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Epigenetic regulation of matrix metalloproteinases and their collagen substrates in cancer

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Abstract

Our review covers the recent epigenetic data that are focused on matrix metalloproteinases (MMPs), their inhibitors (tissue inhibitors of MMPs; TIMPs) and collagen substrates. Twenty-four MMPs, four TIMPs and at least 28 collagen types are known in humans. The MMP activity regulates the functionality of multiple extracellular matrix proteins, cytokines, growth factors and cell signaling and adhesion receptors. Aberrantly enhanced MMP proteolysis affects multiple cell functions, including proliferation, migration and invasion. This aberrant MMP proteolysis is frequently recorded in cancer. Recent evidence, however, indicates that several MMPs function as tumor suppressors in cancer. Their inhibition could have pro-tumorigenic effects (making them anti-targets), counterbalancing the benefits of target inhibition and leading to adverse effects in cancer patients. The current epigenetic data suggest that there are distinct multi-layered epigenetic mechanisms that regulate MMPs, TIMPs and collagens. We show that in certain cancer types, epigenetic signatures of selected MMPs exhibit stem cell-like characteristics. Epigenetic mechanisms appear to play an especially important role in glioblastoma multiforme. Glioblastomas/gliomas synthesize *de novo* and then deposit collagens into the brain parenchyma. The collagen deposition, combined with an enhanced MMP activity in glioblastomas/gliomas, facilitates rapid invasion of tumor cells through the brain. It is tempting to hypothesize that the epigenetic mechanisms which control MMPs, TIMPs and collagens and, consequently, tumor cell invasion, represent promising drug targets and that in the near future these targets will be challenged pharmacologically.

Keywords

cancer; cell migration; collagen; DNA methylation; epigenetics; extracellular matrix; glioblastoma; glioma; histone modification; MMP

Introduction

Gene products involved in cell locomotion, angiogenesis, tumor progression and survival are all potential targets of epigenetic regulation *via* DNA methylation and histone modification mechanisms (1). In malignancies, DNA methylation is frequently dysregulated. Methylation of CpG islands (CpGI) inhibits transcription and represses tumor suppressor genes. Acetylation of the core histones H3, H4, H2A and H2B are normally associated with the activation of gene transcription (2). Methylation of histone lysines occurs in a form of mono-, di- and trimethylation and is reversed by enzymatic demethylation (3, 4). Methylation of the H3K4, H3K36 and H3K79 residues attracts the RNA polymerase II

complex and, as a result, up-regulates gene expression (5, 6). Methylation of the H3K9, H3K27 and H4K20 lysine residues, however, leads to gene silencing (7-10). It was unclear, until recently, if and how pro-migratory genes including the extracellular matrix (ECM) proteins, matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) are controlled epigenetically (11).

ECM and collagens

A three-dimensional architecture of the ECM, a complex structural entity surrounding and supporting cells within mammalian tissues, includes structural proteins (collagen and elastin) and specialized proteins (fibronectin, laminin and tenascin) and proteoglycans (hyaluronan, chondroitin, keratin and dermatan sulfates). Complex multi-factorial relations among these proteins result in the diverse mechanical characteristics of the ECM. The ECM is modified in hypoxia (12, 13), inflammation (14) and in pathologies, including malignancy, atherosclerosis, arthritis, osteoporosis and fibrosis. Collagens are the most abundant proteins in both the ECM and the human body. There are at least 28 collagen types encoded by 49 COL genes (15) including fibril-forming (types I, II, III, V, VI and XI), fibril-associated (types IX, XII and XIV), anchoring (type VII) and network-forming collagens (types IV, VIII and X). There are multiple genetic diseases in humans which are caused by mutations in collagens including osteogenesis imperfecta (type I), Ehler-Danlos syndrome (types I and IV), arterial aneurysms (type III), Alport syndrome (type IV), Ullrich molecular dystrophy (type VI), certain dysplasias (types II, IX and XI) and additional pathologies.

The MMP family

MMPs play a well-documented role in the collagenolysis and in the general ECM proteolysis in disease. Recent scientific discoveries directly implicate a number of MMPs in multiple diseases of the cardiovascular, pulmonary, renal, endocrine, gastrointestinal, musculoskeletal, visual and hematopoietic systems in humans. Elevated MMP activity and the resulting aberrant ECM proteolysis are also characteristics of malignant lesions (16).

MMPs belong to a zinc endopeptidase, metzincin superfamily (Figure 1A) (17). This superfamily is distinguished by the presence of a conserved HEXXHXXGXX(H/D) sequence motif in the active site. The human MMP family is comprised of 24 zinc-containing enzymes which share several functional domains (18, 19). Six membrane type MMPs (MT-MMPs) are distinguished from soluble MMPs by an additional transmembrane domain and a short cytoplasmic tail (MT1-, MT2-, MT3- and MT5-MMP) or by a glycosylphosphatidyl inositol anchor (MT4 and MT6-MMPs). MMPs are multifunctional enzymes which degrade the ECM components (collagens, laminin, fibronectin, vitronectin, aggrecan, enactin, versican, perlecan, tenascin, elastin and many others), growth factors, cytokines and cell surface-associated adhesion and signaling receptors. Because of their potentially disastrous effect on the cell microenvironment, MMPs are normally expressed in small amounts. Their cellular localization and activity are tightly controlled at both the transcriptional and post-transcriptional levels by cytokines, including interleukins (IL-1, IL-4 and IL-6), growth factors (epidermal growth factor, hepatocyte growth factor and transforming growth factor- β), and tumor necrosis factor- α (20, 21). In a feedback loop, some of these regulatory factors are proteolytically regulated by MMPs (22).

Activation and inhibition of MMPs

MMPs are synthesized as latent proenzymes. In the proenzymes, the active site zinc is coordinated by the three active site histidines and also by the cysteine residue of the N-terminal inhibitory prodomain (23). To become active proteinases, the proenzymes require

proteolytic processing that removes the N-terminal inhibitory prodomain and exposes the catalytic site of the MMP enzyme. The activation of MMPs occurs both intracellularly and extracellularly (24, 25). Several MMPs (MMP-11, MMP-28 and MT-MMPs), exhibit the furin cleavage motif RXX/RR in their propeptides. These MMPs are processed by the furin-like proprotein convertases in the trans-Golgi network (Figure 1A). Additional proteolytic cleavages, however, are required to inactivate the inhibitory prodomain. If the prodomain is released by furin alone, the resulting MT1-MMP enzyme remains inhibited by its non-covalently associated intact prodomain (26). Activation of soluble MMPs is mediated by serine proteases, including plasmin, by MT-MMPs (e.g., activation of the MMP-2 proenzyme by MT1-MMP) or by other active MMPs (e.g., activation of the proenzymes of MMP-1 and -9 by MMP-3).

Because of the overlapping cleavage preferences, there is functional redundancy among MMPs. As a result, MMP knockouts in mice, with the exception of MT1-MMP, are non-lethal and do not exhibit a strong phenotype. MT1-MMP knockout mice, however, develop dwarfism, bone malformations and die before adulthood, thus supporting the role of MT1-MMP in both cell migration during gastrulation and collagen turnover (26-30). Mice lacking both MMP-2 and MT1-MMP die immediately after birth (31).

Once activated, MMPs are inhibited by TIMPs (Figure 1B). Four different TIMPs are known in humans (TIMP-1, -2, -3 and -4) (32, 33). MMP/TIMP balance is a significant factor in the regulation of the net proteolytic activity of MMPs. Structurally, TIMPs contain two domains. The inhibitory N-terminal domain binds non-covalently to the active site of the active MMPs, blocking access of substrates to the catalytic site. The C-terminal domain of TIMP-1 and -2 binds to the hemopexin domain of the proenzymes of MMP-9 and -2, respectively.

Because of their important functions, MMPs are considered promising drug targets in cancer. To date, all clinical trials of wide-specificity inhibitors of MMPs, however, have failed with the exception of doxycycline for periodontal disease (34). In these trials, the inhibition of the tumor-suppressing MMPs might counterbalance the benefits of suppressing the tumor-promoting MMPs. Only after the failure of clinical trials, MMP-8 and -26 and, albeit less conclusively, MMP-3, -9, -11, -12 and -19, have been identified as potential tumor suppressors (35-43).

DNA methylation

The major epigenetic cues include DNA methylation and histone modification. DNA methyltransferases (DNMT1, DNMT3a and DNMT3b) (44, 45) recognize CpG dinucleotides and methylate cytosines on either one (hemimethylated DNA) or both DNA strands. DNA methylation can be reestablished on an unmethylated strand after DNA replication is completed because of the ability of DNMT1 to methylate hemimethylated CpG sites. Because of the high mutation rate leading to the CG suppression in the course of eukaryotic evolution, CpGs are under-represented in the eukaryotic genomes compared with other dinucleotide combinations (46, 47). CpGs are frequently clustered in the specific regions called CpGIs. CpGIs are considered strong if the observed: expected CpG ratio exceeds 0.6 (48). The promoter sequence of 70% of the annotated human genes contains CpGI regions (48, 49). The binding of the Sp1, RARE and GATA methylation-sensitive transcription factors with the methylated DNA sequences is normally repressed. As a result, methylation of CpGIs causes transcriptional silencing of the down-stream genes. Genome-wide methylation profiling at base resolution allows establishing the genomic distribution of methylated sequences – the methylome. The methylome pattern is unique in the individual cell types and altered in malignancies relative to normal cells (50, 51).

Histone modifications

Covalent modifications of the core histones represent a chromatin-level epigenetic regulation. There are several histone modifications: lysine acetylation, lysine and arginine methylation, serine and threonine phosphorylation, lysine ubiquitination, proline isomerization and arginine deimination (5). Acetylation of H3, H4, H2A and H2B is normally associated with the activation of gene transcription (2). Acetyl groups are added by histone acetyltransferases (HATs). Histone acetylation can be removed by histone deacetylases (HDACs). Methylation of the lysine and arginine residues in the histone tails may lead to either transcriptional activation or repression depending on the position of the modified residue on the histone tail. Methylation of histone lysines occurs as mono-, di- and trimethylation and is reversed by enzymatic demethylation (3, 4). Methylation of the H3K4, H3K36 and H3K79 residues attracts the RNA polymerase II complex (POL2) and, as a result, up-regulates gene expression (5, 6). In contrast, methylation of the H3K9, H3K27 and H4K20 lysine residues promotes interactions of the modified histones with the heterochromatin protein 1 (HP1) or its homologues. These events normally lead to gene silencing (7, 8). The co-existing H3K27 and H3K4 methylation constitutes a bivalent mark, a specific characteristic of the temporarily silenced developmental genes. This bivalent mark creates a balance that can be readily misbalanced by a developmental stimulus resulting in a rapid activation of the respective loci (52-54). This bivalent epigenetic mark is a known feature of embryonic stem (EC) cells (52, 53).

The work of Yan et al. (55) was the first demonstrating that recruitment of HDAC2 at the promoter of MMP-9 reduced H3/H4 acetylation levels and MMP-9 expression. As a result of the more recent massive research efforts, volumes of the whole genome epigenetic information, including those on MMPs, have been deposited in the public databases, such as the Gene Expression Omnibus (GEO) repository (56). Specifically for this review, we have analyzed these available, albeit incomplete, epigenetic data for selected MMPs, TIMPs and collagens. We now conclude that epigenetic cues play a significant role in regulating the ECM functionality in malignancy.

Epigenetic control of MMPs in the 11q22.3 genomic region

Knowledge of epigenetic regulation of MMPs is still limited. Genomic data indicate that the promoter region of 13 out of 24 MMPs exhibit pronounced CpGIs (an observed:expected CpG ratio >0.6) (Table 1). Nine MMPs (MMP-1, -3, -7, -8, -10, -12, -13, -20, and -27) are clustered in the 11q22.3 region of chromosome 11 (Figure 2A). Chromosomal abnormalities and multiple polymorphisms in the 11q22.3 region predispose to prostate and lung cancer (57, 58) and coronary artery aneurysm formation in patients with Kawasaki's disease (59). The approximately 0.43 Mb 11q22.3 region is largely deficient of CpGIs. This parameter suggests a limited effect of DNA methylation on MMPs from this cluster. However, there is evidence that methylation of CpGIs is sufficient to impose the selective epigenetic control on MMPs. Thus, the DNA methyltransferase inhibitor, 5-aza-2'-deoxycytidine (5-Aza), reduced the level of methylation of a single CpG site in the MMP-1 promoter and increased the expression of MMP-1 in human amnion fibroblasts. Intriguingly, this CpG site overlaps with the single nucleotide polymorphism (SNP) that is associated with the risk of premature fetal membrane rupture (60). A dual knock-out of the DNA methyltransferases DNMT1 and DNMT3b in mice induced the expression of MMP-3 but not of MMP-1 and -2. The DNA methyltransferase inhibitors, 5-Aza and zebularine, also selectively induced MMP-3 in colon cancer cells (61). The similar treatment of lymphoma cells was without any effect (61).

DNA methylation of the regulatory genes may indirectly affect the expression of MMPs in malignancy. Thus, aberrant methylation of fibulin-5 selectively increases the level of MMP-7 and stimulates the migration of lung cancer cells (62). Similarly, protein kinase PKD1 is highly expressed in the normal ductal epithelial mammary cells in which it performs as a suppressor of MMP-2, -7, -9, -10, -11, -13, -14 and -15. In turn, the PDK1 gene is highly methylated and repressed in invasive breast tumors, while the MMP expression and the invasive cell phenotype are stimulated (63).

Epigenetic profiling of the 11q22.3 MMP cluster in highly migratory glioma U251 cells recorded its selective epigenetic stimulation, including histone H3 hyperacetylation and a gain in the H3K4me2 mark, of the actively transcribed MMP-7, -10 and -13 genes, but not of the MMP-1, -3, -8, -13, -20, and -27 genes. In the non-migratory breast carcinoma MCF-7 cells, in turn, hypoacetylation, a loss of H3K4me2 and a gain in the repressive H3K27me3 mark were observed resulting in epigenetic silencing of MMPs of the 11q22.3 locus (64). In contrast with other MMPs, MMP-8 was epigenetically silenced in both breast carcinoma and glioma cells, providing evidence for the stringent epigenetic control of this proteinase. The global epigenetic profiling data of human H1 embryonic stem cell line (H1 ES), mammary epithelial cells (HMEC), normal epidermal keratinocytes (NHEK), umbilical vein endothelial cell (HUVEC) and leukemia cells (K562) are now available from the Broad Institute and the ENCODE group (53, 65-67). According to these data, there is a differential deposition of the H3K4 methylation marks in the H1 ES and K562 cells relative to HMEC, NHEK and HUVEC (Figure 2A). In NHEK and HUVEC, H3K4me1, a marker of the transcriptionally active genes, strongly associates with the POL2 sites. The H3K4me1 profiles are similar in H1 ES and K562 cells. This similarity is not entirely surprising because K562 cells have multiple features of the multipotent hematopoietic stem cells. The density of the repressive histone marks, including H3K27 methylation, is limited in the 11q22.3 locus (data not shown). Overall, epigenetic signatures of MMPs of the 11q22.3 genomic region exhibit striking similarity with the developmentally regulated genes in ES cells.

Epigenetic regulation of the pro-invasive MT1-MMP/MMP2 axis

Experimental evidence suggests that there is an inverse correlation between the promoter methylation and both the expression levels of the pro-invasive MT1-MMP and MMP-2 and cell migration efficiency. The MT1-MMP and MMP-2 promoter regions are hyper-methylated in non-migratory MCF-7 cells. As a result, these cells do not synthesize MT1-MMP and MMP-2. In contrast, hypo-methylation was recorded in the MT1-MMP and MMP-2 promoter regions in the highly migratory glioma cells (11). According to the methylated DNA immunoprecipitation (MeDIP) profiling, the promoter methylation also inversely correlates with the MT1-MMP expression in prostate and breast cancer cells (Figure 2B) (68). These data correlate well with the inverse correlation between promoter methylation and expression levels of MMP-2 in prostate cancer cells reported by Shukeir et al. (69).

In agreement, 5-Aza increases both the levels of MT1-MMP and MMP-2 and cell invasion in pancreatic cancer cells (70). Furthermore, H3K27 methylation is a clear mark of the inactive MMP-2 gene in non-migratory MCF-7 cells, but not in migratory U251 cells. Interestingly, the role of histone H3 acetylation (H3ac) and H3K4 methylation appears to be limited in regulating MMP-2 and MT1-MMP (11). In agreement, trichostatin A, an inhibitor of HDACs, had no effect on MT1-MMP (71). Hyperacetylation of migration-associated MMP genes explains why HDAC inhibitors were inefficient in repressing migration and invasion of cancer cells.

Regulation of anti-tumorigenic MMPs

The specific epigenetic regulation of the anti-tumorigenic MMPs remains insufficiently understood. There are no clear repressive epigenetic marks in MMP-3, -8 and -12 (Figure 2A) (64). Stimulatory H3K4me1 mark was observed in several primary cell types and associated with activation of the MMP-3 and -8 transcriptional activity. It is, however, unknown if the H3K4me1 mark alone is sufficient or if other factors play a primary role in transcriptional silencing of these three MMPs in malignancy.

The precise epigenetic regulation of MMP-11 that may perform as both an activator and suppressor in malignancies (72, 73) also remains unclear. The ENCODE data show that there are two H3K4me1 and H3K27me3 deposition sites in the MMP-11 gene (Figure 2C). The first site overlaps with the first exon and the CpGI. Only in H1 ES cells the H3K4me1 and H3K27me3 deposition is co-localized in this region. The second H3K4me1 site is close to exon 6.

The regulation pattern of MMP-9 is likely unique among MMPs. The extensive CpGIs are present in the MMP-9 gene coding region (Figure 2D) suggesting an important role of DNA methylation in regulating MMP-9. In agreement, DNA methylation of the promoter and MMP-9 silencing were reported in lymphomas (74) and carcinomas (75). These early observations correlate well with the more recent MeDIP data (68). According to our data, MMP-9 is epigenetically repressed in glioma U251 cells compared with breast carcinoma MCF-7 cells. In MCF-7 cells the elevated expression of MMP-9 correlated with the presence of H3ac and H3K4 methylation. These data agree with the anti-tumorigenic role of MMP-9 expressed by glioma cells (76).

Epigenetic regulation of TIMPs

The available data strongly suggest that epigenetic regulation of TIMPs is different from that of MMPs. There is also a significant difference in the regulation of the different TIMPs. There are pronounced CpGIs in the TIMPs' promoters. DNA methylation plays a significant role in regulating all TIMP species including the suppression of TIMP-3 in malignancies (77, 78). Because of its localization in the X chromosome, TIMP-1 (but not other TIMPs) is a subject for epigenetic regulation and dosage control primarily among females (79).

In contrast with other TIMPs, there are two CpGIs in the TIMP-2 promoter (Figure 2E). The major CpGI overlaps with the first exon. This CpGI is generally unmethylated, but undergoes aberrant hypermethylation in some cancers (80-82). The additional, highly methylated, CpGI is localized 1.5kb upstream of the promoter (11). The presence of these two CpGIs provides an opportunity to epigenetically balance the levels of TIMP-2 in cancer. Transcriptional activity of the TIMP-2 gene also correlates with the presence of the stimulatory H3ac, H3K4me1 and H3K4me2 marks and low levels of the repressive H3K27me3 (Figure 2E) (64).

Epigenetics of collagens

The presence of pronounced CpGIs near the transcriptional start sites is a feature of many collagen genes. This feature suggests that DNA methylation plays an important role in regulating collagens. Our analysis of the MeDIP data demonstrates that the DNA methylation patterns of COL1A1 and COL4A1/A2 vary in cancer and normal cells (68). On a similar note, a loss of type VII anchoring collagen is associated with an increased risk of epidermal cancer (43). The MeDIP data suggest that DNA hypermethylation is the cause of the COL7A1 silencing the mammary and prostate cancer cell lines (Figure 3).

In contrast to many other tissues, the level of collagen is limited in brain parenchyma (83). Strikingly, collagen expression is significantly up-regulated in GBM (64, 83, 84). The data suggest that this up-regulation and subsequent deposition of collagens by tumor lesions facilitate the invasion and spread of GBM cells through the brain (64, 84). It is becoming increasingly clear that histone modifications (H3ac and H3K4me2) play a significant role in the up-regulation of collagens including type I collagen in GBM (64). Conversely, an abundant H3K27me3 deposition was recorded in the inactive collagen genes in MCF-7 cells.

Transcriptional activity of the collagen genes in glioma cells also correlates with the expression level of TGFBI. TGFBI encodes an RGD-containing adhesion protein that binds to type I, II and IV collagens and that plays an important role in cell-matrix interactions (85, 86). In agreement with the deposition of collagens by GBM, the collagen internalization receptor urokinase-type plasminogen activator receptor-associated protein (UPARAP)/Endo180/CD280 is also highly expressed in GBM (84). UPARAP also mediates the collagenolytic activity of MT1-MMP (87). UPARAP mediates the invasion of GBM cells through collagen-containing matrices. Taking together, these studies suggest that GBM-derived collagen is an important constituent of the tumor microenvironment in the brain and that epigenetic regulation of the ECM collagens plays a role in facilitating GBM cell migration through the brain tissues. It is tempting to hypothesize that the specific epigenetic mechanisms which control collagens and consequently, brain cancer spread, represent novel and promising drug targets and that these targets can be challenged pharmacologically.

MicroRNAs

MicroRNAs (miRNA) are small, approximately 22 nucleotides, endogenous noncoding RNAs. miRNAs modulate target gene expression by binding predominantly with the 3'-untranslated region (UTR) of target mRNA sequences. The mature miRNA species binds an RNA-induced silencing complex and then represses protein synthesis either by blocking translation or by causing transcript degradation (88-91). Because a perfect complementarity is not required for the miRNA binding with a target mRNA, over 1000 currently known miRNAs can regulate a majority of genes in the human genome (1). The miRNA expression profiles are significantly different in cancer vs. normal cells. A growing number of miRNAs have been reported to contribute to the regulation of cell functions, including tumor cell invasion. Many miRNAs function as tumor oncogenes or anti-oncogenes.

Evidence is emerging that miRNAs are directly involved in the regulation of ECM components (92). Our data suggest that miRNAs play an important function in highly migratory glioma cells. The total concentration of miRNAs is three-fold higher in highly migratory glioma U251 cells compared to that in non-migratory breast carcinoma MCF-7 cells (64). It appears that glioma cells efficiently employ the miRNA mechanisms to impose an additional level of control in the process of cell migration.

Some recent data are summarized in Table 2. Thus, miR-21 targets MMPs and, as a result, promotes glioma cell invasion (93). The known targets of miRNAs in cancers include MMP-2, MMP-9 MMP-13, MMP-14, and MMP-16 and also their cleavage targets such as type I, II and IV collagens. Frequently, miRNAs (miR-181b, -21 and -221/222) repress the expression of the genes coding for TIMPs, such as the ECM-associated TIMP-3. The repression of TIMP-3 expression increases the activity of invasion-promoting MMPs. Currently, a more in-depth analysis of miRNA is being performed in many laboratories and volumes of data linking miRNAs with MMPs, TIMPs, collagens and ECM maintenance will be generated in the very near future.

Expert opinion

The available, albeit limited, information suggests that the multiple epigenetic mechanisms are engaged in the selective regulation of the transcriptional activity of MMPs, TIMPs and their ECM collagen substrates in cancer compared with normal tissue. The resulting misbalance among MMPs, TIMPs and collagens contributes to cancer cell migration and invasion. Additional comprehensive epigenetic, genome-wide transcriptional and proteomics profiling studies of the DNA methylation and histone modification cues are required to elucidate further a sophisticated regulatory network that governs ECM homeostasis in both normal tissue and disease.

Outlook

Progress in the development of analytical technologies including DNA methylation, Chip-on-Chip, miRNA and gene expression microarrays and proteomics and bioinformatics will result in the volumes of the novel, systems biology level data. We believe that future genome-wide transcriptional, epigenetic and proteomics studies will lead to a much better understanding of how migration-associated genes are regulated in malignancy. As a result, opportunities will emerge to target these genes pharmacologically. This increased level of understanding will ultimately lead to the development of personalized medicine for cancer patients.

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Highlights

- Epigenetic mechanisms play an important role in the regulation of MMPs, TIMPs and ECM.
- MMPs and TIMPs are regulated differently.
- The epigenetic control plays an important role in the regulation of the invasion-promoting MT1-MMP/MMP-2/TIMP-2 axis.
- The regulation pattern of MMP-9 is likely to be unique among MMPs.
- In certain cancer types epigenetic signatures of selected MMPs exhibit stem cell-like characteristics.
- DNA methylation and histone modification stimulate the expression of collagens in highly migratory cancer cells.
- Glioblastomas/gliomas deposit collagen in the brain to stimulate migration of tumor cells through the brain parenchyma.
- Epigenetic regulation of collagens is an important parameter of the brain tumors.
- Specific epigenetic mechanisms that control collagens represent novel and promising drug targets in glioblastomas/gliomas.

Figure 1.
MMPs and TIMPs.

(A) Domain structure of MMPs. The structure of MMPs is made up of the following homologous domains: (1) a signal peptide; directs MMPs to the secretory or plasma membrane insertion pathway, (2) a prodomain; confers latency to MMPs, (3) a catalytic domain with the active site zinc atom, (4) a hemopexin-like domain; in coordination with the catalytic domain controls the interactions with substrates, (5) a flexible hinge region; links the catalytic and the hemopexin domain and provides each domain mobility relative to the other. The membrane-type MMPs contain an additional transmembrane domain and a short cytoplasmic tail domain (MMP-14, MMP-15, MMP-16 and MMP-24) or a glycosylphosphatidyl inositol linkage, which attaches MMP-17 and MMP-25 to the cell surface. MMP-2 and MMP-9 contain fibronectin-like type II repeats which assist in collagen substrate binding. A hemopexin domain is absent in MMP-7 and MMP-26. Several MMPs are activated in the course of their secretion pathway by the proprotein convertases, including furin, which recognize the RXKR cleavage motif. MMP-1/Collagenase-1, MMP-2/Gelatinase-1, MMP-3/Stromelysin-1, MMP-7/Matrilysin-1, MMP-8/Collagenase-2, MMP-9/Gelatinase-2, MMP-10/Stromelysin-2, MMP-11/Stromelysin-3, MMP-12/Matrilysin-1, MMP-13/Collagenase-3, MMP-14/MT1-MMP, MMP-15/MT2-MMP, MMP-16/MT3-MMP, MMP-17/MT4-MMP, MMP-18/Collagenase-4, MMP-19/RASI-1, MMP-20/Enamelysin, MMP-21/X-MMP, MMP-23/no alternative name, MMP-24/MT5-MMP, MMP-25/MT6-MMP, MMP-26/Matrilysin-2, MMP-27/no alternative name, MMP-28/Epilysin. (B) Structure of TIMPs and MMPs. Left panel, Three-dimensional structure of TIMP-2 (PDB entry 1BR9) (116). The N-terminal, inhibitory domain (residues 1–110) is folded into a beta-barrel. The C-terminal domain (residues 111–194) contains a parallel stranded beta-hairpin and a beta-loop-beta motif. Six disulfide bridges (shown as orange sticks) stabilize the structure. Middle panel, three-dimensional structure of the complex formed by the catalytic domain of MMP-14 with the N-terminal inhibitory domain

of TIMP-2 (PDB entry 1BUV) (117). MMP-14 and TIMP-2 are shown as surface and cartoon models, respectively. Active site zinc is shown as a magenta ball. Disulfide bridges are orange. Right panel, close-up of the MMP-14/TIMP-2 complex. MMP-14, a surface model with three active site histidine residues and active site zinc shown as green sticks and magenta ball, respectively. The N-terminal residues of TIMP-2 are shown as sticks. Disulfide bridges are orange. Recently, MMP-18 has been renamed MMP-19.

Figure 2.

Epigenetic signature of the selected MMPs and TIMP-2 in normal and cancer cells. The H3K4me1 and H3K27me3 methylation profiling data of human embryonic H1 stem cells (H1 ES), mammary epithelial cells (HMEC), normal epidermal keratinocytes (NHEK), umbilical vein endothelial cell (HUVEC) and leukemia cells (K562) are derived from the ENCODE database (53, 65-67). The DNA methylation data of breast carcinoma cells (MCF-7, ZR-75-1 and MDA-MB-468), HMEC, prostate carcinoma cells (PC-3, LnCap, 22Rv1 and Du145) and normal prostate cells (RWPE1) are derived from (68). DNA methylation data of the frontal cortex gray matter from the human brain are derived from (118). Color peaks correspond to the enrichment levels of H3K4me1 (red) and H3K27me3 (blue) across the genome (66). Green color marks the POL2-association sites (66). *POL2 data are not available. (A) The 11q22.3 locus includes MMP-1, -3, -7, -8, -10, -12, -13, -20, and -27. The arrows show the size and the transcription orientation of the MMP genes. The numbers below the line show the coordinates in chromosome 11. (B) DNA methylation profiling of the MT1-MMP gene. Each circle represents the methylation data of the individual probe. Open and closed circles represent unmethylated and methylated sites, respectively (68). Open and closed bars correspond to the non-coding and coding regions of the gene, respectively. The arrowhead indicates the transcriptional start site. The thick gradient bar represents CpGI. The panels (C), (D) and (E) show the epigenetic signature of the MMP-9, MMP-11 and TIMP-2 genes, respectively. MeDIP, methylated DNA immunoprecipitation.

Figure 3.

DNA methylation profiling of the COL1A1, COL4A1/A2 and COL7A1 collagen genes. Each circle represents the methylation data of the individual probe. Open and closed circles represent unmethylated and methylated sites, respectively (68). The arrowhead indicates the transcriptional start site. The thick gradient bar represents CpGI. Open and closed bars correspond to the non-coding and coding regions of the gene, respectively. The cell line abbreviations are shown in the legend of Figure 2.

Table 1

Human MMP and TIMP genes.

Gene	Alternative name	Chromosomal location	CpG1 position ^a
MMP-1	Interstitial collagenase/collagenase-1	11q22.3	–
MMP-2	Gelatinase A	16q13-q21	–0.4 to +1.1 (1.5)
MMP-3	Stromelysin-1	11q22.3	–
MMP-7	Matrilysin-1	11q22.3	–
MMP-8	Neutrophil collagenase/collagenase-2	11q22.3	–
MMP-9	Gelatinase B	20q11.2	+0.95 to +3.7 (4.65) ^b
MMP-10	Stromelysin-2	11q22.3	–
MMP-11	Stromelysin-3	22q11.23	–0.3 to +0.55 (0.85)
MMP-12	Macrophage metalloelastase	11q22.3	–
MMP-13	Collagenase-3	11q22.3	–
MMP-14	MT1-MMP	14q11-q12	–0.1 to +1.3 (1.4)
MMP-15	MT2-MMP	16q13-q21	–0.9 to +2.1 (3.0)
MMP-16	MT3-MMP	8q21	–1.3 to +0.7 (2.0)
MMP-17	MT4-MMP	12q24.3	–0.8 to +1.25 (2.05)
MMP-19	Stromelysin-4	12q14	–
MMP-20	Enamelysin	11q22.3	–
MMP-21		10q26.2	+0.1 to +2.2 (2.3) ^b
MMP-23B		1p36.3	–0.2 to +2.1 (2.3) ^c
MMP-24	MT5-MMP	20q11.2	–0.3 to +0.8 (1.1)
MMP-25	MT6-MMP	16p13.3	–1.7 to +0.5 (2.2)
MMP-26	Matrilysin-2	11p15	–0.8 to –0.45 (0.35)
MMP-27		11q24	–
MMP-28	Epilysin	17q11-q21	–0.3 to +1.3 (1.6)
TIMP-1		Xp11.3-p11.23	+0.3 to +0.6 (0.3)
TIMP-2		17q25	–0.5 to +0.9 (1.4)
TIMP-3		22q12.3	+0.5 to +1.5 (1.0)
TIMP-4		3p25	+0.25 to +0.5 (0.25)

^d CpGI position (in kb) is shown relative to the transcriptional start site. The CpGI size is in parenthesis.

^b Multiple CpGIs exist within the indicated region.

^c CpGI overlaps with the whole gene region.

Table 2

miRNAs, MMPs and TIMPs.

miRNA	Target gene	Cell system	Ref.
miR-10b	MMP-14	GBM	(94)
miR-143	MMP-13	Osteosarcoma	(95)
miR-146b	MMP-16	GBM	(96)
miR-181b	TIMP-3	Hepatocellular carcinoma	(97)
miR-206	MMP-2/MMP-9	Breast carcinoma	(98)
miR-216a	Type II collagen	Renal mesangial cells	(99)
miR-218	MMP-9	GBM	(100)
miR-21	TIMP-3	Cholangiocarcinoma, GBM, breast carcinoma	(93, 101, 102)
miR-212/132	MMP-9	Mammary stromal cells	(103)
miR-221/222	TIMP-3	Hepatocellular and non-small cell lung carcinomas	(104)
miR-27b	MMP-13	Osteoarthritis chondrocytes	(105)
miR-29	Type I/III collagens	Systemic sclerosis	(106)
miR-29a	Type IV collagen	Human kidney HK2 cells	(107)
miR-29b	Multiple collagens and MMP-2	Renal fibrosis	(108, 109)
miR-29b	Type I/IV collagens and MMP-2	Prostate carcinomas	(110)
miR-29b	Type I collagen	Stellate cells	(111)
miR-29b	MMP-2/MMP-9	Human aortic smooth muscle cell	(112)
miR-451	MMP-2/MMP-9	GBM	(113)
miR-488	MMP-2	Mesenchymal cells	(114)
miR-675	Type II collagen	Chondrocytes	(115)