Matrix Metalloproteinases: Promoters of Tumor Progression and Invasiveness

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The last three decades have witnessed the emergence of two major theories related to the budding of tumor cells from their primary site, and their progression through surrounding tissues leading to metastasis: angiogenesis and proteolysis by matrix metalloproteinases. The latter family of over 20 Zn^{+2}-dependent enzymes that cleave various components of the extracellular matrix has been increasingly implicated in the early stages of tumor invasion in addition to the previously known family of serine proteinases (urokinase-type and tissue-type plasminogen activators). Malignant cells depend on these proteinases to disrupt basement membranes, invade neighbor tissues and eventually metastasize to remote organs. As evident in other controlled proteolytic events, MMPs are counter-balanced *in vivo* by natural tissue inhibitors, four of which have been identified thus far and are designated TIMPs. Protease activity of MMPs has been extensively evaluated in various human cancers – including breast [1], colorectal [2], prostate [3], skin [4], ovary [5], bladder [6], oral and head and neck squamous tissue [7,8], stomach and lung [9] – showing a positive correlation of enhanced MMP activity in advanced tumor stages. In some tumors a differential expression of different enzymes of the MMP family may take place following progression of malignancy. For instance, MMP-7, a matrilysin that hydrolyzes mainly proteoglycans is elevated dramatically in benign adenomas as compared to the normal mucosa. However, in colorectal carcinoma, MMP-7 activity is not increased whereas MMP-2 and MMP-9 are significantly elevated over the mild activity of these two gelatinases in the preceding adenomatous tissue [10].

Over 2,500 studies have been published on the subject of MMP involvement in the growth of various tumor types, including only a handful of reports on the relevance of these ECM-degrading enzymes in thyroid cancer. In the current issue of *IMAJ*, Korem and colleagues [11] examined the expression of MMPs and their main inhibitor TIMP-2 in neoplastic and normal thyroid tissues, i.e., papillary, follicular and medullary carcinoma, follicular adenoma and multinodular goiter. Of the two tested gelatinases that display a selective ability to degrade type IV collagen, only MMP-2 was found in that report to be significantly higher in cases of papillary carcinoma as compared to the adjacent normal tissue or to the other tested tumor entities. Immunohistochemical staining located MMP-2 activity in the neoplastic papillary epithelial component, yet gelatin zymography failed to demonstrate a difference in MMP-2 activity in papillary carcinoma tissue in patients with lymph node metastasis or in those without. Moreover, the observation reported here that MMP-2 activity is not increased in medullary and follicular carcinomas of the thyroid is intriguing, inasmuch as the latter two carcinomas are regarded as more aggressive than papillary carcinoma. Thyroid papillary tumors constitute about 80% of all thyroid tumor types and are generally associated with slow growth and good prognosis after surgical treatment. However, a significant number of cases show early recurrence, or evolve malignant phenotypes that do not respond to radioactive iodine therapy, and tend to be invasive and metastasize to the lymph nodes even though their tumor size is small [12]. The study by Korem et al. points to the usefulness of MMP-2 expression as a diagnostic marker to differentiate papillary carcinoma from other thyroid neoplasms, but disputes its prognostic power as no correlation was found with lymph node metastasis.

Only a few studies have been published on the activation and enzymatic activity of gelatinase A (MMP-2) in normal and malignant thyroid tissues, suggesting that increased expression of MMP-2 is indeed correlated with the transformation of thyroid tissue to a malignant phenotype. Most of the measurements were performed by *in situ* zymography demonstrating an over-expression of mRNA of the precursor proMMP-2 enzyme, preferentially in thyroid carcinoma cells but not in the stroma cells [13,14]. Recently, a large-scale survey by Maeta et al. [15], which also included morphometric scoring, revealed that high expression of the two gelatinases MMP-2 and MMP-9 significantly correlated with large thyroid tumor size, presence of lymph node metastasis, high intrathyroidal invasion and clinical stage, and high vascular invasion. Unlike the report by Korem's team, MMP-9 activity was also increased in papillary thyroid carcinoma, and both of the gelatinases were elevated concomitant with metastasis to the lymph nodes [15]. It is also

MMPs = matrix metalloproteins
ECM = extracellular matrix

apparent from the latter study by Maeta et al. that the two main tissue inhibitors of MMPs, TIMP-1 and 2, are over-expressed in papillary thyroid tumors of larger size and in highly invasive cases. Such concomitant up-regulation of both MMP-2 and 9 and their inhibitors in papillary carcinoma is controlled by a subtle interplay between these enzymes and their regulators. Shi and colleagues [16] have demonstrated that the high levels of TIMP-1 transcripts in advanced stages of thyroid carcinoma are likely to derive from the surrounding stroma cells rather than from the thyroid tumor cells, thus containing and suppressing invasion and metastasis. MMPs and TIMPs are tightly regulated at the levels of transcription, release and activation [17], and the seemingly discrepant conclusions concerning their expression in various thyroid cancer states in the few published reports probably reflect differing methodologies.

Another aspect of the evaluation of MMP activity in thyroid neoplasms relates to the differential diagnosis of follicular adenoma and carcinoma, due to the difficulty to distinguish between the two by fine-needle aspiration. Follicular carcinoma is characterized by capsular and vascular invasion followed by metastatic dissemination. As such invasion is preceded by collagen degradation in the capsule and in the sub-endothelial basement membrane, measurement of MMP-9 has indeed demonstrated a significantly higher enzymatic activity in follicular carcinoma as compared to that measured in adenoma cells [18].

Originally it was assumed that cancer cells themselves produce MMPs to promote invasion through primary tumor boundaries. This dogma has been reexamined following *in situ* hybridization studies showing that stromal cells such as fibroblasts and endothelial cells, in addition to inflammatory cells that surround the tumor, are actually producing MMPs. MMP-9 has been localized primarily to inflammatory cells (macrophages and neutrophils) rather than tumor cells in colorectal cancer tissue, to reinforce the concept of docking sites on tumor cells that harbor MMPs secreted by stromal cells. Two examples for such tumor cell surface-binding receptors are CD-44 and $\alpha_v\beta_3$ integrin to which MMP-9 and MMP-2 bind, respectively [19]. Human tumor cells express a cell surface glycoprotein that induces and modulates MMP production by local stromal cells [20], and the secreted MMPs are then accumulated in the cytoplasm of the tumor cells. It is also becoming clear that the view of MMPs as destroyers of the ECM is rather simplistic. It is known that several important growth factors such as fibroblast growth factor and transforming growth factor β are tightly associated with the ECM, hence the dissociation of the matrix by MMPs could release these factors in solubilized and active form. Likewise, MMP-2, 3 and 9 hydrolyze interleukin 1β from its precursor form to the active form. Protease activity may down-regulate mitogenic signals, like the cleavage of IL-2 receptor type-α on T lymphocytes to depress IL-2-induced proliferation [21]. Another proteolytic product of MMPs that may induce tumor cell death rather than propagation is the solubilized form of Fas ligand, which is activated by MMP-7 and appears to be crucial for apoptosis as demonstrated in prostate epithelial cells [21]. Thus, MMPs can modulate the balance between cell growth and apoptosis by cleaving cell surface substances.

The Janus-faced nature of MMPs is exemplified in numerous cellular effects. Proteolysis of matrix components by MMPs releases biologically active cryptic peptides, such as the cleavage by MMP-2 of laminin-5, a component of the ECM that promotes cell migration [22]. Yet excess proteolysis by MMPs might degrade matrix receptors and signals to disrupt matrix interactions and inhibit migration [17]. The consequences of MMP involvement in cellular communication during inflammatory responses can either enhance or abrogate host defense systems, so that certain MMP activities could be beneficial to the host while others can aggravate a given pathologic state [21]. MMPs are implicated in early stages of tumor angiogenesis, and MMP-9 plays a pivotal role in the angiogenic switch by generating bioavailable vascular endothelial growth factor [23]. However, MMP activity could as well down-regulate angiogenesis as plasminogen is hydrolyzed by MMP-3, 7, 9 and 12 to form the anti-angiogenic peptide angiostatin [24]. Depending on the sensitivity of tumor cells to angiostatin, MMPs could either exert stimulatory or inhibitory effects. Similar to the double entente of MMPs, a complex behavior applies also to their natural inhibitors. Although the inhibitory effect of TIMPs has been demonstrated both *in vitro* and *in vivo* on tumor invasion in several malignancies such as bladder cancer, increased TIMP levels are related to poor outcome [25]. It is becoming apparent that the relationship between MMPs and TIMPs in tumor development is more complex than just a simple imbalance in their relative secretion to explain invasiveness and metastatic potential. Indeed, the fact that some malignant tumors are associated with increased TIMP production led to the notion that TIMPs exert growth-promoting effects mediated by tyrosine phosphorylation [26], or by inhibiting apoptosis as shown in B cells [27].

The strategy of administering synthetic inhibitors, which may prevent the initiation of MMP activation cascade and ECM degradation, has attracted growing attention. Currently, several synthetic inhibitors of MMPs are in phase I/II clinical trials in various cancer forms [28]. To mention just a few, BB3103, of the hydroxamate class, inhibits specifically MMP-2 and the invasive behavior of human ovarian cancer cells. A zinc-chelating agent, pyrimidine-2,4,6-trione developed at Hoffmann-LaRoche, proved to be an effective and selective gelatinase inhibitor. Another promising inhibitor of MMP-2 and MMP-9, a decapeptide isolated from a phage display library, has proven to suppress tumor and endothelial cell migration *in vitro*, target tumors *in vivo* and prevent their growth and invasion in mice [29]. Other molecular approaches to inhibit MMP activity are limited to *in vitro* assays and to animal model tumors. These include the introduction of antisense oligonucleotide into MMP mRNA, or the infection of human breast cancer cells with a recombinant adenovirus vector containing the MMP inhibitor TIMP-2 expression cassette, yielding the inhibition of tumor growth and metastasis [26]. A most recent report has demonstrated that a transfection of invasive human head and neck squamous cell carcinoma cells with PDX, a selective furin inhibitor, attenuated and even abolished critical attributes of the advanced malignant phenotype [30]. Furin, a pro-enzyme convertase expressed in many

IL = interleukin

human tumor lines, is known to activate stromelysin-3 and membrane type-1 matrix MMP that activates some MMP species from their zymogen non-active form. Clinical cancer trials with MMP inhibitors began in 1997, yet phase III evaluation in patients with advanced breast, lung, prostate, gastrointestinal tract and brain tumors has not yet proven to be effective [20].

MMPs play a complex and key role in tumor growth and metastasis. Exciting recent discoveries related to the biology of this family of proteases and their inhibitors will help to clarify some of the existing dilemmas. The range of growth factors or cytokines that depend on MMPs activation, and the intricate relation between the MMPs and their target substrates, are far from being elucidated. And perhaps the foremost ongoing efforts of designing therapeutic strategies and drugs to inhibit MMP hold promise for the control of tumor spread.

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The thing that impresses me most about America is the way parents obey their children

King Edward VIII (1894–1972)