive rats and the levels of MMP-9 peak within the time frame of maximal brain damage and correlated with infarct size (111). Using similar protocols in MMP-9 knockout mice, Asahi et al (112, 113) reported that infarct size and BBB damage were both reduced. In addition, mice transgenic for superoxide dismutase (SOD) express less MMP-9 under conditions of cerebral occlusion, suggesting that the oxygen radical production under ischemic conditions may be the primary stimulus for brain cell MMP production (114). It should be appreciated that factors other than MMP activity contribute to the overall pathology of cerebral ischemic events and the plasminogen/plasminogen activator/plasmin system is the focus of much attention because of its multifunctional role in MMP activation, ECM remodeling and turnover, cell signaling, and growth factor activation (reviewed by Rao, 89). Indeed activation of microglial proMMP-3 by plasmin could serve to convert astrocytic and T-cell derived proMMP-9 to its active form. Plasmin can also generate active MT1-MMP (in the presence of TIMP-2), which in turn activates pro-MMP-2. It is this cumulative MMP proteolytic cascade that probably leads to damage of the BBB, brain edema, and intracerebral hemorrhage in stroke victims (88).

Transcriptional regulation is but one of several mechanisms controlling MMP activity in neuronal cell populations. In general, transcription refers to the process of mRNA chain initiation and elongation by RNA polymerase (RNA pol) and a scaffold of proteins binding to the so-called TATA box some 30 odd bp upstream from the transcriptional start site (115). Transcriptional induction is primed and orchestrated by nuclear transcription factors that recognize specific DNA sequences (referred to as enhancer sequences/elements) in the promoter region of target genes. The transactivational capacity of these factors is subject to environmental cues and the attendant cell signaling. They also manifest a tissue specific expression profile (for an exhaustive review see 115).

Several MMP genes are inducible by extracellular stimuli including MMP-1, 3, 7, 9, 10, 12, and 13 while MMP-2, 11, and most MT-MMPs are regulated developmentally and are expressed constitutively in terminally differentiated, phenotypically stable cells (11). The expression of target MMPs is upregulated by extracellular stimuli in the form of proinflammatory cytokines, growth factors, cellular stress, oncogenic transformation, cell-cell interactions, and cell-matrix interactions. Transcription factor proteins respond rapidly to stimulation through post-translational modification (e.g., phosphorylation) by signaling intermediates like mitogen activated protein kinases (e.g., ERK1/2), stress activated kinases (JUN N-terminal kinase, p38 MAP kinase), protein kinase C (PKC), and protein kinase A (PKA), to name just a few.

In both normal brain function and in pathological states, MMP-9 seems to play a prominent role in regulated/disregulated proteolysis (96, 116, 117). The

MMP-9 gene spans 7.7 kb harboring 13 exons and is transcribed into a 2.5 kb mRNA (118). The 5' flanking sequences, which include some 670 nucleotides, contain a TATA box (-29 bp) and putative binding sites for AP-1 (-79 and -533 bp), NF-kB (-600), Sp1 (-558, GC box), PEA3 (-540), and AP-2 (118). In malignant gliomas cells, MMP-9 expression, synthesis and activity are up regulated through changes in cytoskeletal structures, ostensibly mediated by PKC (delta) activation of ERK1/2 pathway and NF-kB (119). Presumably, IkB-alpha is phosphorylated by activation of the PKC cascade with concomitant release of transcriptionally active NF-kB and ubiquitin-dependent, proteosomic degradation of the IkB-alpha. In post-hypoxic human brain capillary ECs, MMP-9 is induced by NF-kB via peroxide production while in IL-1beta-treated rat astrocytes NF-kB activation partially mediates the stimulation of MMP-9 (120). The evidence for transactivation by NF-kB in the above-mentioned studies is circumstantial as no detailed promoter studies were conducted. However, a recent study by Esteve *et al.* (121) provided conclusive evidence that NF-kB transactivation of the MMP-9 promoter in cytokine-stimulated C6 rat gliomas cells was necessary and sufficient to support increased MMP-9 gene expression.

As is the case with most inducible MMP genes, the AP-1 transcription complex appears to play an essential role in stimulating transcriptional activation of MMP-9 (11, 118). In its active form, the AP-1 complex is comprised of homodimers of c-JUN family members (i.e., c-*jun*, *jun*D, *jun*B) and heterodimers of c-*jun* isoforms and members of the c-*fos* group (40). It binds to a consensus 5'TGAC/GTCA-3' palindromic sequence referred to as a phorbol response element (TRE). Parenthetically, the AP-1 site in the c-Jun promoter favours c-Jun/ATF-2 dimer binding for efficient transactivation (40). AP-1 proteins require post-translational modification for activity and c-*jun* is activated by N-terminal phosphorylation of specific serine residues (ser63/73) that can be activated by jun-N-terminal kinase (JNKs). As such, it was suggested that the JNK pathway is necessary in mediating MMP-9 production (122), although an integrin-linked kinase (ILK) has also been shown to regulate MMP-9 expression in human brain tumour cell lines via AP-1 (123). While stimulation of the MMP-9 gene by tumor necrosis factor-alpha (TNF-alpha) is mediated partly through the NF-kB and Sp1 motifs located -600 and -558 nucleotides upstream of the transcriptional start site in gliomas cells (118), mutation of AP-1 and PEA3 motifs located at -553 and -540, respectively, severely impairs the ability of h*Ras* to induce the MMP-9 gene in an ovarian cancer cell line (124). Thus, the cis elements of the promoter and the transacting factors regulating MMP-9 production appear to differ with a given cell phenotype and stimulus.

Conserved polyoma enhancer A binding protein-3 (PEA3) elements that bind members of Ets transcription factors have been found in almost all inducible MMP promoters and are, as mentioned above, generally located adjacent to at least one AP-1 element. Ets transcription factors are members of a family of helix-turn-helix proteins that have a modular domain structure featuring a highly conserved Ets domain, which recognizes the purine-rich PEA3 element A/CGGAA/T (125). Given the absence of leucine zipper domains essential for dimerization, Ets proteins do not usually dimerize and bind to DNA alone, but form complexes with other transcription factors, particularly AP-1 proteins, for which they function as coactivators (126). The cooperative interactions between AP-1 and Ets factors in the regulation of MMP-9 gene expression reveal that *in vivo* interactions between the distinct transcription factors may modulate the response of MMP promoters especially in situations where simultaneous induction of the expression of both of them occurs, such as tumor cell growth and invasion (89).

Interestingly, polymorphisms in the MMP-9 promoter have been identified which alter the responsiveness of the promoter to basal and induced transacting factors (reviewed in 127). Single nucleotide polymorphisms (SNPs) have been identified at position -1562 with a C-to-T transformation or microsatellites $(CA)_n$ at position -90. The former polymorphism is associated with increased risk for intracranial aneurysm while the latter is associated with MS.

Despite the absence of a TATA box, the promoter of MMP-2 contains a high GC content, 2 tandem putative p53 binding sites (-1640 and -1629 bp), a CREB site, sites for AP-2, Sp1, and GC-rich sequences (-89, -69) that harbour 11 CpG islands (128). For the most part, MMP-2 is refractory to extracellular stimuli although a recent report has shown that TGF-beta can activate the MMP-2 promoter through the p53 sites in monocytic/microglia cells (129). The constitutive expression of MMP-2 mRNA was probably maintained by Sp1 and AP-2 binding. In this connection, the MMP-2 promoter (in contrast to other MMP promoters) could be highly methylated (CpG islands) in certain cells types causing gene silencing and low levels of mRNA expression (130, 131). The expression of MMP-2 varies in different parts of the brain normally and under pathological conditions suggesting that changes in endogenous cellular methylation activity could be responsible for the observed differential expression patterns (130, 131).

Membrane type-MMPs, particularly MT1-MMP, are regulated in several cell phenotypes by cytokines, growth factors, mitogens, tumour promoters, extracellular matrix molecules, and intracellular interactions with cytoskeleton (132-134). Recently Lohi *et al.* (135) isolated and characterized a genomic clone of MT1-MMP, which includes 7.2 kilobases (kb) of the 5'-untranslated sequence (i.e. upstream from the translational start site). Of interest, four major and several minor transcriptional start sites were identified, although the significance of these potential variants is currently unknown. Computer analysis of 753 base pairs in the sequence upstream (5') to the first transcription start site for potential consensus transcription factor binding sites, identified a putative Sp1 binding site and three potential sites with high homology to the TGF-beta 1 inhibitory element (TIE-like) (135). The Sp1 site functioned to support basal transcription, to the extent that site-directed mutagenesis of the Sp1 site resulted in a 90%

reduction in luciferase activity. DNA binding gel mobility shift assays also revealed decreased binding profiles Sp1 mutant oligos. The data thus support the importance of this site in MT1-MMP basal promoter activity. Luciferase reporter assays have identified an additional enhancer element between -7.2 and -1.2 kb and the presence of negative regulatory elements between -1.2 and -0.4 kb, where the TIE-like binding sites are located (135). Similar to the MMP2 promoter, MT1-MMP lacks a TATA sequence and an AP-1 consensus binding site and indeed PMA treatment of cells transfected with the MT1-MMP promoter-luciferase constructs failed to increase reporter activity (135). In addition, studies by Han *et al.* (132) showed that treatment of human dermal fibroblasts with TNF-alpha resulted in increased production of proMMP2, which was attributed to transcriptional induction through a p65 NF-kB site. Although the studies used non-neuronal tissue, the work permitted the identification of a p65 NF-kB consensus enhancer site within the MT1-MMP promoter region. It remains to be determined whether the latter enhancer site is functional in neuronal/glial cells either under normal or pathological conditions.

3.4. Cardiovascular development and pathology

The role of MMPs in cardiovascular development and disease is centered on their involvement in the process of vascular remodeling. Vascular remodeling implicates lasting structural changes in the vessel wall in response to hemodynamic stimuli (i.e., flow, circumferential stress) (136). This process is an important part of various developmental and adaptive processes during cardiovascular development, including blood vessel growth and regression during fetal development and growth, wound healing, exercise training and pregnancy (137). However, blood vessel wall remodeling is also implicated in the development of cardiovascular pathologies, particularly atherosclerosis and stenosis.

MMPs, along with the fibrinolytic (plasminogen/plasmin) system, degrade the EC wall ECM and permit cell migration and tissue restructuring required for blood vessel remodeling to occur. The major cellular constituents of normal blood vessels (i.e., ECs and smooth muscle cells (SMCs)) constitutively express MMP-2, (138-140), which is important for physiological vascular remodeling and angiogenesis (141, 142). Han *et al*. (143) displayed that the transcription factor GATA-2 is likely implicated in EC MMP-2 expression *in vivo*. Rat microvasculature ECs cultured within a three-dimensional type I collagen matrix displayed increased GATA-2 protein expression and binding to two regulatory elements situated between nucleotides -1 437 and -1 387 of the MMP-2 promoter.

The role of MMPs in cardiovascular pathology is best illustrated during the atherosclerotic process. Atherosclerosis, the main cause of coronary artery disease, entails the formation of vascular lesions, called atheromatous plaques, in the blood vessel wall. The disease can be viewed as a multistep, chronic inflammatory disease that involves the interplay between various soluble mediators, monocytes, ECs and SMCs. Damage to the ECs lining the blood vessels (often via binding and subsequent oxidation of low density lipoproteins (LDL)) results in release of a variety of inflammatory mediators. Circulating monocytes are attracted to the lesion site by chemokinesis, adhere to the damaged EC wall and migrate across the endothelial barrier into the intima layer. At this stage, monocytes differentiate into macrophages and phagocytise oxidized low-density lipoproteins (ox-LDL), subsequently turning into foam cells. Foam cells secrete a variety of pro-inflammatory factors (i.e., interleukin-1, interleukin-6, TNF-alpha), all of which can contribute to additional leukocyte accumulation and induce smooth muscle proliferation and migration from the medial layer into the intima. As a result, the arterial wall thickens as more LDLs are taken up by macrophages and results in the formation of an atheroma (a core of lipids and necrotic cellular debris resulting from dying foam cells). The smooth muscle cells produce collagen, which forms a protective fibrous cap over the atheroma (144).

MMPs play a prominent role in the growth and rupture of atheromas, as is implied by the induction of MMP expression in atherosclerotic arteries (145-148) and upon arterial morphological changes in experimental models of atherosclerosis and restenosis (149-151). SMC MMP expression at atherosclerotic sites is associated with migration of medial SMCs to the intima, which is a critical event for plaque growth (152). MMP expression by macrophages present at plaque shoulder regions promotes degradation of the protective fibrous cap, which consequently ruptures and leads to the release of thrombotic atheroma tissue fragments that trigger the onset of clinical cardiovascular disease.

Up-regulation of MMP gene transcription in atherosclerosis is commonly attributed to aberrant expression and activation of NF-kB (153, 154) and AP-1 transcription factors. Bond *et al*. (155) reported that up-regulation of MMP-1, -3 and -9 by pro-inflammatory cytokines and growth factors expressed locally in atherosclerotic plaques occurs by NF-kB-dependent mechanisms. Further studies on MMP-9, whose up-regulation is commonly associated with the progression of atherosclerotic lesions (156, 157), have demonstrated that ox-LDL can induce MMP-9 production in normal human monocyte-derived macrophages (158). Elevated levels of ox-LDL, which is formed by mild oxidation in the arterial wall by cell-associated lipoxygenase and/or myeloperoxidase, induce the growth and development of atherosclerotic plaques by stimulating monocyte infiltration and SMC migration at lesion sites (159). Using electromobility shift assays (EMSA), Xu *et al.* (158) have demonstrated that up-regulation of MMP-9 by oxidized-LDL was associated to increased nuclear binding of NF-kB and AP-1. However, none of the above-cited studies demonstrated direct transcriptional activation of the MMP-9 promoter by either NF-kB or AP-1. More convincing evidence for direct NF-kB/AP-1-mediated transcriptional regulation of MMP-9 in atherosclerosis was provided in a study of the mechanisms behind the athero-protective effects of epigallocatechin-3-gallate (EGCG) (a major catechin found in green tea) (160). In this study (160), Kim

and Moon demonstrated that TNF-alpha-induced MMP-9 promoter activation in human aortic smooth muscle cells (VSMCs) is mediated through the MMP-9 NF-kB and AP-1 *cis*-regulatory elements and that its suppression by EGCG involves decreased binding of NF-kB and AP-1. This group went on to show a similar suppression of MMP-9 expression in TNF-alpha-stimulated mouse VSMCs by the diganglisoide GD3, the levels of which are elevated during atherosclerosis (161, 162). As with EGCG, overexpression of the GD3 synthase gene in mouse VSMCs reversed binding of NF-kB and AP-1 to regulatory elements in the MMP-9 promoter (163). Direct MMP-9 transactivation by NF-kB and AP-1 was confirmed in a subsequent study by gene reporter assay, which revealed that mutation of the AP-1 and/or NF-kB sites of the MMP-9 promoter completely abrogated TNF-alpha-induced promoter activity in human VSMC (164). Taken together, these data show a prominent role for NF-kB and AP-1 in the direct transactivation of the MMP-9 promoter during atherosclerosis.

Another factor associated with MMP expression in cardiovascular remodeling and development is Ets-1. The role of Ets-1 in angiogenesis has long been reported to be important because. Ets-1 converts ECs to an angiogenic phenotype in response to angiogenic factors by inducing MMP-1, -3 and -9 (along with urokinase plasminogen activator and integrins beta 2 and 3) (165, 166). Induction of these MMPs by Ets-1 in platelet-derived growth factor-BB (PDGF-BB)-stimulated VSMCs has also been reported to promote SMC migration and proliferation, which as previously mentioned, is a key step in the development of atherosclerotic plaques (167-172). However, although MMP-1, -3 and -9 gene promoters contain Ets binding elements, it must be noted that these data are at most correlative and by no means evidence for direct transcriptional activation.

The transcription factor Smad3, in conjunction with AP-1, has been shown to counter-regulate MMP-12 expression in human peripheral blood-derived macrophages. Using EMSA and promoter reporter assays, Feinberg *et al*. (173) reported that AP-1 is responsible for MMP-12 up-regulation by pro-atherosclerotic cytokines and growth factors and that TGF-beta1 abrogates this response by preventing binding of AP-1 to the MMP-12 promoter. This inhibitory effect was dependent on the transcription factor Smad3, which can sequester c-jun and/or transcriptional coactivators CREB-binding protein and p300 and thus prevent the assembly of a functional AP-1 transactivation complex.

Transcriptional regulation of MMPs during cardiovascular development and pathology is also influenced by SNPs in MMP gene promoters. With the development of extremely accurate genotyping technology, it has become apparent that MMP SNPs can protect against or contribute to the development of cardiovascular disease (174) and also explain the inter-individual heterogeneity in response to therapy (175). A common “6A” SNP in the MMP-3 promoter, which creates an allele containing a run of six adenosines at nucleotide position -1 171, is linked to reduced MMP-3 expression and faster progression of angiographically detectable lesions in coronary artery disease patients homozygous for the 6A allele (176). A cytidine (C)-to-thymidine (T) transition at nucleotide position -1 562 of the MMP-9 promoter was shown to up-regulate promoter activity and increase the likeliness of more severe coronary atherosclerosis (i.e., triple vessel disease) (177). Using EMSA, Zhang *et al*. displayed the disappearance of a specific DNA-protein complex when using an oligonucleotide sequence containing the T allele. In light of this finding, the authors argued that the C-to-T polymorphism abolishes the binding element for an unidentified transcriptional repressor, thus explaining elevated MMP-9 transcription for the T allele.

In some cases, certain MMP SNPs are only influential in individuals displaying specific cardiovascular risk factors. For example, -181 adenosine (A)/guanosine (G) and -153 C/T polymorphisms in the MMP-7 promoter influenced coronary arterial dimensions solely in hypercholesterolemic patients with manifest coronary artery disease (178). Hypercholesterolemic patients carrying the -181G or -153T allele had smaller reference luminal diameters before percutaneous transluminal coronary angioplasty (PTCA) than did patients homozygous for the -181A or -153C allele, respectively. Basal promoter activity was higher in promoter constructs harboring both less common SNPs (i.e., -181G and -153T) in transient transfection studies. EMSA analysis using U937 nuclear proteins (human monocyte/macrophage cell line) demonstrated different protein binding profiles and affinities for the allelic variants. Although the proteins found to differentially bind to each allele were not identified, it is likely that they contribute the allele-specific effects on MMP-7 transcription. In other cases, the presence of other risk factors can add to and even exacerbate the effects of some of the MMP SNPs on remodeling before or in response to interventions. For instance, a common functional polymorphism within the MMP-12 promoter (A-to-G at nucleotide position -82) decreases binding affinity of AP-1 and is associated with lower MMP-12 promoter activity in transient transfection studies in insulin-stimulated U937 and murine lung macrophage (MALU) cells (179). An allele-specific difference in reference diameter was found in diabetic patients, with the A allele associated with a smaller luminal diameter coronary artery disease in diabetics.

3.5. Cancer and metastasis

A more in depth understanding of the fundamental molecular processes underpinning cancer has led to the identification of MMPs as fundamental players in oncogenesis. The role of MMPs in the various stages of cancer is related to their ability to degrade the ECM, cytokines and their receptors, growth factor receptors and cell surface proteins that enable cell-cell and cell-ECM interactions (i.e., catenins/cadherins and integrins, respectively). The potent and broad-range catabolic activity of MMPs directly or indirectly influences a variety of processes that are central to the initiation, progression and metastasis of malignancies, including dissemination and invasion of transformed cells, angiogenesis and dysregulation of cell death and cell

division. Consequently, it is not surprising that the expression of various MMPs is irregular in different forms of malignant cancers (180, 181).

Structural and functional analysis of various human MMP gene promoters has provided great insight into the mechanisms that regulate MMP gene expression and has facilitated our understanding of how transcription of such genes is disrupted in cancer. Such studies have demonstrated that the promoters of most inducible MMPs harbor a proximal AP-1-binding TRE site approximately at nucleotide position -70 with respect to the transcription start site (Figure 1). AP-1 transcription factors (TFs) are dimeric complexes composed of proteins from the proto-oncogene families jun (i.e., c-jun, junB, junD) and fos (i.e., c-fos, fosB, fra-1, fra-2), and thus provide an interesting link between TFs related to oncogenesis and MMP expression. Jun family proteins can bind to DNA either as Jun/Jun homodimers or Jun/Fos heterodimers, whereas Fos proteins cannot bind DNA as homodimers (40, 182). Although increased AP-1 gene expression has been demonstrated during growth of malignant tumors, data about regulation of their activity in malignancies in vivo is limited. Strong evidence for the involvement of AP-1 TFs in oncogenic MMP expression came from studies with c-fos knockout mice. The studies revealed that c-fos is necessary for malignant and invasive progression of skin papillomas and for induction of mouse MMP-3 and MMP-13 gene expression by platelet-derived growth factor and epidermal growth factor, but not by phorbol esters (183, 184). Stable transfection and gene silencing of the Fos family member Fra-1 in breast cancer cell lines demonstrated that Fra-1 expression directly regulates MMP-1 and -9 transcription and is directly correlated with the degree of cell proliferation and motility (185). Interestingly, pharmacological inhibition of MMP activity in a nonmalignant human bronchial epithelial cell line prevented cigarette smoked-induced Fra-1 expression, thus revealing the existence of an autoregulatory loop implicating MMP and Fra-1 expression (186).

It has been suggested that AP-1-mediated MMP transactivation requires the interaction of AP-1 dimers with other TFs (187). One very important interaction occurs with Ets TFs, which predominantly bind DNA upon association with other TFs. Ets family members bind to conserved polyoma enhancer A binding protein-3 (PEA3) elements, which like AP-1, are found in most inducible MMP gene promoters. More importantly, the AP-1 and PEA3 sites in MMP promoters are usually adjacent or located in very close proximity, thus allowing for the physical interaction of AP-1 and Ets factors that is required for cooperative MMP promoter activation (Figure 1 and Figure 2). There have been numerous reports demonstrating the importance of the Ets family members in MMP gene activation and subsequent tumorogenesis. Immunohistochemical and in situ mRNA analyses have shown that expression of Ets-1 colocalizes with MMP-1, -3 and -9 to the stromal fibroblasts adjacent to the invasive edge of several types of tumors (i.e. lung carcinomas (188), angiosarcomas of the skin (189), breast carcinomas (190)). Furthermore, overexpression of Ets factors Ets-1, Ets-2 and E1AF/PEA3 enhances the activities of MMP-1, MMP-3 and MMP-9 gene promoters in various tumor cell lines (122, 191, 192). Similarly, a tight correlation between Ets factors E1AF/PEA3 and Ets-1 and MMP-2 mRNA levels was shown in breast carcinoma cells in effusions by in situ hybridization (193). E1AF/PEA3-induced MMP-9 expression in non-invasive MCF-7 cells was also shown to confer a motile and invasive phenotype (194). Additional experimental support for Ets-1's involvement in the regulation of oncogenic MMP expression came from the discovery of single nucleotide polymorphism (SNP) at nucleotide position -1 607 of the MMP-1 promoter (i.e. the "2G" SNP). This polymorphism, which results from the insertion of an additional guanosine, creates an Ets binding site that subsequently promotes Ets-1 binding and enhances MMP-1 promoter activity (53). The presence of the 2G allele is tightly correlated to several malignant tumors (55, 195-198).

In addition to promoting oncogenesis, Ets-1-mediated transactivation of MMP genes has also been associated to the maintenance of tumor growth and viability by favoring angiogenesis. In one study, overexpression of Ets-1 converted quiescent mouse ECs into an invasive, angiogenic phenotype by inducing the expression of MMP-1, -3 and -9 (165). In a second study, 17-beta-estradiol-activated Ets-1 expression in a mammary tumor/EC co-culture system was linked to increased MMP-1 and -9 expression and new capillary formation (199). However, neither study provided evidence of direct transcriptional activation of MMP genes by Ets-1.

The functional interplay between AP-1 and Ets factors in the regulation of MMP gene expression *in vivo* is of extreme complexity, as demonstrated by the differential modulation of AP-1-dependent activation of MMP-1 gene expression in NIH3T3 fibroblasts by structurally distinct Ets factors (i.e., Ets-1, ERGB/Fli-1, PU.1) (200). While Ets-1 potentiated the ability of both c-Jun and JunB to activate the MMP-1 promoter, ERGB/Fli-1 alone failed to activate MMP-1 gene expression and only enhanced c-Jun-mediated MMP-1 transcription. Conversely, overexpression of PU.1 had a potent inhibitory effect on AP-1-mediated MMP-1 transactivation. Further adding to the complexity of this regulatory paradigm is a report demonstrating Ets-1's involvement in the repression of AP-1-mediated MMP-1 transcription by p21SNFT (21 kDa small nuclear factor isolated from T cells) (201). The latter mechanism was demonstrated in p21SNFT-transfected hepatocarcinoma cells and resulted in reduced invasiveness of these cells through type I collagen and reconstituted basement membrane. Similarly, NIH3T3 cells stably transfected with the Ets factor PU.1 exhibited weaker binding of transactivating c-Jun/JunD containing AP-1 complexes to the MMP-1 promoter AP-1 element (200). Taken together, these results would suggest that regulation of MMP promoter activity by these transcription factors is specific to particular AP-1/Ets complexes formed in different tumor cells. Hence, AP-1/Ets-mediated MMP gene expression is cell and tissue specific and confers both transcriptional induction and suppression.

The promoters of various MMP genes also contain one or more GC-rich sequences termed "GC-rich

boxes". These elements bind zinc-finger transcription factors, including Sp factors and the early growth response factor-1 (Egr-1). However, only a subset of the GC-box-containing MMP promoters has actually been shown to be activated during oncogenesis by Sp1 or Egr-1. The Sp1 TF has been reported to function as a basal regulator of MT1-MMP and MMP-2 transcription. MT1-MMP is responsible for cleavage and activation of pro-MMP-2, whose overexpression has been tightly correlated to metastasis. In Hippel-Lindau disease, loss of the von Hippel-Lindau (VHL) tumor suppressor leads to stabilization of the normally short-lived transcription factor HIF-2 (hypoxia-inducible factor). Increased HIF-2 activity promotes metastatic renal cell carcinoma in 45% of VHL cases. Petrella *et al*. (202) identified a HIF binding site (nucleotide position -125) upstream and adjacent to the Sp1 element (nucleotide position -92) of the MT1-MMP promoter and showed that these two factors function in a cooperative fashion to up-regulate overexpression of the MT1-MMP gene in VHL renal carcinomas. The MT1-MMP gene is also activated by Egr-1 in ECs stimulated to undergo angiogensis by culture in a three-dimensional extracellular matrix environment (203). Constitutive expression of MMP-2 in invasive astrogliomal (204) and lung cancer (205) cells has also been attributed to GC-box-binding factors Sp1 and Sp3. In the latter report, NSAIDs were shown to suppress MMP-2 expression via inhibition of Sp1 and Sp3 activation. Two C-to-T nucleotide polymorphisms in the MMP-2 promoter at nucleotide positions -1 306 and -735 have been shown reduce the predisposition to and metastasis of cancers of the lung (206), gastric cardia (207), breast (208), colon (209) and esophagus (210). Studies have demonstrated that the protective effect conferred by these polymorphisms is related to the abolishment of Sp1 regulatory elements and subsequent decrease in MMP-2 expression.

Another TF involved in altered MMP expression in cancer is NF-kB. Aberrant activation of this TF has been implicated in the pathogenesis of several human malignancies. The list of MMP promoters harboring NF-kB binding sequences is significantly shorter than that for promoters containing AP-1/ PEA3 and Sp1 sites. NF-kB regulatory elements are found in the promoters of MMP-1 (nucleotide position -3 029) (50), MMP-9 (nucleotide position -600) and MT1-MMP (nucleotide position -1 142) (211), the latter whose functional importance must still be validated. In addition, a polymorphism in the SIRE (stromelysin interleukin (IL)-1 responsive element) site located at nucleotide position -1 595 of the MMP-3 promoter causes association of NF-kB proteins. However, only the MMP-9 NF-kB response element has been linked to dysregulated MMP expression in cancer. Using promoter mutation and mobility shift analyses, Ricca *et al*. (212) specifically demonstrated that the increased metastatic potential of the human breast cancer cell line MCF-7 (ADR) observed upon bcl-2 overexpression was related to NF-kB-induced MMP-9 transactivation. Although other studies (listed below) point to an important role for NF-kB in malignant MMP-9 expression, the data do not demonstrate direct transactivation of the MMP-9 promoter by this TF. Yamanaka *et al*. (213) demonstrated that NF-kB regulates the increased invasive phenotype of gastric carcinoma cells following IL-1beta stimulation by increasing MMP-9 expression. Inhibition of NF-kB activity in highly malignant human adenocarcinoma cell line resulted in reduced MMP-9 mRNA expression and suppressed invasiveness, tumorogenicity, metastasis and angiogenesis upon injection into nude mice prostate glands (214). In addition, NF-kB inhibition in these cells stopped invasion in a chicken chorioallantoic membrane extravasation model and prevented bone resorption when co-cultured with rat bone marrow cells (215).

4. PERSPECTIVE

The last decade has seen a remarkable increase in the number and quality of publications on the importance of MMPs both in physiological and pathological states. Our knowledge concerning mechanisms of cell/tissue-specific transcriptional regulation of MMP gene expression and the attendant signal transduction pathways holds hope for the rational design of pharmaceuticals that control the production of MMPs in a targeted fashion. Adding such agents to our armamentarium of drugs will not only be of great value for understanding the basic biology of matrix structure and remodeling but also for the palliation of diseases resulting from aberrant ECM degradation.

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Abbreviations: AD:Alzheimer's disease, ADAMs:A disintegrin and metalloproteases , ALS:Amyotrophic lateral sclerosis , AP:Activator protein, ATF-2:Activating transcription factor-2, BBB:Blood brain barrier, bFGF:Basic fibroblast growth factor , BMP:Bone morphogenic protein, bp:Base pairs, c/EBP:CCATT/enhancer binding protein, Cbfa1:Core binding factor alpha 1, CREB:cAMP response element binding protein, EAE:Experimental allergic encephalomyelitis , EC:Endothelial cell, ECM:Extracellular matrix, EGCG:Epigallocatechin-3-gallate, Egr-1:Early growth response factor-1, EMSA:Electromobility shift assay, ERK:Extracellular signal-regulated kinase, Ets:Erythroblastosis twenty-six, GBM:Gliomblastoma multiforme, GR:Glucocorticoid receptor, GRE:Glucocorticoid response elements, HIF-2:Hypoxia-inducible factor , hnRNA:Heterogeneous nuclear RNA, IkB:Inhibitor of NF-kB, IL:Interleukin, IL-1Ra:IL-1 receptor antagonist , ILK:Integrin-linked kinase, JNK:Jun-N-terminal kinase, kb:Kilobase pairs, kDa:Kilodaltons, LBP-1:Leader binding protein-1, LDL:Low density lipoproteins, MAPK:Miotgen-activated protein kinase, MMP:Matrix metalloprotease, MS:Multiple sclerosis, MT-MMP:Membrane type MMP, NF-1:Nuclear factor-1, NF-kB:Nuclear factor-kappaB , nt:Nucleotides, OA:Osteoarthritis, Ob:Osteoblast, OSE-2:Osteoblast specific element-2, ox-LDL:Oxidized low density lipoproteins, p21SNFT:21 kDa small nuclear factor isolated from T cells, PDGF-BB:Platelet-derived growth factor-BB, PEA3:Polyoma enhancer A binding protein-3, PKA:Protein kinase A, PKC:Protein kinase C, PGE2:Prostaglandin E2, PTCA:Percutaneous transluminal coronary angioplasty, PTH:Parathyroid hormone, RA:Rheumatoid arthritis, RAR:Retinoic acid receptor, RARE:Retinoic acid responsive element, RNA pol:RNA polymerase , RXR:Retinoid X receptor, SBE:SIAT binding element, Sil:Silencer, SIRE:Stromelysin IL-1 responsive element, SNP:Single nucleotide polymorphism, SOD:Superoxide dismutase, Sp:Stimulatory protein, SPRE:Stromelysin-1 PDGF response element, TF:Transcription factor, TGF-beta:Transforming growth factor-beta, TIE:TGF-beta 1 inhibitory element, TIMP:Tissue inhibitor of metalloprotease, TNF:Tumor necrosis factor, TRE:Phorbol-ester response element, TRF:Octamer binding protein regulatory element, VHL:von Hippel-Lindau, VSMC:vascular smooth muscle cell

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