

Metalloproteinases involvement in liver tumoral pathology- an update

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Abstract

MMPs and TIMPs involvement in tissue destruction may be incriminated in malignant invasion and metastasis, showing correlations between the overexpression, aggressiveness, tumor stage and prognosis. Recent data provide evidence of their complex role in creating an auspicious microenvironment for tumor growth in primary and metastatic sites. The investigation of MMPs and TIMPs functional interdependency is an important direction, useful in carcinogenesis intrinsic mechanisms deciphering, its apparently paradoxical role being incompletely defined. Literature review regarding MMPs and TIMPs study in primary and secondary hepatic tumors allows us to affirm that defining a precise profile of their activities is extremely difficult. The most studied metalloproteinase, in liver tumoral microenvironment, were MMP2 and MMP9 together with their inhibitors TIMP 2 and TIMP1, respectively. The expression variability of both MMPs and TIMPs is associated to promoter or inhibitor action of stromal cells and / or tumor cells, as liver microenvironment has a modulatory action for MMPs and TIMPs. MMPs capacity to intervene in many biological processes is attributed to their ability of ECM proteolysis, as a possible initiator of unrevealed functions. The understanding of biochemical and structural aspects of MMPs, and the capacity to form molecular complexes with TIMPs open the perspectives of design of potent specific inhibitors for MMPs and, thus, the development of new therapies for primary and metastatic liver cancers.

Keywords: *matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), HCC, liver metastasis, carcinogenesis.*

Introduction

Tumor microenvironment is a continuously changing concept in defining malignant behavior not only by genetic mutations but also in the cellular and molecular context of their particular medium which provides cells

the opportunities for growth, proliferation, and/or metastasation [1, 2].

Tumor microenvironment is a dynamic system, mostly orchestrated by inflammatory cells, including: (i) cellular component represented by tumor cells, immune cells: T cytotoxic and regulatory cells, fibroblasts, myofibroblasts, macrophages, and endothelial cells; (ii) a non-cellular component represented by growth factors, such as TGF- β and PDGF, proteolytic enzymes, such as matrix metalloproteinases (MMPs) and their specific inhibitors (TIMPs), extracellular matrix proteins (ECM), and inflammatory cytokines [3-5]; all these elements form a cellular and

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molecular complex in a continuous functional interrelation [5, 6].

Recent studies highlight the microenvironment role in initiation, onset, and development of primary and secondary liver cancer, firstly reflecting the direct relationship between the chronic inflammatory status generated by B and C viruses, by production of cytokines and growth factors, and the appearance of carcinogenic process [4, 5].

Macroregenerative nodules of cirrhotic liver contain foci of dysplastic hepatocytes. Histologically, these dysplastic lesions are classified: "with small cells", "with large cells", and "adenomatous hyperplasia foci"; between these, dysplastic lesions with small cells and adenomatous hyperplastic lesions are considered as precursor lesions of hepatocellular carcinoma (HCC) [7].

Liver malignancies represent the fifth most frequent type of cancer and the third cause of death worldwide [8]. HCC is the most common liver cancer in adult representing more than 90% of all primary liver cancers [9]. The majority of patients diagnosed with HCC have a background chronic inflammatory disease this being considered the main cause of primary liver tumor.

The general accepted paradigm related to carcinogenesis is that the malignant transformation is produced by the progressive accumulation of genetic and epigenetic alterations which result in malignant phenotype achievement. On the other hand, recent studies have launched the idea of a minimum number of molecular changes responsible for the acquisition of a key characteristic of malignant phenotype, such as, among others, unrestricted cellular proliferation [10, 11].

The lack of understanding of intimate substrate and of a clear delimitation of some steps in hepatic carcinogenesis may be partially attributed to tumor profile versatility; this might be initiated in variable genetic and medium contexts and almost sure developed due to denaturation the regulatory action of some signaling pathways [12]. In a complete scenario of pathogenesis, this limitation restrains the development of some efficient interventions, regarding prevention or therapy.

Recent analyses using genomic approaches and improved animal models tried new and promising subclassification of the largely heterogeneous molecular and prognosis tumor subtypes [13, 14].

About the MMP Family

Matrix degrading metalloenzymes, matrixins or matrix metalloproteinases, metalloproteinases (MMPs) [15-18] are part of a multigenic family of proteolytic enzymes, firstly described half century ago [19], functioning at neutral pH [20] which are present in hepatic microenvironment. MMPs are produced in their latent form, as proenzymes (inactive zymogens) or pro-MMPs and, thus, require a process of proteolytic activation [21].

Matrix metalloproteinases (MMPs) family consists of endopeptidases that share homologous protein sequences, being composed of conserved domains and specific domains [20]. Their great variability results in their complex intervention in large panel of pathophysiological conditions. MMPs display a key role in embryogenesis and in a large spectrum of physiological activities, such as cell motility, proliferation, remodeling, healing, angiogenesis, and reproductive events [22-25].

MMPs activity is closely regulated by their endogenous inhibitors, tissue inhibitors of MMPs (TIMPs) [21]. MMP / TIMP imbalance may result various inflammatory, neoplastic, and degenerative conditions [18, 26].

Due to structural and substrate specificity, MMPs are currently divided into seven classes: collagenases (MMP1, MMP8, MMP13, and MMP18), gelatinases (MMP2 and MMP9), stromelysins (MMP3, MMP10), stromelysin like (MMP11 and MMP12), matrilysins (MMP7 and MMP26), membrane type (MMP14, MMP15, MMP16, MMP17, MMP24, and MMP25), and others (MMP19, MMP20, MMP21, MMP22, MMP23, MMP27, and MMP28) [18, 27, 28].

ADAM family proteinases (a disintegrin and metalloproteinase) and ADAMTS (a disintegrin and metalloproteinase with thrombospondin motifs) are also associated,

being involved in a large spectrum of activities, such as fertilization, development, and carcinogenesis [29].

MMPs tissue inhibitors (TIMPs) belong to a family of multifunctional molecules [30, 31] which act as specific endogenous MMPs regulators, expressing an important role in adaptive modulation of ECM by their action on cellular adhesion molecules, cytokines, chemokines, and growth factors [31].

There are 4 known members of TIMPs family, able to inhibit all known MMPs [30, 32, 33], however their inhibitory action is variable for each member in correlation to MMPs substrate. For instance, TIMP1 strongly inhibits many MMPs excepting membranar type, including MMP14,-15,-16,-19 and -24 [30, 32]. TIMP1 and TIMP3 inhibitory activity register latency in correlation to MMP9, while TIMP2, -3 and -4 are able to interact even with pro-MMP2 [32, 34].

TIMP1 overexpression is associated with a significant reduction of degradative capacity of mesangial cells on the matrix while the addition of a neutralizing anti-TIMP1 antibody results in increased matrix degradation ability [34, 35].

The known literature data are demonstrating the protective ECM role of TIMP2 for excessive proteolytic effects seen in some fractures [36], keloids [37], hepatic lesions [38, 39], kidney [40], Dupuytren contractures [41], and heart lesions [42].

TIMP3 has more variable functions, including inflammation regulation by ADAM17 inhibition [43]. There are powerful proofs correlated to its function, suggesting that TIMP3 is a key inhibitor in ECM remodeling [34].

TIMP4 prevents ECM proteolysis in lungs [44], female genital tract, [45], eyes [46], and in transplanted tissues [47].

The investigation of MMPs and TIMPs functional interdependency is an important direction, useful in carcinogenesis intrinsic mechanisms deciphering, its apparently paradoxical role being incompletely defined [30].

MMPs intervene in tumor genesis and invasiveness by their powerful proteolytic action on ECM, initially demonstrated by *in*

vitro experiments [48]. Beside their ECM degradation role, MMPs possess other biological roles, such as; promoter of tumor cells, proliferation stimulator, apoptosis inhibitor, pro- and antiangiogenic action [49-51]. Numerous researcher teams have demonstrated a direct association between their action and tumors development [23, 52].

MMPs are overexpressed in large spectrum of human malignant tumors, including cutaneous [53], colorectal [54], breast, lung and prostate [55-57], esophagus [58], stomach [59, 60], endometrial [61], and ovarian [62]. Occasional controversial reports are supported by other authors [63].

MMPs and TIMPs expression evaluation is mainly focused on stromal cells and less on tumor cells. MMPs and TIMPs are largely produced by reactive stromal cells previously recruited in tumor microenvironment [64]. As a response to microenvironment stimuli, stromal cells develop secretory capacities [65]. MMPs, together with cysteine proteinases, aspartic proteinases, and serine proteinases are proteolytic enzymes involved in extracellular matrix (ECM) and basement membranes (BMs) degradation [66].

For instance, MMP9 involvement in collagen IV degradation, resulting in basement membrane loss of integrity, is well known [52]. As a consequence of its activation and basement membrane degradation favors metastatic process, supplemented by amplification of vascular permeability, as powerful machinery of matrix degradation [48]. The correlation between MMPs and TIMPs proved to be a useful tool in prognosis evaluation.

Overview of MMPs and TIMPs in tumoral pathology

MMPs involvement in tissue destruction may be incriminated in malignant invasion and metastasis [20], showing correlations between the overexpression, aggressiveness, tumor stage and prognosis [67], periodontitis, bullous dermatitis, osteoarthritis, chronic ulcers, nephritis [66], photo aging, fibrosis, endometriosis [18, 26], osteoarthritis, rheumatoid arthritis, [68, 69], decubitus ulcer,

gastric ulcer [70], corneal ulceration, brain injury [71], and neuroinflammatory diseases [72]. MMPs have been variably associated to atherosclerosis, fibrotic lung disease, liver cirrhosis, otosclerosis, and multiple sclerosis [73]. Furthermore, MMPs have been considered as important players in weakening of the matrix, as in aortic aneurysm [74], dilated cardiomyopathy, epidermolysis bullosa, and restenotic lesions [75].

MMPs involvement in tumor invasion and metastasis

Although the mechanism of degradation of extracellular matrix involved in tumor invasion and by default in circulation intravasation, extravasation and migration to metastatic sites [76] has been initially considered, recent data provide evidence of their complex role in creating an auspicious microenvironment for tumor growth in primary and metastatic sites [67]. Removal of ectodomains in the pericellular space, by cleavage of a transmembrane molecule in the juxtamembrane region of the extracellular domain, alters the signaling on the cell surface, a mechanism involved in tumor proliferation and angiogenesis. Thus, HB-EGF is removed by MMP3 and MMP7 in tumor proliferation and angiogenesis, E-cadherin by MMP3 and MMP7, in tumor invasion, and TNF- α by MMP7, in tumor apoptosis [67].

By the release of soluble E-cadherin, a transmembrane cell adhesion protein, the inhibition of MMP3 and MMP7 is carried out, in a paracrine manner, acting as a promoter of the migration and invasion [77]. MMP7 converts the activator of nuclear factor kappa B ligand receptor (RANKL) in a soluble form that promotes osteoclast activation in metastatic prostate cancer [78]. Experiments with transgenic mice show complex and somewhat paradoxical effects of MMP system in tumor carcinogenesis. Experiments which dosed the levels of TIMP1 and TIMP2 production, demonstrate high levels of TIMP1 in all cancerous tissues [79]. Imbalance between MMPs (MMP1, MMP2, MMP3, MMP7, MMP8, MMP9, and MMP13), TIMP1 and TIMP2 was found with no correlation

between the molar ratio and cancer progression or metastasis [79]. TIMP-1 can act as a growth promoter and / or antiapoptotic factor for cancer cells [80].

MMPs interaction with non-extracellular matrix proteins in tumor invasion

Although extracellular matrix degradation has been considered as the main MMPs mechanism of involvement in tumor invasion, followed by intravasation into circulation, extravasation, and migration to metastatic sites [76]. Cytokines, growth factors, and cell adhesion molecules exhibit a complex control on MMPs. MMP3, MMP7, MMP9, and MMP19 stimulate tumor growth by releasing IGF (insulin-like growth factor) [20].

The process of invasiveness in tumors has been attributed to fibroblasts enzymes [81] and subsequently isolated and identified as serine proteinases, hyaluronidases, and MMPs. MMPs are responsible for stimulation of neoangiogenesis [23], for tissue breakdown and remodeling, followed by intravasation into circulation, extravasation, and metastasis to distant sites [82]. Consequently, MMPs are overexpressed in a wide panel of tumors in correlation to their stage, aggressiveness, and prognosis [20].

MMP expression seems to be more complex than simple tumoral cell secretion, being characterized by metalloproteinase expression induction in "host" stromal cells [83], corresponding also to the fibroblastic population described in the original postulate [81].

MMP9 overexpression in tumor cells induced by oncogene products, growth factors, and cytokines and loss of the metastatic phenotype due to its inhibition, lead to the concept of MMP9 key role in the invasion and metastatic processes [84]. The activation ratios of pro-MMP2 correlate with lymph node metastasis in lung, breast, thyroid, and digestive tract carcinomas [79]. Moreover, pro-MMP2 overexpressed in stromal fibroblasts or serum derived is captured and activated on the surface of cancer cells by MT1-MMP. Thus, MT1-MMP overexpression is correlated to that of MMP2 in carcinomas [79]. As TIMP2

is required for the efficient activation of pro-MMP2 by MT1-MMP, immunohistochemistry [79] and *in situ* zymography [79] demonstrated their co-localization in epithelial carcinomatous and adjacent stromal cells.

The principal role of ECM degradation by tumor-derived proteases has been recently enhanced by profound effects on cell adhesion and migration related to gelatinase A activation on various tissue culture substrates including gelatin, fibronectin, and vitronectin [85]. Inhibition of the endogenous gelatinase A with either neutralizing antibody or TIMP2 in these cultures results in enhanced attachment to these substrates. The altered production of TIMP2 modulates ECM proteolysis, cell adhesive and spreading properties, resulting in modified cell morphology.

MMP2 promotes the migration of breast malignant carcinomatous cells by cleaving and regulating laminin-5 (Ln-5), as a specific ECM glycoprotein [86]. Tumoral cells adhere or migrate on BM components, such as collagen type IV, fibronectin, and laminin-1 [87, 88], using integrin receptors [32]. The Ln-5 subunit is cleaved by collagenase A at residue 587, exposing a putative cryptic pro-migratory site or may mask a site that suppresses cell motility. The pro-motility cryptic site does not support adhesion suggesting that the gelatinase A proteolytic activity may provide a signaling mechanism for tumoral cells. Consequently, migration is initiated during mammary gland morphogenesis or tumorigenesis, as demonstrated by an enhanced migratory response to gelatinase A, in the breast carcinomatous cell line, MCF-7 [89].

An excessive proteolysis impairs tumor cell adhesion or disrupts and degrades the cell-matrix interactions or matrix signals required for migration and invasion, suggesting a critical range or gelatinase A / TIMP2 ratio necessary for tumor invasion and angiogenesis [90]. A correlation between gelatinase A overexpression and tumor grade, in carcinomas of the colon, prostate, bladder, pancreas [91], breast, ovary, and skin (squamous and basal cell histological types) has been demonstrated in numerous experimental studies. MT5-MMP activates pro-

MMP2, exhibiting a strong expression in brain tumors [92].

The expression of both MMPs and their specific TIMPs has been associated to tumor progression and clinical outcome, exhibiting an increased level of TIMPs in malignant tissues [79]. Thus, antiapoptotic and/or growth promotor capacities have been attributed to TIMP1 [80]. An enhanced expression of gelatinase B mRNA has been associated to an early relapse and poor survival [93] and a strong TIMP1 expression has been correlated to metastatic spread and an early relapse in colorectal cancer [94].

Similarly, other MMPs or TIMPs have been associated to poor prognosis in malignancies with different locations, as following: activated gelatinase A in stomach [95], stromelysin-3 in breast [96], TIMP1 in lung (small cell type) [97], and TIMP2 in bladder [98].

The high level of regulatory activity associated to an amplified metalloproteinase expression results in both a high expression of MMP and the specific inhibitor. Local remodeling, by angiogenesis and stromal tissue transformation is generated by interaction between MMPs and TIMPs produced by the tumor cells and microenvironment.

Collagenases, gelatinases, stromelysin-3, matrilysin, MT1-MMP [99], and stromelysin-1 [100] are considered the most closely associated to the invasive phenotype. The mechanism of tumor progression promotion is performed by MMPs by disrupting local tissue architecture, by stimulation growth *via* angiogenesis, and disrupting the basement membranes to allow metastatic spread. The tumoral cells produce collagenase-3, gelatinase A, and matrilysin, while most other MMPs are produced by stromal host cells stimulated by tumoral cytokines. For example, gelatinase A binds to endothelial cell, promoting tumor angiogenesis [101].

The efficacy of MMP inhibitors is highly variable, more efficient in nude mice models and less efficient in case of numerous tumor associated macrophages, possible as a property of macrophage elastase. Macrophage elastase is exclusively expressed in host

macrophages, as demonstrated in human breast cancer [102]. Moreover, macrophage elastase is responsible for dormancy status of lung metastases in murine models of lung cell carcinoma [103].

Consequently, macrophage elastase prevents metastatic growth, while other MMPs are tumoral promoters. MMPs interact with endothelial cells, promoting tumor angiogenesis. Oppositely, macrophage elastase produced by tumor-associated macrophages cleaves plasminogen, thrombospondin, and type XVIII collagen generating anti-angiogenic molecules, such as angiostatin, thrombospondin fragments, and endostatin, respectively. Macrophage elastase expresses a higher binding affinity for elastin and other matrix components than other MMPs and consequently may have more potent proteolytic capacity. Moreover, the free C terminal of metalloelastase competes for endothelial cell binding with "pro-angiogenic" MMPs [104].

MMP expression in tumor and stromal cells is tumor-type dependent [90]. MMP3 is expressed in tumoral stroma of invasive breast cancer along with MT1-MMP, MMP1, MMP2, and MMP11 [105]. MMP9, MMP12, and MMP13 have a focal location: endothelial cells MMP9 expression, isolated tumor cells MMP13 association, and macrophage-like cells MMP12 expression [105]. MMP1, MMP3, MMP7, and MMP10 are expressed by head and neck carcinoma. MMP2 and MMP3 are expressed by squamous cell carcinomas of esophagus. MMP7 is expressed by colon and gastric carcinomas and MMP3 is produced by stromal component of some colorectal carcinomas. The normal mucosa adjacent to bronchial lesions, intraepithelial lesions, and squamous carcinomas of the lung express MMP3 transcripts [90], while a primarily MMP3 expression is observed in microinvasive and invasive bronchial lesions.

The correlation between invasive tumoral behavior and the expression of stromelysins is suggested by variable MMP3 and MMP10 expression in transformed rat embryo cell lines. The isolated expression of a single matrixin, MMP3, is insufficient for the progression to an invasive and metastatic

breast cancer phenotype, in transgenic mice models but the process is amplified by the matrix degradation resulting in cell proliferation and apoptosis alteration [90].

Progelatinase A is activated by forming a trimeric complex with MT1-MMP and TIMP2 [106]. TIMP2 may inhibit the cell surface activation of progelatinase A or facilitates progelatinase A binding to the cell surface by progelatinase A-TIMP2 complex formation. The variable ability of tumor cell lines to bind progelatinase A with or without enzyme activation suggests the presence of a progelatinase A receptor distinct from MT1-MMP receptor, such as integrin $\alpha\beta 3$ of the surface of cultured melanoma cells [101].

An excess TIMP2 over the levels of gelatinase A-TIMP2 complex in the culture fluid of many human tumor cells from melanoma, fibrosarcoma, breast carcinoma, lung carcinoma, and pancreatic carcinoma was observed in sensitive and specific sandwich ELISAs [90]. The great variability of secreted TIMP2 / gelatinase A ratio in culture may have a variable influence on the invasive behavior of tumor cells. The progelatinase A-TIMP2 complex has to be activated, as most of the progelatinase A produced by cells is immediately complexed with TIMP2.

A positive cells and a lower percentage of TIMP2 positive cells were found in final stages of gastric cancers compared to survivors.

The mean gelatinase A / TIMP2 ratio in patients with recurrent urothelial cancer showing muscular invasion or lymph node metastasis is significantly higher than in patients without recurrence [107]. The disease-free survival is inversely correlated to gelatinase A: TIMP2 ratio [107]. The gelatinase A / TIMP2 ratio measured by RT-PCR suggests enhanced gelatinase A expression relative to TIMP2 in patients with lymph-node metastasis of breast cancers [108], without correlation between the magnitude of this ratio and the frequency of positive lymph nodes or to relapse status. Thus, an early prognostic indicator in various types of human cancer may be obtained by evaluation of gelatinase A / TIMP2 mRNA balance and/or serum level.

MMP3 and MMP7 capacity to release soluble E-cadherin, a cell adhesion transmembrane protein, results in paracrine inhibition of its function and consequently acting as a promoter of tumor cell migration and invasion.

MMP7 promotes the osteoclast activation mechanism in metastatic prostate cancer due to its ability to transform the receptor activator of RANKL (nuclear factor-kappa B ligand) to a soluble form [78].

MMPs exhibit complex and somehow paradoxical effects in carcinogenesis, as demonstrated by transgenic or knock-out mice experiments. Haptoglobin-MMP1 stimulates hyperkeratosis and acanthosis, promoting skin carcinogenesis, a process enhanced by MMP8 absence in defective inflammatory responses, and inhibited MMP9 absence (a mechanism involved in prolonged contact dermatitis) [23, 109, 110]. MMP3 involvement in epithelial cell apoptosis and MMP14 in breast hyperplastic processes stimulate breast carcinogenesis. Due to MMP11 involvement in neoangiogenesis, its absence results in breast carcinogenesis inhibition and similarly pancreatic carcinogenesis is reduced by the lack of MMP2 and by of MMP9 [23, 109, 110]. MMP2, MMP7, MMP11 absence results in carcinogenesis inhibition; the lack of MMP9 results in reduced metastatic process and MMP-11 absence stimulates the metastatic mechanism [23, 109, 110]. MT4-MMP has an unknown mechanism of involvement in breast carcinoma, being also expressed in breast cancer cell lines.

MMPs involvement in tumor angiogenesis

Angiogenesis anomalies occur in many pathological processes, such as rheumatoid arthritis, diabetic retinopathy, psoriasis, hemangiomas and cancer [111]. MMP2 causes cleavage of collagen IV, by engaging the PEX domain, to expose cryptic sites $\alpha\beta 3$ (by coupling loss to integrin $\alpha 1\beta 1$ that promotes tumor angiogenesis) [111]. MMP2, MMP9, MMP7 are expressed in tumor endothelial vascular cells [111]. Additionally, MMP7 stimulates the vascular proliferation [111]. Conversely, oligonucleotides anti-

matrilysin inhibits tumor angiogenesis [111]. Generation of MMP9 in macrophages and tumor endothelial cells opens new perspectives for correlations between MMPs [23]. MMP9 can generate tumstatin by proteolysis of collagen IV, with suppression of angiogenesis and tumor growth [23].

MMPs are necessary for cell migration and tube formation due to their direct effects on endothelial cells [111]. MT1-MMP exhibits a fibrinolytic activity, being involved in invasion of fibrin barriers and endothelial cell migration [111].

In experiments using cultures of endothelial cells on matrigel, the addition of recombinant gelatinase A is followed by an amplification of tube networks, a process inhibited both by neutralizing antibody and TIMP2, demonstrating the MMPs key role. The process has a complex regulation mechanism, as excessive levels of gelatinase A are inhibitory and TIMP2 may both stimulate or inhibit the angiogenic response according to the level of protease expression.

Angiogenesis and tumor invasion are stimulated by TGF- β (transforming growth factor- β) activation by surfaced-anchored MMP9 [112]. Angiostatin is released by MMP2, MMP3, MMP7, MMP9, MMP12, MMP13 and MMP20 [20]. VEGF (vascular endothelial growth factor) stimulates tumor angiogenesis, being released by MMP3, MMP7, MMP9, and MMP19 [20].

The ectodomain shedding interferes with cell surface signaling by proteolytic cleavage of the extracellular domain in the juxtamembrane region of a transmembrane molecule. This process results in release of a soluble ectodomain into the pericellular space and subsequent cell-tissue interaction. The ectodomain shedding mechanism is involved in angiogenesis and tumor proliferation, as a result of MMP3 and MMP7 action on HB-EGF, in tumor invasion, by E-cadherin shed by MMP3 and MMP7, and in tumor apoptosis by MMP7 action result on TNF- α (tumor necrosis factor- α) [20].

MMP7 induces angiogenesis in vivo, by enhancing the endothelial cell proliferation and up-regulates endothelial expression of MMP1 and MMP2 [113].

The endothelial cells express MMP2, MMP9, and MT1-MMP membrane vesicles are located near pseudopodia. The stimulation of angiogenesis by bFGF or VEGF results in shedding of vesicles. Supplementary, MMP9 stimulation of bFGF enhances *in vitro* endothelial cell growth [114].

The endothelial cells of tumors express MMP2, MMP7, and MMP9 [115]. Moreover, MMP7 stimulates vascular proliferation [113], while matrix-specific antisense oligonucleotides inhibit tumoral angiogenesis.

MMPs disrupt cell-to-cell adhesion by cleaving the ectodomain of VE-cadherin [111]. MMP3, MMP7, MMP9, and MMP19 have the ability to cleave matrix-bound isoforms of VEGF, releasing it as soluble fragments which are less effective in angiogenesis than matrix-bound VEGF despite their common cell surface receptor (VEGFR2) [116].

The induction of MMP9 expression in tumor macrophages and endothelial cells creates new hypothesis related to the correlation between MMPs and angiogenesis. MMPs also exhibit an inhibitory action on angiogenesis. MMP2, MMP7, MMP9 [114], and MMP12 [117] cleave plasminogen into angiostatin which promotes endothelial apoptosis and inhibits endothelial cell proliferation.

Another endogenous inhibitor of angiogenesis, endostatin is generated by MMP3, MMP7, MMP9, MMP13, and MMP20 cleavage of collagen type XVIII [118, 119]. MMPs are also capable to cleave the precursor of endostatin. Supplementary, MMP7 generates neostatin-7, the C-terminal 28-kDa endostatin-spanning proteolytic fragment, by proteolysis of collagen XVIII [120]. Tumstatin may be generated by MMP9 proteolysis of type IV collagen, resulting in angiogenesis and tumor growth inhibition [121].

MMPs influences vascular stability and permeability, mainly by MMP14 which mediates the vascular response following tissue injuries and modulates tumor progression, by TGF- β activation [122]. The relationship between lymphangiogenesis and tumor progression is well-known and MMPs involvement in the modulation of the above

process has been experimentally demonstrated [123]. MMP1, MMP2 enhanced expression [124], along with that of MMP3 [125] are correlated to lymphatic invasion and lymph nodes metastases. MMP2, -9, and -14 inhibition reduces angiogenesis and lymphangiogenesis, and lymph nodes metastases [126].

Apoptosis and MMPs in tumoral tissues

MMPs are involved in apoptosis regulation. By MMP7 ability to generate sFasL (soluble FasL) and subsequent Fas activation, apoptosis is stimulated [127]. This process is prevented by protein anomalies of malignant cells which result in disruptions in the signal transduction cascade of apoptosis [127]. FasL also exhibit a protective function for malignant cells from chemotherapeutic drug toxicity [128]. MMP7 expression is considered as a predictive marker for chemoresistance in patients with lung cancer [129].

MMP11 has an inhibitory action on tumor cell apoptosis but, paradoxically, an unknown mechanism of metastasis prevention has been demonstrated in experiments in transgenic mice [130].

MMPs in colorectal hepatic metastases

Metastatic and non-metastatic colorectal cancers variably express MMPs along with their specific inhibitors, such as MMP1, MMP2, MMP3, MMP7, MMP9, MMP10, MMP11, and MMP13 [54]. Thus, MMP / TIMP expression has potential value as a prognosis marker, some of them being directed toward hepatic metastases of colorectal cancers [131].

The epithelial and stromal colorectal cancer cells are responsible for production of molecules involved in matrix degradation and thus in local invasion and metastatic process [48]. MMPs produced by colorectal tumor stromal cells show contradictory expression [63, 132]. A reason of this variability is the promoter or inhibitory action of the microenvironment.

The balances between MMP2 / TIMP2 and MMP9 / TIMP1 have been extensively studied in primary and secondary colorectal cancers

beginning with the 90's [64, 93, 133-136]. MMP9 seems to be more involved than MMP2 [54], showing a general low MMP / strong TIMP expression in metastatic carcinomas. MMP9 positive stromal cells at the invasion front in primary carcinomas may suggest a potential direct or immune inhibitory function in hematogenous metastatic process [63].

The tumoral areas rich in MMP9 positive cells elicit an inflammatory reaction resulting in tumoral necrosis [63]. Another view is that MMP9 positive cells from the tumoral front are stimulating the inflammation, desmoplasia, and neovascularization, resulting in tumoral dissemination [132]. A similar role is attributed to stromal macrophages MMP9 positive from the periphery of hepatic metastatic nodules [48] although its expression seems to be weaker than in the primary site [132].

Starting with the 90's, strong serum and tissular MMPs and TIMPs expression result in a shorter disease-free interval and survival [93, 134, 135]. Moreover, MMP9 expression is considered as an early event in adenoma-carcinoma progression [133]. Another opinion was that of a marker of intratumoral and peritumoral inflammation more likely than direct involvement in tumoral progression [137].

MMPs and TIMPs expression have been associated to useful markers of hepatic metastases [48, 138, 139], MMP2 being associated with as much as 99% predictive value for hepatic metastatic process [139]. Thus, MMPs and TIMPs seem to be associated to the development of an aggressive phenotype, with poor prognosis and short survival [136]. Post-ablative metastatic hepatic tissue exhibits an increases expression of both MMP2 and MMP9 in the transition area [65].

Histologic growth pattern of secondary hepatic tumors is determined by the response ability of metastatic cells to the hepatic microenvironment characteristics; thus, there are three growing patterns: desmoplastic, pushing, and replacement. Plasmatic level for TIMP1 is a prognosis marker for colorectal cancer, being significantly higher in patients with metastatic disease [140].

Our experience in prognosis value of MMP9/TIMP1 (Figure 1) justifies our conclusions that our study supports MMP9 and TIMP1 potential to influence the tumor progression in liver metastases. However, the confirmation of MMP9 and TIMP1 value as prognostic factors, based only on immunohistochemical expression evaluation, requires a threshold validation [141].

MMPs and TIMPs in HCC

MMPs are involved in variable normal processes, such as growth and remodeling, or pathological, such as inflammation and tumor cells migration, invasion, and metastasation. Consequently, MMPs are major components of tumor microenvironment and strong modulators of key events in liver carcinogenesis [25].

MMPs are involved in liver cirrhosis development, a well-known precursor of HCC. In experimental models, MMP9 mutation result in inhibition of fibrogenesis, with marked collagen accumulation in portal and periportal spaces and also of the stellate liver cells trans-differentiation in myofibroblast-type phenotype. Moreover, an increased activated stellate liver cell apoptosis may result by viral vector induced mutations (MMP9-H401A and MMP9-E402Q) [142].

MMP9 overexpression is associated to the activation of PI3K/PTEN/AKT pathways in HCC [143, 144]. Fas ligand is cleaved by MMP7 and becomes unable to initiate apoptosis [128]. MMP2 and MMP9 are able to modulate VEGF biodisponibility and to promote angiogenesis in HCC [145, 146].

MMPs also participate in the regulation of inflammatory response by cytokines and chemokines, which are also involved in cancer progression [147-149]. MMP9 is strongly expressed in HCC, showing an expression correlated to the capsular invasion [150]. By the means of osteopontin precursor cleavage and its transformation in an active form, MMP9 acts as a promoter of invasion and metastasation [151].

MMPs are liberated in their inactive forms and become activated by their catalytic site interaction [25]. By several mechanisms, such

as coiling in position 1, interaction with X HBV protein, plasmin, furin, focal adhesion molecule (FAK), claudin-1, or other active MMPs results in their activation, thus promoting the process of liver fibrosis and HCC progression [152-155].

The use of statins as chemopreventive agents against HCC seems to be mediated by MMP2 and MMP9 inhibition and MMP14 and TIMP2 reduced expression [156].

Active MMPs are inhibited by a negative feedback loop, as a protective mechanism against excessive deterioration and tissue inflammation. MMPs activity is also regulated in genetic and transcriptional levels. TIMPs have complex roles in regulation of proliferation, apoptosis, MMPs activation, and

angiogenesis. TIMPs also prevent excessive ECM degradation. TIMP3 exerts an inhibitory effect on MMPs and has a limitative action in liver tumoral cells progression, invasion, and metastasation [49, 157]. TIMP1 overexpression inhibits the proliferative and invasive capacity of liver tumor cells lines [158, 159]. TIMP2 has a dual capacity to activate and inhibit MMPs. Reduced TIMP2 concentration activate MMP2 and MT1-MMP binding, as a critical step in MMP2 activation, while high TIMP2 concentrations inhibit MMP2 activation [106, 160]. MMP2 and MMP9 hyperactivation is associated to tumor invasion, metastasation, and an unfavorable prognosis in HCC evolution, as an expression of unbalanced MMPs and TIMPs levels [144].

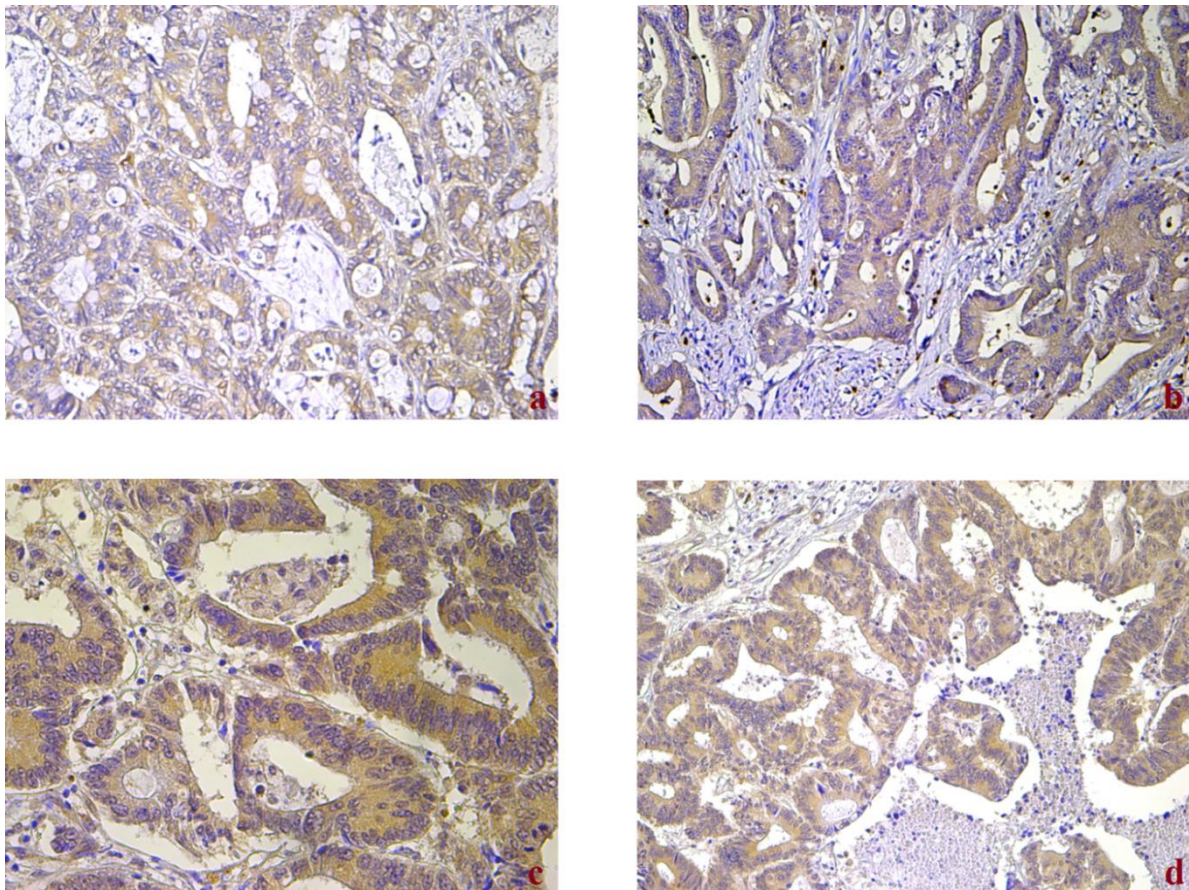


Fig. 1. MMP9 and TIMP1 expressions in liver metastases: (a) MMP9 - moderate staining intensity, 80% positive tumor cells, IHC – anti MMP9, X200; (b) TIMP1 – moderate staining intensity, 90% positive tumor cells, IHC – anti TIMP1, X200; (c) MMP9 - strong staining intensity, 100% positive tumor cells, IHC – anti MMP9, X400; (d)TIMP1 – strong staining intensity, 100% positive tumor cells, IHC – anti TIMP1, X200.

Conclusions and future perspective

MMP9 overexpression is a poor prognosis factor in hepatic metastases of colorectal cancer [63,132, 161]. Supplementary, MMP9 overexpression in HCC is associated with a poor prognosis [162] and similarly for MMP2 [163] and MMP12 [164], in experimental tumor development [165]. However, a recent study demonstrated a statistically significant longer overall survival in patients with increased serological MMP9 / TIMP1 ratio in comparison to that of patients with reduced MMP9 / TIMP1 ratio [166].

Literature review regarding MMPs and TIMPs study in primary and secondary hepatic tumors allows us to affirm that defining of a precise profile of their activities is extremely difficult [48, 131, 132, 161, 166-171]. The

expression variability of both MMPs and TIMPs is associated to promoter or inhibitor action of stromal cells and / or tumor cells, as liver microenvironment has a modulatory action for MMPs and TIMPs.

MMPs capacity to intervene in many biological processes is attributed to their ability of ECM proteolysis, as a possible initiator of unrevealed functions. The understanding of biochemical and structural aspects of MMPs, and the capacity to form molecular complexes with TIMPs open the perspectives of design of potent specific inhibitors for MMPs and, thus, the development of new therapies.

The elucidation of the complex interactions between molecules involved in proliferation and apoptosis, by future research, creates new perspectives in the early diagnosis and treatment of liver neoplasia.

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