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REVIEW

Matrix metalloproteinases in head and neck cancer: current perspectives

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Abstract: Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases encoded by 24 distinct genes. Their functions have been implicated in numerous normal and pathologic processes, including uterine involution and organogenesis, inflammation and wound healing, vascular and autoimmune disease progression. Pertinent to this review, the role of MMPs in cancer biology is fairly well researched and documented, and remains a subject of continuing intense investigation. Not only are several MMPs overexpressed in head and neck squamous cell carcinomas (HNSCCs), expression has been correlated with salient tumorigenic hallmarks, such as cell proliferation, angiogenesis, invasion, and metastasis. The utility of changes in the expression profile, as well as various MMP polymorphisms as potential prognostic markers in oral cancers and oral premalignant lesions, have been investigated. Furthermore, the potential therapeutic utility of targeting MMPs in cancer remains attractive, although outcomes in this respect appear so far to be less encouraging with respect to HNSCCs. Because of the disappointing results observed in clinical trials where MMP-targeting regimens for HNSCCs utilized broad-spectrum small MMP catalytic site inhibitors, investigators now envision new strategies for MMP-specific targeting based on the recognition of new noncatalytic MMP domains with distinct functions. This review provides an overview of MMP activities in general and in cancers, and an update of their activities in HNSCC. Specifically, their role in the development and progression of HNSCC and their function as signaling molecules is discussed. Finally, their role as potential prognostic biomarkers and therapeutic targets in HNSCC is revisited.

Keywords: MMPs, head and neck cancers, HNSCCs, oral squamous cell carcinoma, prognosis, therapy

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth-most common cancer worldwide, accounting for more than 550,000 cases and approximately 300,000 deaths annually.[1] HNSCCs are dominantly neoplasms arising from the squamous mucosae of the upper aerodigestive tract, accessory salivary glands, oropharynx, nasopharynx, and hypopharynx.[2] The 5-year survival rate for patients with HNSCC depends on the tumor stage at the time of diagnosis, but overall is approximately 50%, and has not improved significantly over the past five decades, despite advances in treatment techniques and modalities.[3] The molecular basis of HNSCC has been extensively studied, and several genetic and epigenetic alterations have been characterized and associated with cancer-cell proliferation, survival, differentiation, and invasion/metastasis, in an effort to identify novel diagnostic and prognostic markers and therapeutic targets.[2,4]

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For the most part, HNSCCs are local diseases, which may spread to regional lymph nodes, with distant metastasis often a late event with fatal outcome.[5] Characteristics of malignant neoplasms in general and typical of epithelial malignant neoplasms, local, regional, or metastatic spread depend on a series of interactions and a successful breach of the epithelial–mesenchymal interface comprising the basement membrane, fibroblasts, extracellular matrix (ECM), immune cells, and vasculature. These epithelial–mesenchymal interactions involving cell–cell, cell–ECM, and angiogenesis constitute vital components in the multistep process of carcinogenesis. Notably, ECM remodeling during tumor progression is mediated by MMPs.[6–8]

Here, we provide an overview and update of MMP activities in cancer biology, with specific focus on their activities in HNSCCs. Their role in the development and progression of HNSCC, their function as signaling molecules, and their potential prognostic utility and continued targets for new therapeutic designs for HNSCCs are revisited. Finally, their role as potential prognostic biomarkers and therapeutic targets in HNSCC is revisited.

Matrix metalloproteinases

Classification of MMPs

As summarized in Figure 1, MMPs are classified into collagenases, stromelysins, gelatinases, matrilysins, metalloelastase, membrane-type MMPs, and others on the basis of their substrate specificity.[9,10] Collagenases (MMP1, -8, and -13) are secreted MMPs that cleave different types of collagen at specific sites prior to degradation by other MMPs.[11] Gelatinases (MMP2 and MMP9) degrade gelatin, collagens, precursors of TNFα and IL-1β, elastin, proteoglycan, and fibrillin 1.[12] They are expressed by endothelial cells, osteoclasts, chondrocytes, osteoblasts, and malignant cells. MMP12 degrades elastin, type IV collagen, type I gelatin, myelin basic protein, and α_1-antitrypsin.[13] MMP12 is mainly expressed by macrophages, and is associated with varied pathologic conditions, including inflammation.[13]

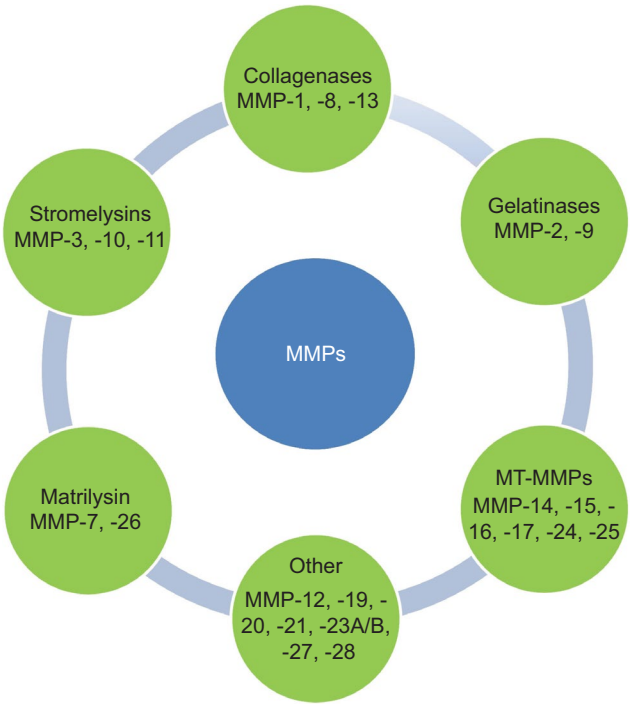

Figure 1 Classification of MMPs.
Abbreviations: MMPs, matrix metalloproteinases; MT, membrane-type.

The domain structure, substrate activities, and biologic functions of the membrane-type MMPs (MT-MMPs) were recently described in an elaborate and elegant review by Itoh.[14] Briefly, MT-MMPs have either a transmembrane domain or a glycosylphosphatidylinositol anchor.[14] MT-MMPs include MMP14 (MT1-MMP), -15 (MT2-MMP), -16 (MT3-MMP), -17 (MT4-MMP), -24 (MT5-MMP), and -26 (MT6-MMP).[14] Whereas MT1-, MT2-, MT3-, and MT5-MMPs are secured as transmembrane domains, MT4-MMP and MT6-MMP are anchored to the cell membrane via a glycosylphosphatidylinositol anchor.[14] Pro-MT-MMPs are activated by proprotein convertases, such as furin.[14] With respect to their substrate activities, MT1-MMP demonstrates an extensive substrate of targets that include the degradation of fibrillary collagens: types I, II, and III (but not type IV) collagens.[14] While other MT-MMPs exhibit varying degrees of collagenolysis and specificity, only MT1-MMP degrades fibrillary collagen.[14] MT1-, MT2-, MT3-, and MT5-MMPs activate pro-MMP2 on the cell surface and degrade laminin and fibronectin.[14] The degradation of fibrin by MT1-, MT2-, and MT3-MMPs has been shown to promote cellular invasion into matrices,[15–17] a process that may promote cancer-cell activity.

MMP20 (enamelysin), first cloned from odontoblasts, was until recently widely regarded as tooth-specific.[18–22] It is a proteolytic enzyme critical for proper dental enamel formation.[18–22] Recently, we reported the expression of MMP20 in oral squamous cell carcinomas (OSCCs) and metabolically active duct epithelial systems of the salivary gland and nephron, indicating that the tissue distribution of MMP20 under physiologic and pathologic conditions may be wider than previously thought.[22–24]

On the basis of mechanism of regulation, MMPs are classified into three groups: group 1 (MMP1, -3, -7, -9, -12, -19, and -26) contains a TATA box and an activator protein, AP1, which binds to the Fos and Jun family of transcription factors;

group 2 MMPs (MMP8, -11, and -21) have the TATA box, but lack the AP1 site; and group 3 MMPs have neither the TATA box nor the AP1 site.[25] Notwithstanding the presence or absence of TATA box or AP1 site, all MMPs are regulated by various factors. MMP activity may be induced by cytokines, growth factors, and oncogenes. Signal-transduction pathways, such as MAPKs (eg, ERK1 and ERK2) can inhibit or stimulate the transcription of MMPs.[26] The ETS family,[27] NFκB,[28] STATs,[11] CIZ, and p53[29] serve as positive modulators, while TGFβ-inhibitory elements or AG-rich elements serve as negative modulators of MMP transcription.[30,31]

Structure of MMPs

MMPs are zinc metalloenzymes and encoded by at least 26 distinct genes.[6–8] Although structural similarity exists among members of the MMP family, subtle but significant differences have been identified. As summarized in Figure 2, all MMPs have the propeptide (prodomain) region responsible for maintaining the latency of the inactive enzyme. This is followed by a catalytic domain harboring the zinc (Zn^{2+}) active site that binds to three histidine residues and a conserved methionine, and the hemopexin-like C-terminal domain linked to the catalytic domain by a hinge region.[32,33] The latter domain determines the substrate specificity and interactions with inhibitors.[34] Activation of MMPs takes place in the pericellular space with the involvement of integrins (eg, pro-MMP2) or it may happen intracellularly with furin-like proprotein convertases (eg, MT-MMPs), cleaving the prodomain from the catalytic domain, thereby rendering MMPs active.[35]

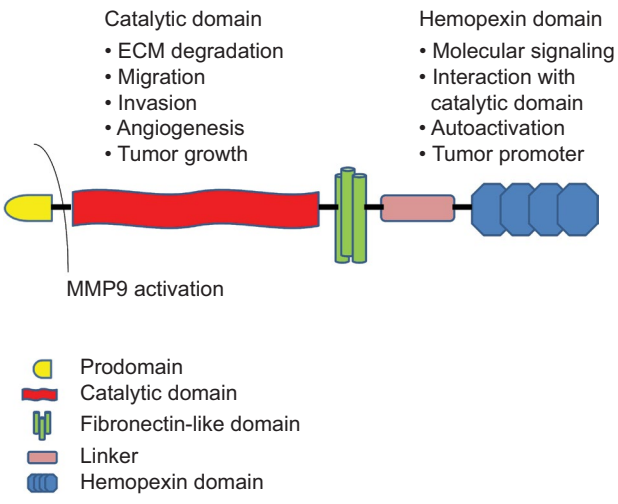

Figure 2 Structure of MMPs.
Notes: Schematics of MMP9 structure, showing the various domains common to all MMPs. Differences however exist among members of the MMP family.
Abbreviations: MMPs, matrix metalloproteinases; ECM, extracellular matrix.

SIBLINGs as activators of MMPs

A universal classic dogma of latent MMP activation usually entails a cleaving of the propeptide as a necessary step to MMP enzymatic activation. In 2004, Fedarko et al[36] reported that three members of the SIBLING family – BSP, DMP1, and OPN – bind and activate three specific MMPs in vitro: BSP-MMP2, DMP1-MMP9, and OPN-MMP3. The other two members of the SIBLING family are DSPP and MEPE.[37] Although the cognate MMP partner for MEPE, if any, is yet to be identified, we recently identified MMP20 as the cognate MMP for DSPP.[22–24]

As depicted in Figure 3, this activation is significant, because the bound SIBLINGs activate their cognate MMP partners, not by removing the inhibitory propeptides, but by inducing a conformational change that lowers the propeptides' affinity for their own binding domain.[36] In consequence, the propeptides vacate the active sites, thereby allowing substrates to be digested (Figure 3A). While the resultant SIBLING–pro-MMP pairs exhibit resistance to the inhibitory activities of TIMPs, activated MMPs previously inhibited by TIMPs become reactivated upon binding of their cognate MMP partner (Figure 3B).[36] The check and balance necessary for reversing these activation pathways are provided by circulating complement factor H, which has a much higher affinity (~100-fold) for BSP, DMP1, and OPN than the SIBLINGs have for their cognate MMP partners.[36] Therefore, complement factor H binds to SIBLINGs on the active and latent (Figure 3C) MMPs, in order to disengage them from the protease complex, allowing the propeptide and TIMP to reinhibit protease activity (Figure 3D).

Subsequent studies showed that the SIBLING–MMP pairing observed in vitro also obtains in vivo, suggesting that SIBLING–MMP interaction may be biologically important.[22–24] Published results from our laboratory show SIBLINGs with known MMP partners coexpressing and colocalizing with their cognate MMP partners in highly metabolic duct epithelial systems of the salivary gland and nephron, and in OSCC cells (DSPP-MMP20 in Figure 3E).[22–24]

MMPs and cancer

Cancer research has traditionally highlighted the role of overexpressed molecules that contribute to the survival and proliferation of cancer cells. At the same time, several studies have presented compelling evidence that the tumor microenvironment, particularly that of the ECM, plays a crucial role in cancer progression.[33] In this context, the role of MMPs in ECM remodeling, cancer invasion, angiogenesis, and metastasis has been investigated and continues to be so.[7] The expression/

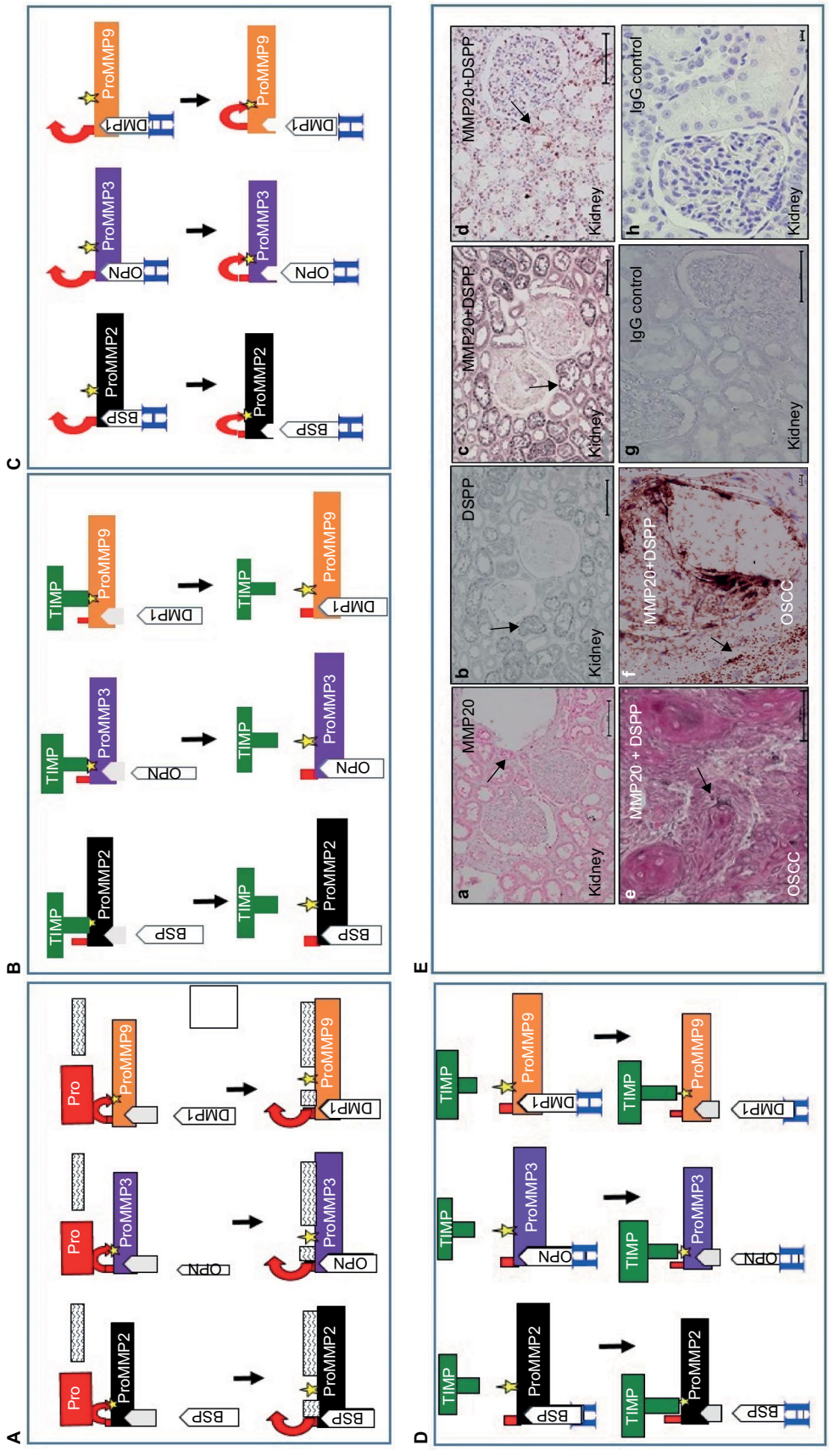

Figure 3 SIBLINGs as activators of MMP.

Notes: (**A**) When proMMP with active site (top) binds to cognate SIBLING, it undergoes conformational change necessary to expose the active site to substrates (bottom). (**B**) MMP without propeptide, inhibited by TIMP, binds SIBLINGs to bring about conformational change (top). TIMP is then released (bottom), resulting in MMP activation. (**C**) When factor H binds to SIBLING (top), SIBLING is disengaged from the MMP, allowing the propeptide to fit back into the active site (bottom). (**D**) Factor H engages (top) and extricates SIBLING from the MMP–SIBLING complex, allowing TIMP to bind and inhibit MMP (bottom). Stars in (**A–D**) indicate active sites.[167] (**E**) Illustrative coexpression and colocalization of DSPP–MMP20 in human kidney and OSCC. MMP20 positive immunoreactivity (red, arrow, a and e [×20]). DSPP-positive immunoreactivity (black, arrow, b [×20]). MMP20–DSPP coimmunoreactivity (reddish black, arrow), and colocalization of MMP20–DSPP by in situ proximity ligation assay (brown dots, arrow, d [×20] and f [×10]). Ogbureke KU, Koli K, Saxena G. *J Histochem Cytochem*. 64(10):623–636. Copyright © 2016. Reprinted by Permission of SAGE Publications, Inc.[24] Negative controls for immunohistochemistry (g [×40]) and in situ proximity ligation assay (h [×40]) are also shown. Bar 100 µm, except for "f" panel (substitute), which is 10 µm.

Abbreviations: MMPs, matrix metalloproteinases; OSCC, oral squamous cell carcinoma; DSPP, dentin sialophosphoprotein; IgG, immunoglobulin G.

upregulation of specific MMPs, including MMP1, -2, -7, -9, and -13, in several human cancers has been demonstrated, with expression/upregulation correlated with tumor aggression, tumor stage, and poor patient prognosis.[33,34] It stands to reason, therefore, that they have been proposed as potential diagnostic and prognostic biomarkers in many types of cancer,[6] with research efforts intensifying toward establishing any diagnostic and prognostic values of clinical importance. In addition, a growing number of therapeutic anticancer strategies continue to focus on MMPs as attractive targets.[6,34,35,38]

Currently, it is fairly well established that polymorphisms in MMP genes are associated with progression of a number of cancers. For example, meta-analysis revealed the *MMP7*$^{-181A/G}$ polymorphism as a low-penetrant risk factor for cancer development in the East Asian population.[39] *MMP2*-promoter polymorphism has been associated with invasive cervical carcinoma in Mexican women.[40] Furthermore, polymorphisms in *MMP2* in nonsmokers, and *MMP12* and *MMP13* in smokers, have been linked to gastric cardia adenocarcinoma in a high-incidence region of north China.[41] In a large meta-analysis, Peng et al found that *MMP2* (–1306C>T and –735C>T) and *MMP7*$^{-181A>G}$ polymorphisms play allele-specific roles in cancer development, while the *MMP9*$^{-1562C>T}$ polymorphism did not appear to be a major risk factor for cancer.[42] Another meta-analysis by Li et al,[43] focusing on digestive tract cancers, demonstrated an association of polymorphisms in the promoter regions of specific MMPs with increased (MMP1 and -7) or decreased (MMP2 and -9) susceptibility, while Yang et al[44] further suggested that the *MMP7*$^{-181A>G}$ polymorphism may contribute to susceptibility to gastric cancer. In their meta-analysis of studies investigating the role of the –1171(5A>6A) polymorphism in the promoter region of *MMP3*, Yang et al[45] did not find an association with overall cancer risk, suggesting that this polymorphism may be related to a decreased cancer risk in general in Asian populations when specifically compared with gastrointestinal cancers.[45]

MMPs in cancer invasion and metastasis

Invasion is a localized process that occurs at the tumor–host interface, where tumor and stromal cells exchange biologic molecules (enzymes and cytokines) that modulate the local ECM and stimulate cell migration. The invasive front consists of a unique subset of tumor cells interfacing with bone marrow-derived and organ-specific supportive cells. During invasion, MMPs modulate the availability of growth factors and cell-surface receptors, while driving the formation of specialized structures called invadopodia. Invadopodia utilize several secreted and activated MMPs to degrade ECM macromolecules, modulate shedding of membrane-anchored ligands (eg, epidermal growth factor receptor [EGFR]), control integrin-proliferation effects, alter antiapoptotic signals by cleaving Fas ligands, and regulate tumor vasculature.[6] MMPs also may dampen chemotactic and inflammatory responses by inactivating MCP3.[46] Therefore, MMPs not only aid in cell invasion but also in many cases may inhibit invasion, depending on a balance of factors expressed. For example, T cells and macrophages produce the TNFα-related cytokine RANKL, which interacts with its receptor and activates IKKα.[47] IKKα inhibits tumor progression by the expression of maspin. Maspin in turn alters the expression of integrin-adhesion molecules restricting cell mobility.[47] Metastasis, a major cause of death in cancer, is a multistep process that involves the stroma, blood vessels, and other associated factors. Successful tumor metastasis depends on several parameters, such as cell invasion, migration, angiogenesis, host immune escape, and extravasation. In all, MMPs are overt or covert crucial players in several of these metastatic processes.[48]

MMPs in HNSCC

MMP-expression profile in HNSCCs

Several published studies have demonstrated that certain MMPs are upregulated in HNSCC, and elucidated their role in multiple aspects of tumor formation and progression.[49] MMP1, -2, -3, -7, -8, -9, -10, -11, -13, and -14 are most commonly noted to be overexpressed in head and neck cancers.[50] Specifically, gene microarray-expression analysis in whole HNSCC-tumor samples detected overexpression of MMP1, -2, and -3,[51,52] whereas another comprehensive review indicated that MMP1, -3, -7, -10, -12, and -13 expressions were significantly increased in almost all investigated microarray data sets of HNSCC.[49] Immunohistochemical studies have also confirmed the results of microarray analysis. For example, Ye et al[53] detected upregulated expression of MMP1, -3, -7, -9, -10, -11, -12, and -13 in tongue squamous cell carcinoma (SCC).

Omar et al[54] reported higher MMP7-expression levels in the invasive portion of oral and cutaneous SCCs that was more intense in oral tumors, and without MMP8 expression in either cancer type. Ogbureke et al[55] reported that BSP and OPN are overexpressed in OSCCs, accompanied by their known cognate MMP partners: MMP2 and MMP3, respectively. Recently, Saxena et al[22] reported the expression of MMP20 in OSCCs and dysplastic oral premalignant lesions using archived human tissues and cell lines. Hitherto this report, MMP20 expression had been widely regarded as tooth-specific, and thus temporally limited to the odontogenic

apparatus during odontogenesis, where it participates with MMP2 in processing DSPP into DSP, DPP, and DGP.[22,56]

MMPs are elaborated by malignant epithelial cells, as well as cells of the surrounding stroma, including endothelial cells, fibroblasts, and inflammatory cells.[57] As a result, many studies have examined MMP-expression levels in body fluid, notably serum, as potential diagnostic and prognostic indicators in HNSCC. Lotfi et al[58] indicated that serum levels of MMP2 and MMP9 were higher in OSCC patients compared with levels in healthy subjects. Tadbir et al[59] reported high MMP3 serum levels in patients with OSCC, and suggested that MMP3 serum concentration may aid diagnosis, but not to predict prognosis in OSCC. Elevated serum levels of MMP2 and MMP9, along with TGFβ_1, E-selectin, and CRP, have been reported in patients with leukoplakia.[60]

MMP polymorphisms in HNSCCs

Genetic studies have reported that MMP single-nucleotide gene polymorphisms in the promoter regions of MMP1, -2, -3, and -9 are associated with OSCC.[61,62] In another study, by Chaudhary et al, the authors detected a higher frequency of MMP1-promoter genotypes with the 2G allele, which is associated with higher enzymatic activity, in patients with HNSCC or the preneoplastic condition, oral submucous fibrosis.[63] In addition, elevated expression of the 5A allele of the *MMP3* gene has been associated with an increased risk of oral SCC development.[64] It has also been reported that the *MMP9*^{P574R} polymorphism (GG genotype) may contain a genetic risk factor for esophageal SCC,[65] and the C>T polymorphism in the MMP9 promoter is associated with the risk of developing OSCC.[66,67] On the other hand, Lin et al[68] demonstrated that the –1306C>T polymorphism in the MMP2 promoter, which eliminates the Sp1-binding site and downregulates the expression of the *MMP2* gene, is associated with a decreased susceptibility for developing OSCC. In consequence, patients carrying the CC genotype had almost twice the risk compared with the CT or TT genotype.[69]

MMPs in HNSCC invasion

The activities of several proteases, particularly MMPs, have been implicated in HNSCC invasion.[50] AP1 has been reported to increase the transcription of MMP9,[69,70] while EGFR and integrins enhance MMP9 activity.[71–75] Furthermore, MMP9 degrades type IV collagen and promotes HNSCC invasion.[76,77] Indeed, immunohistochemical studies correlated MMP expression with loss of type IV collagen α-chain areas in OSCC, and MMP9 has been proposed as an invasion- and infiltration-pattern marker of OSCC at the invasive front.[78] MMP9 is able to cleave several proteins including TGFβ and chemokines, E-cadherin, and certain cell-surface receptors.[79,80] MMP2 and MT1-MMP have also been implicated in HNSCC invasion.[81] Moreover, MT1-MMP, along with TIMP2, has been shown to contribute to secreted protease activation, such as MMP2.[82,83] In addition, EMMPRIN is another protein that has been proposed to increase the protease activity of MMPs, cathepsin B, and uPAR, in order to enhance HNSCC invasion.[72,84] In concert with other players, MMP10 and MMP13 have been associated with HNSCC invasion.[85–89]

MMPs and angiogenesis

MMPs play a key role in ECM remodeling. In addition, they release growth factors and unmask cryptic sites, which help malignant cells to elude homeostatic control.[90] For example, following activation by plasmin, MMPs bind to docking sites on cell surfaces. Plasmin activates MMP2 and MMP9, and multiple MT-MMPs process the pro-MMP2 to its active form.[77] Activated MMP9 induces angiogenic switch, increases the availability of growth factors, and plays an important role in recruiting pericytes from the bone marrow. It also converts the Kit ligand from a membrane-bound molecule to a soluble survival/mitogenic factor soluble Kit ligand and promotes tumor angiogenesis through the release of ECM-bound angiogenic factors, such as VEGF.[91] MMP2, -3, and -7 induce the release of TGFβ_1 from decorin, a proteoglycan that sequesters TGFβ in the matrix.[92] CD44 and $\alpha_v\beta_3$ localize MMP2 and -9 to the migrating invasive front and provide a docking site for the proteinases.[93]

Collagen remodeling in perivascular stroma is associated with angiogenesis. The first step in endothelial morphogenesis is the cleavage of type IV collagen of the basement membrane by MMP2 and -9 in vivo (as well as MMP1 in vitro). MT1-MMP breaks down collagen types I–III, gelatin, laminin, and other ECM components, in addition to activating pro-MMP2.[94] Collagen cross-linking induces tumor progression by activating FAK, PI3K, and Akt.[95] MMP1 induces epidermal hyperplasia and increases the susceptibility to tumorigenesis, invasion, and angiogenesis.[49] Since MMP3 is expressed by fibroblasts and tumor cells, it regulates cancer stem cells during tumor initiation and metastasis.[90]

MMPs and angiogenesis in HNSCC

MMPs are upregulated by Notch1, EGFR, TGFβ, HGF, and GM-CSF, which are commonly overexpressed in HNSCC.

MMP7 expressed in OSCC of the buccal mucosa is mostly affected by *PTEN* mutation.[96] It also enhances endothelial cell proliferation and upregulates expression of MMP1 and -2.[97] MMP10 plays an important role in the invasion and metastasis of HNSCC, and is associated with p38 MAPK inhibition.[98] Epithelial dedifferentiation and histologic aggression, extracapsular spread, and nodal metastasis of HNSCCs are associated with MMP12 expression.[99] Invasive cells that express periostin, IFITM1, and Wnt5b induce MMP10 and -13 expression.

MMP13 produced from stromal fibroblasts promotes angiogenesis and aggression of HNSCC through increased secretion of VEGFA and VEGF-2 from fibroblasts and endothelial cells, also activating latent MMP9.[49] MMP19, found in the tumor-invasive fronts, facilitates HNSCC invasiveness.[100] MT1-MMP regulates VEGF expression and activates the Akt and mTOR pathways. It also stimulates cell migration through a nonproteolytic mechanism, involving MEK1/2–ERK1/2–p90RSK signaling.[101] MMP14 facilitates endothelial migration by shedding Tie2.[102] It also activates pro-MMP13 by cleaving the signal peptide.[102] MT4-MMP increases metastatic intravasation by increasing vascular leakage.[92]

MMPs as signaling molecules

Noncatalytic functions

Prior to recent understandings and updates on the relevance of minute structural difference in the various MMPs to function, studies focused on the catalytic role of MMPs in many important physiological and pathological processes.[103] It is well understood that the MMP-family structure includes a hydrophobic signal peptide, a propeptide domain for enzyme latency, a catalytic domain, and a hemopexin-like C-terminal domain, hemopexin (PEX), connected to the catalytic region via a flexible hinge domain.[80] Indeed, the majority of MMPs, excluding the smaller matrilysins MMP7, -23, and -26, have a COOH-terminal PEX domain with a distinct four-bladed β-propeller structure.[104] The PEX domain of MMPs is now considered to be responsible for their noncatalytic activity, as it is capable of interacting with receptors, inhibitors, and substrates.[105–107] For example, the PEX domain interacts with cell-surface receptors, including LRP1 and megalin/LRP2,[108,109] as well as with inhibitors, such as TIMP1[110] and TIMP3.[111] Besides receptor and inhibitor binding, the PEX region also interacts with substrates, including gelatin, collagen types I and IV, elastin, and fibrinogen.[112,113] Recently, it has been proposed that the PEX domain is involved in autoactivation, by guiding the activation of the MMP catalytic domain.[104]

Although published data on the tumorigenic contribution of noncatalytic functions of MMPs are not derived from head and neck cancer cases, these studies of cancers of other regions implicate MMPs that also are highly expressed in HNSCC. The hemopexin domains of MMP9 (PEX9) have been extensively studied. Sequence-alignment studies of human MMP9 revealed that its PEX9 domain shows low homology with other MMP PEX domains (25%–30% amino acid identity), thereby suggesting PEX9 as a potential therapeutic target for selective MMP9 inhibition.[114,115] Ugarte-Berzal et al also have shown that although MMP9 degrades gelatin, the PEX9 domain inhibits this degradation by shielding gelatin and averting its interaction with the MMP9 catalytic site.[114,115] The PEX9 domain may regulate intracellular signaling and survival in chronic lymphocytic leukemia (CLL) cells.[114,115] Ugarte-Berzal et al reported that the PEX9 domain contributes to CLL progression, and found a connection between blade B4 and the $\alpha_4\beta_1$ integrin.[114,115] These authors recently proposed a novel PEX9 sequence involved in CLL PEX9–pro-MMP9 binding and interaction with CD44.[115]

In other relevant studies, pro-MMP9 was shown to bind with the Ku protein through its PEX domain to promote the migration of acute myeloid leukemia cells,[116] whereas PEX9 and CD44 interaction in COS1 monkey kidney cells induced cell migration.[117] Furthermore, thrombin-mediated invasion of U2 osteosarcoma cells involved a PEX9 and β_1-integrin association,[118] while MMP9 catalytic and PEX domains have been reported to induce FGF2-mediated angiogenesis in neutrophils.[119]

With respect to other MMPs, Suenaga et al[120] reported that binding of CD44 to the MMP14 PEX domain is critical for shedding of human fibrosarcoma and breast carcinoma cells. In addition, Eisenach et al described an MMP14–VEGFR2–Src complex formation that controls VEGFR2 cell-surface localization through hemopexin-dependent activity in breast cancer cells.[121] As a result of this complex formation, Akt and mTOR are activated, leading to enhanced VEGFA transcription.[121] Cross-talk signaling between MMP14 and CD44 has also been proposed for phosphorylation of the EGF receptor, leading to the activation of the MAPK and PI3K signaling cascade and consequent migration of Cos1 cells.[122]

The MMP3 PEX domain has been found to induce hyperplastic growth in orthotopic transplants of lentivirally transduced mammary epithelial cells, even in the complete absence of its active domain, resulting in a nonproteolytic interaction with the Wnt ligand.[104] A similar function of MMP3 PEX has also been suggested to enhance invasiveness

of breast cancer cells.[123] Extracellular interaction of MMP3 PEX with HSP90β has been reported to be critical for invasion and morphogenesis of mouse mammary epithelial cells.[123] Furthermore, extracellular Hsp90α stabilizes and protects MMP2 from degradation in human breast cancer cells through a regulatory mechanism mediated by interaction of Hsp90α with the MMP2 C-terminal hemopexin domain.[124] This interaction enhances the proteolytic activity of MMP2, and thus promotes tumor angiogenesis.

MMP23 contains a noncatalytic region different from hemopexin. This region presents a small toxin-like domain and an immunoglobulin-like cell-adhesion molecule domain. These two domains were found to interact with potassium channels in the endoplasmic reticulum. Notably, MMP23 and potassium-channel coexpression has been reported in several diseases, including cancer and inflammatory disorders.[125]

In summary, findings of a new hemopexin-dependent role for MMPs have shifted the focus from their proteolytic activity, and added a new dimension in the role of MMPs as drug targets and a novel direction for therapeutic strategies aimed at interfering with MMP function in cancer.

MMPs as tumor biomarkers

The detection of MMPs in HNSCC tissues by such techniques as immunohistochemistry, DNA/RNA analysis, and zymography has prompted analysis of potential diagnostic and prognostic significance of MMP expression in HNSCCs. However, cautionary notes, informed by inherent limitation with these techniques, temper the outcomes of these analysis. In gene-expression studies, for example, the detected MMP-transcription levels may not reflect the biological active protein levels. Therefore, while immunohistochemical techniques may provide information on protein-expression levels of active MMPs in the tissues, antibody–antigen cross-reactivity and/or interactions with latent or inhibited enzyme complexes may lead to false positives or exaggerated positive immunostain results.[91] Advances in antibody technology, resulting in the production of highly specific and sensitive antibodies, are however greatly mitigating this challenge.

For the most part, reports tend to suggest that the bulk of MMPs in the tumor environment are produced by the surrounding stroma cells, and tumors may be exposed to circulating MMPs in normal serum.[57] Furthermore, it is difficult to draw assertive conclusions on the significance of positive MMP immunostains based on meta-analysis, because of the heterogeneity of both data collection and statistical methods employed. Standardization of staining procedures and evaluation protocols does help to compare the variability of published data and extract valid results.[126] Nevertheless, the existence of a considerable number of conflicting studies should be acknowledged, as well as the possibility that some studies indicating negative MMP expression in tumors are unlikely to be published.[50]

In spite of these limitations, results of several studies evaluating MMP expression in HNSCCs provide insight into their prognostic importance. The results of MMP gene-expression profiling recently carried out by Iizuka et al suggested that MMP1, -3, -7, -10, -12, and -13 expressions are significant prognostic markers for HNSCC tumorigenicity and malignant progression.[49] Earlier studies by Rosenthal and Matrisian proposed that MMP1, -2, -9, and -14 expression in HNSCC were related to disease progression.[50] Lotfi et al[58] investigated MMP2 and MMP9 serum levels in OSCC patients, and concluded that both were significantly elevated in OSCC patients compared with their healthy counterparts. Significantly, the authors considered MMP2 a better marker for assessing lymph-node metastasis and tumor grade.[58] A meta-analysis of laryngeal cancers demonstrated that MMP2 expression was higher in cases with lymph-node metastasis, and suggested that upregulation of MMP2 could be instrumental in tumorigenesis, progression, and prognosis in laryngeal cancer.[127]

Zhang et al investigated MMP2, -3, and -9 single-nucleotide polymorphisms in esophageal SCC.[128] The authors suggested that *MMP2*$^{-1306TT}$ and *MMP9*$^{-1562CC}$ single-nucleotide polymorphisms correlated with increased esophageal SCC risk and significantly high death odds.[128] In a study by Virós et al,[129] the prognostic value of MMP2- and -9-expression levels in patients with HNSCC following treatment with radiotherapy or chemotherapy was analyzed by multivariate analysis. The authors determined that MMP9 expression was the only significant factor related to adjusted survival.[129] Patients with low and high MMP9-expression levels had 5-year survival rates of 92.9% and 61%, respectively. Significantly, patients with elevated MMP9 expression had a 6.1-fold higher death risk compared with patients with low MMP9 expression. The authors thus suggested that elevated MMP9 is associated with poor local disease control, and that increased MMP9 mRNA levels may represent a treatment-response marker in chemotherapy and/or radiotherapy in HNSCC patients.[129]

In an analysis of histologically negative surgical margins of OSCC, Ogbureke et al suggested MMP9 as a preferred predictor of tumor recurrence at histologically negative resection margins of primary OSCC.[130] The authors recommended a redefinition of true-negative (tumor-free) margins in OSCC to incorporate the MMP9 status of histologically negative margins.[130] It has also been reported that the immunoexpression of VEGFR2 and MMP9 in oral dysplastic lesions correlated

with the degree of dysplasia, and appeared to be higher in more severe dysplasias.[131] Furthermore, Smith et al[132] noted that MMP9 may serve as a marker for increased risk of transition from oral dysplasia to frank OSCC. Similarly, Chang et al[60] proposed MMP9 expression as the most relevant index of progression from leukoplakia to cancer, and suggested that physicians take into account elevated MMP9 levels in deciding treatment modality in any given case and frequency of patient follow-up.

Zhou et al[133] proposed serum autoantibody levels of MMP7 as a diagnostic biomarker for esophageal SCC. MMP7 was also found to mediate metastasis in laryngeal carcinoma.[134] Elevated MMP7 levels have also been associated with negative survival in patients with OSCC.[135] However, in their immunohistochemical analysis of expression levels of MMP7, -8, and -9 in OSCC and cutaneous SCCs, Omar et al[54] did not find any association of MMP levels with overall survival rates.

Mäkinen et al[136] investigated the potential prognostic values of MMP2, -8, -9, and -13 in OSCC of the tongue. MMP8 levels, though elevated and previously suggested to play a protective role in tongue OSCC,[137] did not correlate with protection from tongue OSCC.[136] Similarly, there was no correlation between MMP2 and -9 levels with known clinicopathologic and/or prognostic variables.[136] On the other hand, the authors showed that high nuclear MMP13 expression was associated with increased invasion depth, tumor size, and poor survival in tongue OSCC.[136] The authors thus suggested that elevated serum levels of MMP13 may serve as a tumor biomarker in HNSCC.[136] In contrast, MMP13 was not shown to correlate with tumor recurrence in HNSCC cases.[138]

In summary, there is accumulating evidence that specific MMPs can serve as potential prognostic markers in HNSCC. However, conflicting data and results attributable to finesse and other variabilities associated with experimental procedures employed and statistical analytic methods remain significant confounding factors in the interpretation of results. In addition, HNSCCs are a notoriously heterogeneous population, wherein tumor behavior is intricately related to specific sites as much as it is related to biologic molecules (such as MMPs) that may be expressed at that site.

Therapeutic approaches in targeting MMPs in HNSCC

With increasing understanding of the biology of MMPs, therapeutic targeting of MMPs remains a very attractive strategy for the treatment of many cancer types, including HNSCCs. Although early concepts and designs of biomimetic targeting of MMPs in cancers held lofty promise, disappointment with patient outcomes following clinical trial treatments soon followed.[38,91] The failure (or modest success) of early anti-MMP drugs for cancers is at least in part attributable to hitherto-limited understanding of the structural diversity of MMPs and the significance of this diversity to the working of different MMPs. As summarized in Figure 4, anti-MMP drug-design strategies largely aim for interference with either catalytic or noncatalytic MMP activity.

Targeting MMP catalytic activity

Early drug research on MMP inhibitors (MMPIs) targeted binding to the MMP catalytic domain.[38] MMPIs used in head and neck cancer treatment have been formulated as peptidomimetics (batimastat, marimastat), nonpeptidic MMPIs (prinomastat, tanomastat, rebimastat), natural MMPIs (silibinin, Neovastat), tetracycline derivatives, and bisphosphonates.[74,139,140] Marimastat is a widely tested MMPI and the first orally bioavailable MMPI for clinical trials. The effects of marimastat were based on its ability to inhibit the release of major C-*ERBB* ligands, such as TGF, β-cellulin, and heregulin.[74]

Nafamostat mesylate (FUT175), a serine protease inhibitor, has been shown to perturb MMP2 and MMP9 activity through the downregulation of TGFβ in HNSCC.[141] Treatment with α-mangostin in vitro has been shown to decrease MMP2 and MMP9 expression and to inhibit HNSCC growth in a concentration-dependent manner, possibly through a JNK and ERK1/2 signaling pathway.[142,143] Also, proteasome inhibitors, including ALLN and lactacystin, caused suppression of TNFα-induced migration of OSCC cells, via interruption of

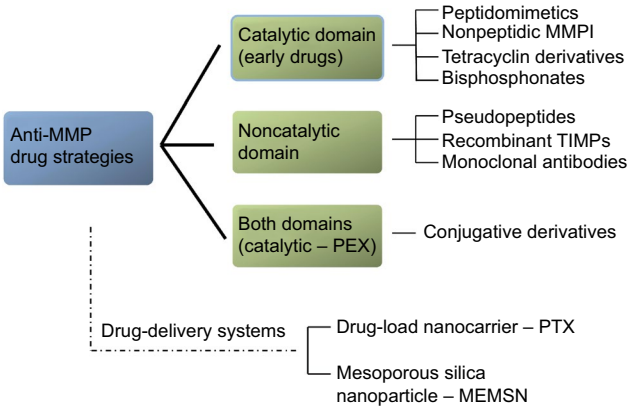

Figure 4 Schematic of drug-therapy strategies against MMPs.
Notes: Anti-MMP drug-design strategies target either catalytic or noncatalytic MMP activity.
Abbreviations: MMP, metalloproteinase; MMPI, MMP inhibitor; PTX, paclitaxel; MEMSN, multifunctional envelope-type mesoporous silica nanoparticle.

NFκB activation and MMP9 production.[144] Tranexamic acid and SLPI have been evaluated for their potential to downregulate MMP activity by inhibiting plasminogen activation.[145,146]

Other design strategies targeting the MMP catalytic domain

Following disappointments with treatment outcomes with earlier anti-MMP regimes, design strategies soon shifted to more sophisticated, selective inhibitors, with fewer side effects. One such strategy focused on designing small-molecule inhibitors that fit in size and shape with the variable S1′ deep cavity of the catalytic domain as an alternative approach to disrupting the strong catalytic zinc-binding activity using MMP12-inhibition models.[147,148] Pseudopeptides with the general formula X-l-Glu-NH_2 that affected zinc ion binding were recognized as MMP12 inhibitors.[149]

More recently, two monoclonal antibodies have been designed to inhibit substrate activation by binding to the catalytic domain without affecting the catalytic zinc region.[38] The first, DX2400, is a specific inhibitor of MMP14, known to activate pro-MMP2, and promotes angiogenesis, cell invasion, and metastasis in breast cancer cells.[150] The second antibody, REGA3G12, a murine monoclonal antibody, is designed to inhibit the human MMP9 catalytic domain secreted by neutrophils.[151] Alternatives to synthetic antibodies have been proposed, namely natural human TIMPs. Paradoxically, TIMP expression is generally increased in OSCC, and is associated with increased metastatic risk and tumor-cell migration.[152,153] However, recombinant TIMPs are novel promising variants, such as that developed for selective inhibition of MMP14 resulting in decreased MT1-MMP activity and CD44 shedding in breast cancer and fibrosarcoma cells.[154]

Currently, doxycycline hyclate (Periostat; Galderma Laboratories LP, Fort Worth, TX, USA) is the only MMPI approved by the US Food and Drug Administration, and is used for the treatment of periodontal diseases. At the moment, it is not clear whether or not doxycycline, an MMP1 inhibitor, possesses chelating activity that targets the catalytic domain or whether it targets the hemopexin-like domain.[50,155,156] With respect to MMP7, doxycycline is considered to act on hydrophobic tryptophan residues of the catalytic domain proximal to the zinc ion.[157]

Targeting the MMP noncatalytic domain

Recent recognition of the MMP hemopexin (noncatalytic) region, providing new insights into the functional role and structural homology of MMPs, have spurred a fresh quest for new inhibitors targeting the hemopexin domain. In both in vitro and in vivo systems, designed small molecules and peptides exert their inhibitory effects by preventing dimerization, thereby reducing tumor size, MMP-induced migration, and angiogenesis.[158–160] Promising studies have shown that these selective compounds could bind on specific sites for each MMP inside their hemopexin domain and prevent dimer-induced functions of several MMPs, including MMP14 and -9.[158–162]

A more sophisticated approach proposed a bifunctional fusion protein able to bind and inactivate both the catalytic and hemopexin domains of MMP2.[163] This macromolecular protein was made by conjugation of an MMP2-selective inhibitory peptide (APP-IP) to the N-terminus of TIMP2.[163] Double binding of this recombinant protein inhibited activation of pro-MMP2 and reduced the degradation of type IV collagen and the migration potential of human fibrosarcoma cells.[163]

Given the desire to target cancer cells specifically with any therapeutic regimen, attention is also being focused on drug-delivery systems for anti-MMP drugs. Liposomes are small vesicles able to conjugate with several compounds, including selective markers or peptides, and transfer them intracellularly. Zhu et al[164] developed such a drug-load nanocarrier containing paclitaxel (PTX) (conjugate/prodrug, $PEG_{2,000}$ peptide–PTX) and tested its activity against MMP2. The results were very promising, as the investigators reported high cellular uptake and antitumor efficacy combined with low side toxicity in a non-small-cell lung cancer mouse xenograft model.[164] Similarly, a multifunctional envelope-type mesoporous silica nanoparticle was developed for selective intracellular drug delivery, containing an RGD motif and an MMP-substrate peptide, Pro-Leu-Gly-Val-Arg.[165] Treatment of SCC7, HT29, and 293T cells with this drug-delivery system resulted in increased cell death, with low cytotoxic effects.[165]

Taken together, MMPs remain hopeful targets for anticancer therapy in various types of solid and hematological malignancies, in spite of disappointments with earlier regimens.[166] Therefore, ongoing research continues to explore modern therapeutic approaches combining small molecules and macromolecular inhibitors with novel drug-delivery systems in HNSCCs.

Conclusion

The prognosis of patients with HNSCC remains dismal, in spite of continuing advances in various modes of therapy. Surgery remains the mainstay of treatment of head and neck cancers. Particularly disappointing is the slow pace in discovering drugs that make for an effective chemothera-

peutic approach to the treatment of head and neck cancers. The discovery of such drugs will obviate the morbidity and mortality often associated with extensive cancer surgery of the head and neck.

MMPs have well-established complex and key roles in HNSCC. Specific MMPs, including MMP1, -2, -3, -7, -8, -9, -10, -11, -13, and -14, show aberrant expression in cancer tissues, and stand as potential diagnostic and prognostic biomarkers. However, conflicting data and results attributable to finesse and other variabilities associated with experimental procedures and statistical analytic methods confound the results. The heterogeneity of HNSCCs related to specific sites further complicates the interpretation of the biologic significance of expressed molecules, such as MMPs.

Earlier anti-MMP drug designs focused on the catalytic role of MMPs ostensibly responsible for matrix remodeling, angiogenesis, and cancer invasion. However, it is now well known that the structure of MMP-family members includes the hitherto less-emphasized hemopexin-like C-terminal domain, which mediates proteolysis-independent MMP activities in several important physiological and pathological processes. This latter domain now presents itself as an exciting new frontier for MMP cancer research. The noncatalytic functions of MMPs commonly found in head and neck cancers are now well elucidated, as in other system malignancies. This provides the justification to intensify research on potential anti-MMP biomimetic-based targeting of MMP noncatalytic domains. Therefore, MMPs remain a viable target for HNSCC therapy, with the added opportunity to enhance the concept of the personalized medicine approach for effective treatment of HNSCC patients.

Disclosure

The authors report no conflicts of interest in this work.

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