Egr-1 Mediates Extracellular Matrix-driven Transcription of Membrane Type 1 Matrix Metalloproteinase in Endothelium*

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Matrix metalloproteinase activity is instrumental in processes of cellular invasion. The interstitial invasion of endothelial cells during angiogenesis is accompanied by up-regulation of several matrix metalloproteinases, including membrane type 1 matrix metalloproteinase (MT1-MMP). In this study, we show that endothelial cells stimulated to undergo angiogenesis by a three-dimensional extracellular matrix environment increase production of the transcription factor Egr-1. Increased binding of Egr-1 to the MT1-MMP promoter correlates with enhanced transcriptional activity, whereas mutations in the Egr-1 binding site abrogate the increased transcription of MT1-MMP in the stimulated cells. These data identify Egr-1-mediated transcription of MT1-MMP as a mechanism by which endothelial cells can initiate an invasive phenotype in response to an alteration in extracellular matrix environment, thus functionally associating MT1-MMP with a growing number of proteins known to be up-regulated by Egr-1 in response to tissue injury or mechanical stress.

Angiogenesis is a critical component of the adaptations that occur in response to chronic increases in metabolic activity within tissues or tumors (1, 2). Defining the regulation of genes involved in early stages of angiogenesis is fundamental to our understanding of this process and to the selection of target control points for therapeutics intended either to augment or to inhibit growth of new blood vessels. Proteolysis of the capillary basement membrane is thought to be a prerequisite for subsequent invasion and migration of capillary endothelial cells into the interstitium, where new capillaries are established (1). Thus, regulation of protease production by endothelial cells represents a potentially powerful control point in the angiogenesis pathway. Matrix metalloproteinases (MMPs)1 comprise a family of structurally related zinc endopeptidases capable of proteolysis of numerous components of the extracellular matrix as well as nonmatrix molecules (3). Production and activation of MMPs correlates strongly with migratory and invasive behavior in many cell types, including endothelium (4, 5).

The membrane-type matrix metalloproteinases (MT-MMPs), which contain a membrane spanning domain, play a unique role compared with secreted MMPs because their cell membrane location focuses extracellular matrix proteolysis on the cell surface, and perhaps specific subdomains of the cell surface, such as the leading edge of a migrating cell (6). The most ubiquitous and well characterized MT-MMP, MT1-MMP (MMP-14), is known to have substrate specificity for diverse extracellular matrices including collagens type I and III, fibronectin, and tenascin (7). MT1-MMP was demonstrated recently to be an effective activator of fibrin, implicating it as an important player in fibrinolytic cascades (8). In addition to its direct proteolysis of extracellular matrix components, MT1-MMP, together with tissue inhibitor of matrix metalloproteinases-2, has been shown to play a pivotal role in the cell surface tethering and activation of pro-MMP-2 (9, 10). In many tissues, MMP-2 is produced constitutively, but is found almost entirely in latent form. MT1-MMP mRNA and protein levels are very low in most noninvasive cells but are up-regulated in invasive cells, and this up-regulation correlates with increased activation of MMP-2 (11). Thus, production of MT1-MMP likely is a major rate-limiting component of MMP-2 activation.

Despite the correlation between MT1-MMP levels and invasive phenotype, very little is known about the mechanisms underlying transcriptional regulation of MT1-MMP mRNA. Our previous studies showed that up-regulation of MT1-MMP mRNA and protein occurs in microvascular endothelial cells cultured in a malleable three-dimensional (3D) type I collagen matrix (12). Under these conditions, the cells adhere to and reorganize the matrix, establishing tractional forces that, in turn, elicit invasive and angiogenic behaviors including cell elongation and migration within the extracellular matrix to form multicell networks and tubular structures. Production of MT1-MMP correlates with this phenotype, and treatment of these cells with MMP inhibitors or antibodies greatly reduces the ability of these cells to form capillary-like structures (12–14). Furthermore, invasion and migration of fibroblasts and carcinoma cells have been attributed to three-dimensional type I collagen-induced up-regulation of MMPs, including MT1-MMP, implying that extracellular matrix-dependent signaling may be an important means by which multiple cell types regulate production of MT1-MMP (15–17). Taken together with other studies that have examined the effects of tractional force-dependent mechanical stress on cell behavior (18, 19), there is extensive evidence that adhesion-mediated mechanical forces play a critical role in determining cell phenotype, and in particular, affecting protease production.

In this study, we investigated the regulatory mechanisms underlying transcriptional up-regulation of MT1-MMP in endothelial cells under noninvasive or invasive conditions. We found that 3D collagen matrix-induced MT1-MMP transcriptional activity occurred as a consequence of increased production and promoter binding of the transcription factor Egr-1. This finding is notable because it defines a mechanism for...
extracellular matrix/mechanical force-sensitive transcriptional regulation of MT1-MMP production and it links MT1-MMP to numerous vascular cell gene products involved in tissue remodeling that also are up-regulated by Egr-1 as a result of mechanical stress.

**EXPERIMENTAL PROCEDURES**

*Analysis of Genomic Clone—*A genomic clone of murine MT1-MMP (pml145) in pBluescript SK containing 4.7 kilobases of 5'-flanking and untranslated sequence, exon 1 and part of intron 1 (20) was sequenced bidirectionally using automated dye-labeled sequencing (Keck Laboratory, Yale University). The sequence was analyzed using the TRANSCRIPTS database (21) to identify potential transcription factor binding sites. Ribonuclease protection assays were performed to locate the 5'-transcription start site(s). Probes were generated by subcloning a Pst I-Sac II fragment of pml145 (360-base pair fragment from -395 to -39 from ATG) into Bluescript followed by transcription with either T7 or T3 polymerase to generate sense and antisense riboprobes labeled with [32P]UTP, which were gel-purified prior to use in ribonuclease protection assays. The RPA II kit (Ambion) was used according to manufacturer's directions, using 20–40 μg of total RNA (isolated from NIH 3T3 cells, day 11 mouse embryo, or mouse placenta) and 10× 106 cpm of probe per sample. Samples were analyzed first on a 11 × 15 cm 5% acrylamide sequencing gel and then on a 4.5% acrylamide gel to provide better size resolution of the protected fragments. Ambion century markers and [γ-32P]dATP end-labeled 10- and 25-base pair DNA ladders were used as size markers. The assay was repeated five times using multiple preparations of RNA and probes. Start sites were calculated by approximating the band size of each protected fragment and calculating their corresponding distances from the ATG codon, with the assumption that RNAse degradation occurred only from the 5'-end of the probe.

**MT1-MMP mRNA Half-life Estimation—**MT1-MMP mRNA half-life was estimated using RNA harvested from cultured rat microvascular endothelial cells exposed to actinomycin D (ActD) (10 μg/ml). Microvascular endothelial cells were isolated from rat epididymal fat pad and cultured as described previously (22). For experiments, cells were either plated as a monolayer on type I collagen (2D culture) or embedded within a three-dimensional collagen matrix (3D culture) (23). After 1 day of culture in these conditions, ActD was added to all cultures. RNA was harvested from cells after 0, 4, 8, 12, and 18 h of ActD treatment, and samples were analyzed by Northern blotting (12). Following autoradiography, films were scanned, and MT1-MMP mRNA band intensity was quantitated using imaging software and normalized to 28S ribosomal RNA. Intensities were expressed as a ratio to the 0 h samples. Results from four experiments were averaged and expressed as mean ± S.E. mRNA half-life was estimated using a linear regression best-fit to mathematically define the decay profile (Cricket Graph 1.1).

**Constructs—**Restriction enzyme-generated fragments of the MT1-MMP genomic clone (Sac II site at position −39 as the 3'-end) were subcloned into the pG3L Basic reporter vector (Promega) for use in transient transfection assays. Additional truncation constructs of the MT1-MMP-luciferase constructs were generated using Erase-a-Base (Promega) and sequenced to define the sites of the resultant truncations. Mutations of the Egr-1 and Sp1 binding sites (refer to Fig. 4A) were made to the −300-base pair truncation construct using site-directed mutagenesis (Stratagene) and confirmed by sequencing.

**β-Galactosidase CDNA (originating from pCMVβ) (CLONTECH) was subcloned into pCDNA3 (Invitrogen) for use as a normalization vector for transient transfections (β-galactosidase-pCDNA3). An Egr-1-pCDNA expression vector was generated using the full-length human Egr-1 coding sequence kindly provided by Dr. Vikas Sukhatme (Harvard Medical School, Boston, MA).**

**Transient Transfections—**Lipofectamine-based transient transfections of MT1-MMP promoter sequences and β-galactosidase-pCDNA3 were performed on primary cultures of rat microvascular endothelial cells (passages 5–9), according to manufacturer’s directions (Life Technologies, Inc.). On day 2, cells were trypsinized and split into 2D and 3D culture conditions (23). On day 3, cells were lysed in reporter lysis technologies, Inc.). On day 2, cells were trypsinized and split into 2D and 3D cultures (passages 5–9), according to manufacturer's directions (Life Technologies). Western blotting was done using polyclonal antibodies against Sp1 and Egr-1 (Santa Cruz Biotechnology). Histone H1 (anti-histone H1; Pharmingen) or vimentin levels in the lysates were used to normalize loading variations among samples. Enhanced chemiluminescence detection (Pierce) was performed according to the manufacturer’s directions.

**Electrophoretic Mobility Shift Assays—**Consensus Sp1 and Egr-1 binding double-stranded oligonucleotides (Santa Cruz Biotechnology), and complimentary oligonucleotides corresponding to base pairs −303 to −284 (wild-type) and −309 to −276 (mutants) (synthesized by Critical Chemicals Laboratory, Yale University; see Fig. 4A for sequence details) of the MT1-MMP promoter were annealed and end-labeled using [32P]ATP. Enriched sources of Egr-1 (in vitro transcribed and translated using Promega Tnt system) and recombinant Sp1 (Promega) were used to define the binding patterns of these transcription factors to wild-type and mutated MT1-MMP oligonucleotide sequences.

**RESULTS**

Sequencing of a genomic clone containing 3.3 kilobases of the murine MT1-MMP 5' noncoding region showed that this gene lacks a TATA box (Fig. 1A). Consensus binding sites for transcription factors include Sp1, Egr-1, AP1, AP2, NFκB, and Nkx-2 (murine tinman homolog) but not AP-1 or AP-2, and there are no typical transforming growth factor-β, hypoxia, or thrombin response elements. The apparent absence of both a TATA box and common phorbol ester- and growth factor-inducible elements within the murine MT1-MMP promoter region are characteristics shared with the MMP-2 promoter but contrast with most other MMP genes, which are known to be regulated strongly by AP-1, AP-2, and transforming growth
factor-β responses (25, 26). Multiple transcription start sites, as identified by ribonuclease protection assay, were localized to a region 220 to 2200 base pairs 5′ of the ATG codon (Fig. 1B). The use of a cluster of multiple start sites is consistent with a TATA-less promoter.

Previously, we demonstrated a significant 3.2 ± 1.1-fold increase in MT1-MMP mRNA in primary cultures of rat microvascular endothelial cells stimulated to an invasive phenotype by culture within a 3D type I collagen matrix as compared with noninvasive cells cultured on a planar coating of type I collagen (2D) (12). Estimations of MT1-MMP mRNA half-life showed no significant differences when comparing 2D and 3D cultured cells (t1/2 5 26 and 27 h, respectively), suggesting that transcriptional control of MT1-MMP is likely a major contributor to the difference in mRNA levels seen between 2D and 3D cultures (Fig. 2A).

Transcriptional activity of the MT1-MMP promoter region was assessed in endothelial cells using full-length and truncated promoter sequences coupled to the reporter gene encoding luciferase. Comparisons were made between the transcriptional activity of cells cultured in 2D with that of cells in 3D. The full-length MT1-MMP promoter activity was 2.4-fold higher in 3D compared with 2D cells (Fig. 2B). Although transcriptional activity varied moderately with truncations of distal 5′ noncoding regions, the region between –300 and –220 base pair was sufficient to provide both a low level of basal activity in 2D and 2.2-fold enhanced transcriptional activity in 3D, and thus was considered to contain the nominal elements necessary to enhance transcription in 3D-stimulated endothelial cells.

Within the region between –300 and –220 lies a GC-rich sequence (–288 to –275) that contains overlapping consensus binding sites for Sp1 and Egr-1. It has been demonstrated that Sp1 and Egr-1 compete for binding to such regions, with higher levels of transcription occurring when Egr-1 rather than Sp1 is bound to the promoter (27). Applying the hypothesis that a similar pattern of Sp1 and Egr-1 control would be involved in regulation of the MT1-MMP promoter, the endogenous levels of Sp1 and Egr-1 were assessed in 2D and 3D cultures of rat microvascular endothelial cells. Egr-1 and Sp1 mRNA and protein products were detectable in both 2D and 3D conditions.

After 20 h of 3D culture, Egr-1 mRNA was 2.2 ± 0.2-fold greater than in 2D cultured cells, whereas Sp1 mRNA was 0.62 ± 0.13 that of 2D cultured cells (Fig. 3A). Similarly, Sp1 protein levels in 3D were unchanged (0.99 ± 0.14), whereas Egr-1 protein levels were increased 2.2 ± 0.18-fold in 3D (Fig. 3B), providing support for the involvement of Egr-1 rather than Sp1 in mediating the 3D collagen matrix induction of MT1-MMP.

**Fig. 1. Analysis of the murine MT1-MMP 5′ noncoding region.** A, the sequence of the noncoding region of MT1-MMP and locations of consensus transcription factor binding sites and transcription start sites (arrows) are shown. B, transcription start sites were defined using ribonuclease protection assay. Multiple protected fragments (arrows) correspond to a cluster of start sites at the positions indicated (relative to the ATG codon). Blot shown is representative of five experiments.
Egr-1 Enhances Transcription of MT1-MMP

FIG. 2. MT1-MMP mRNA half-life and transcriptional activity in 2D- and 3D-stimulated cells. A, MT1-MMP mRNA half-life in 2D and 3D endothelial cells was estimated by analysis of mRNA decay time course following ActD treatment of 2D and 3D cells. Open circles and closed circles delineate 2D and 3D MT1-MMP mRNA profiles, respectively. RNA blots (n = 4) were probed with a MT1-MMP cDNA probe and analyzed using densitometry, with the band intensity at each time point calculated as a ratio to the time 0 point (mean ± S.E.). Estimated MT1-MMP mRNA decay curves were not significantly different between 2D (t1/2 = 26 h) and 3D (t1/2 = 27 h) cultured cells (p > 0.05). B, transcriptional activities of full-length and truncated MT1-MMP promoter-luciferase constructs were tested in 2D (open bars) and 3D (hatched bars)-stimulated endothelial cells. Luciferase activities of each sample were normalized to β-galactosidase activity and then expressed as fold increase above promoterless luciferase activity (Basic). Vertical bar to the left of the graph shows the location of consensus transcription factor sites relative to the truncations. Results are presented as mean ± S.E. of four independent experiments.

EMSA analyses, using oligonucleotides containing either wild-type or mutated MT1-MMP promoter sequences (Fig. 4A), were performed to determine Egr-1 and Sp1 binding profiles. Recombinant Egr-1 interacted with the wild-type MT1-MMP oligonucleotide and could be supershifted with specific antibodies to Egr-1 (Fig. 4B). Mutant oligonucleotides 1 and 3, both containing a GG to TA mutation within the Egr-1 binding site, blocked Egr-1 binding. Binding of Egr-1 to mutant 2 (GG to TA mutation in the 5′ Sp1 site) was unaffected. Mutant 4 (multiple G to T point mutations within both Sp1 and Egr-1 sites) allowed partial binding of Egr-1. Recombinant Sp1 also bound to the MT1-MMP wild-type oligonucleotide (Fig. 4B). Antibodies to Sp1 depleted the Sp1 gel shift band. Sp1 interacted strongly with mutant sequences 2 and 3, very weakly with mutant 1, and not at all with mutant 4. Recombinant AP2, which also binds to a GC-rich sequence (5′-CCCCAGGC-3′) did not shift the MT1-MMP oligonucleotide (data not shown).

We next assessed the gel-shift profiles of nuclear extracts from 2D and 3D endothelial cells. A gel-shift complex that could be supershifted with an Egr-1 antibody was detectable in nuclear extracts of both 2D and 3D cells (Fig. 4C). However, the Egr-1 gel shift band was much more prominent in the lanes of 3D-stimulated extract. Antibodies to Sp1, AP2α, or WT (Wilms’ tumor product) did not compete off or supershift the gel shifted complexes.

Evidence for functional involvement of Egr-1 in enhancing MT1-MMP transcription was obtained using two approaches. First, COS-1 cells were transiently transfected with the MT1-MMP(−300)-luciferase construct in combination with either cDNA encoding full-length Egr-1 (Egr-1-pcDNA3) or the empty expression vector (pcDNA3). Co-transfection with the Egr-1-pcDNA3 construct resulted in 2.7-fold higher activity of the MT1-MMP promoter than was seen for the MT1-MMP promoter in combination with pcDNA3 alone (Fig. 5A).

Secondly, we tested the role of endogenous Egr-1 in enhancing endothelial cell transcription of MT1-MMP in 3D culture. Rat microvascular endothelial cells were transfected with the wild-type MT1-MMP(−300) promoter-luciferase construct or one of four mutated MT1-MMP(−300) promoter constructs, which contained the same series of mutations in the Egr-1/Sp1 binding sites as used in EMSA (Fig. 4A). Luciferase activities of the mutated constructs were compared with the wild-type construct following culture of the endothelial cells in 2D or 3D. As seen in Fig. 2B, wild-type MT1-MMP promoter constructs exhibited approximately 2.5-fold greater activity in 3D compared with 2D cells. Mutants 1 and 3, which by EMSA lacked the ability to interact with Egr-1, failed to exhibit significantly greater luciferase activity in 3D-stimulated cells. However, mutants 2 and 4, which provided full or partial binding of Egr-1 by EMSA, both exhibited significant increases in luciferase activity in 3D-stimulated cells (Fig. 5B). The ability to bind Sp1 (mutants 2 and 3) or reduced ability to bind Sp1 (mutants 1 and 4) did not correlate with enhancement of transcription in 3D. Thus, enhanced MT1-MMP transcription upon 3D stimulation appears to be dependent on and entirely attributable to binding of Egr-1.

FIG. 3. Egr-1 but not Sp1 levels increase in 3D-stimulated endothelial cells. A, endogenous Egr-1 and Sp1 mRNA levels were assessed in 2D and 3D cells. 28S ribosomal RNA was used to normalize loading for total RNA loaded. On the right, band intensities, as assessed by densitometry and normalized for loading, are plotted as a ratio of 3D level compared with 2D. B, endogenous Egr-1 and Sp1 protein levels were assessed in 2D and 3D cells, using vimentin to normalize for protein loading. On the right, band intensities, as assessed by densitometry and normalized for loading, are plotted as a ratio of 3D level compared with 2D. For A and B, blots are representative of three individual experiments. * p < 0.05 (3D compared with 2D).
DISCUSSION

Mechanisms underlying transcriptional regulation of MT1-MMP are relevant to understanding the cellular events that trigger the invasive phenotype displayed by endothelial cells during angiogenesis. In particular, the role of matrix-initiated signaling in angiogenesis is recognized but not well understood. We present the novel finding that the transcription factor Egr-1 acts to enhance transcription of MT1-MMP in endothelial cells under conditions that involve mechanical stress exerted via the extracellular matrix and that mimic events involved in angiogenesis.

Egr-1 is known to be an important activator for multiple endothelial cell genes transcribed during vascular remodeling, including platelet-derived growth factors A and B, tissue factor, and transforming growth factor-β (27, 28). Those studies demonstrated that increasing levels of Egr-1 displaced the basal transcription factor Sp1 from the promoter, resulting in a significantly greater rate of transcription. The results of our analysis suggest that transcriptional regulation of MT1-MMP in endothelial cells is similar to but not entirely consistent with this paradigm. Although both recombinant Sp1 and Egr-1 interacted with the MT1-MMP promoter in EMSAs, we did not see the expected band corresponding to Sp1 binding to the MT1-MMP promoter in 2D cell extracts. Rather, these assays detected a faint Egr-1/DNA complex in gel shift in the 2D extracts and a prominent Egr-1/DNA complex in the 3D-stimulated extracts. This observation implies that the amount of Egr-1 present rather than the Sp1 to Egr-1 ratio determines transcription rate of MT1-MMP in these cells. Thus, the increased level of Egr-1 in 3D collagen-stimulated cells results in enhanced transcription of MT1-MMP. However, because the contribution of surrounding DNA sequences on transcription factor binding is removed in EMSAs, we do not rule out the possible involvement of Sp1 in binding to the MT1-MMP promoter and regulating basal transcription rates. The potential also exists for modulation of Egr-1-induced transcriptional activity via factors binding to sites currently uncharacterized in the surrounding regions, as has been observed with other promoters (28).

Egr-1 levels increase in endothelial cells upon wounding or increased shear stress, or during cyclic mechanical stretching of mesangial cells (27, 29, 30). It is well established that tractional forces develop within collagen matrices as cells attach, elongate, and migrate (19, 31, 32), and it is likely that these forces provide the trigger for production of Egr-1 in our model. Consistent with a mechanical stress induction of Egr-1, and subsequent transcription of MT1-MMP, the time course of increase in Egr-1 and MT1-MMP mRNA upon 3D stimulation (hours rather than minutes) correlates more closely with the processes of cell elongation and matrix contraction rather than with the initial adhesion event. Notably, both shear stress and mechanical stretch are hypothesized to trigger angiogenesis in skeletal and coronary muscle in vivo (33), and increased Egr-1 levels are detectable in skeletal muscle under angiogenesis-promoting conditions (34).

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**Fig. 4.** Egr-1 and Sp1 binding profiles using EMSA. A, the sequences of wild-type and mutated MT1-MMP oligonucleotides used for EMSA show two putative Sp1 sites (underlined) and an Egr-1 site (boxed). Mutated bases are delineated by boldface lowercase letters. B, EMSAs were used to define binding of both purified Egr-1 and Sp1 to the MT1-MMP promoter. Identities of the gel-shifted bands were confirmed by supershifting the Egr-1 complex and by depleting the Sp1 complex with appropriate antibodies. C, EMSAs using nuclear extracts from 2D (2) and 3D (3)-stimulated endothelial cells detected a gel-shifted complex (arrow) that was more prominent in 3D compared with 2D extracts and that could be supershifted by an anti-Egr-1 antibody. The arrowheads in B and C point to nonspecific bands that did not involve either Egr-1 or Sp1 binding. Each blot in B and C is representative of four EMSAs performed using independent preparations of probe and extracts.
The corresponding promoter region of the human MT1-MMP is not yet published, and thus the extent of species-specific differences in transcriptional regulation remains to be determined. It is known that phorbol ester treatment enhances MT1-MMP synthesis 2–3-fold in endothelial cells of human origin but not in rodent cells (12, 14, 35), implying the existence of some unique transcriptional control elements.

Because of the apparent co-regulated production of MT1-MMP and MMP-2 in many cell types, including endothelial cells in 3D culture, the use of common regulatory mechanisms to drive transcription of both genes has been hypothesized. However, we have not found evidence for consensus Egr-1 sites in the rat MMP-2 promoter, and neither are there consensus binding sites within the MT1-MMP promoter for the transcription factor YB1, which is an essential factor for MMP-2 transcription in mesangial cells (36). Thus, although common intracellular signaling pathways may initiate up-regulation of transcription factors required for production of MT1-MMP and MMP-2, it is unlikely that a common transcription factor coordinates the production of both enzymes. This divergence in signaling could account for the constitutively high levels of MMP-2 and low but inducible levels of MT1-MMP present in many cells versus the constitutively high levels of MT1-MMP and inducible levels of MMP-2 found in other cell types, such as murine T lymphocytes (37). Similarly, production of MT1-MMP and the tissue inhibitor of matrix metalloproteinases-2 is both spatially and temporally co-ordinated during mouse embryogenesis (20), but a review of the human tissue inhibitor of matrix metalloproteinases-2 promoter (38) failed to identify consensus Egr-1 binding sites.

By demonstrating MT1-MMP transcriptional regulation via Egr-1, we add support to the preexisting evidence that Egr-1 is a key transcription factor involved in the initiation of a migratory and invasive phenotype in endothelium. We predict that Egr-1-mediated enhancement of MT1-MMP transcription is not endothelial cell-specific but that similar control occurs in other cell types that induce MT1-MMP mRNA in response to a change in extracellular matrix environment (15–17). Will this include regulation of MT1-MMP production in metastatic tumor cells? Studies to date on the role of Egr-1 in tumor cell phenotype are contradictory. One study has linked increased Egr-1 levels to increased malignancy of prostate tumors (39). However, the majority of studies define Egr-1 as having a tumor suppressor effect, likely by means of its antiproliferative and differentiating effects mediated through induction of transforming growth factor-β (40).

Given our evidence for Egr-1 induction of MT1-MMP in endothelial cells, it will be worthwhile to examine the possibility that Egr-1 is involved in maintaining the abnormally high levels of MT1-MMP so frequently observed in invasive tumor cells.

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