ROLE OF METALLOPROTEINS IN THE CLINICAL MANAGEMENT OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Abstract: Metalloproteins are a group of catalytic proteins, which play significant roles in cell cycle and death. Matrix metalloproteinases (MMPs) are a family of endopeptidases that are capable of digesting extracellular matrix components. They have been implicated in carcinogenesis and recent developments have been made to use MMPs clinically to predict outcomes. In the future, selective inhibition of these proteins and their regulatory pathways may prove useful in anticancer therapeutics. We present a review article on the clinical applications of metalloproteins in head and neck squamous cell carcinoma (HNSCC). Metalopanstimulin is highlighted as a putative metalloprotein of interest for those treating HNSCC. Expression of particular metalloproteins has correlation with lymph node metastasis, tumor invasiveness, and overall prognosis in HNSCC.

Keywords: metalloproteins; metalomatrix proteins; MMP; metalloproteases; matrixins; metalopanstimulin; MPS; inhibitors; head and neck cancer

One third of all proteins are “metalloproteins,” chemical combinations of protein atoms (carbon, nitrogen, oxygen, hydrogen, sulfur) with ions of alkaline earth metals such as iron, calcium, copper, and zinc. The number of peptides reported as metalloproteins continues to grow. They are an attractive subject to study for discovering putative control mechanisms in uncontrolled cell division (oncogenesis), tissue invasion, metastasis, or cell death (apoptosis). All metalloproteins have in common stability and activity when containing a metal cation at its core, highly conserved linker regions between cation domains, and some function they perform. Some have hypothesized that the conserved sequences may be common among many gene control proteins.

Examples of human metalloproteins include metalopanstimulin (MPS) and matrix metalloproteinases (MMPs or matrixins). MPS has been shown to be an important biomarker for Head and neck squamous cell carcinoma (HNSCC) as it is found in significantly increased quantities in the sera of patients with new or recurrent HNSCC.1–4 MPS is a DNA repair protein that has antiapoptotic activity. It is expressed in benign and malignant tis-
sues throughout the human body, embryogenesis, and is phylogenetically conserved. MMPs are a family of secreted zinc-dependent endopeptidases that are capable of digesting extracellular matrix (ECM) and basement membrane components. These ubiquitous proteins play roles in many major physiological processes like embryonic development, morphogenesis, reproduction, and tissue resorption and remodeling. Matrixins also play significant roles in pathological processes such as arthritis, cancer, cardiovascular disease, nephritis, neurological disease, and fibrotic lung disease. Recently, these proteins have been discovered to play a significant role in metastasis. In the process of carcinogenesis, MMPs enable local tumor invasion and the spread of tumor to adjacent and distant tissues. Although MMP’s and MPS have primarily distinct functions, their similarities as catalytic metalloproteins justifies their being treated in a single article, and they will be discussed together since they are both metalloproteins and may have other similarities in structure and function that are yet to be appreciated.

METALLOPANSTIMULIN

MPS is homologous to the rat S27 ribosomal protein. It is a 9.5-kD, 84 amino acid peptide, ribosomal subunit zinc finger protein that is present in all tissues and expressed in large quantities in a wide spectrum of proliferating tissues and oncogenic processes. MPS can also be elevated in pregnancy and chronic medical conditions such as cirrhosis. When MPS is over-expressed, it is released down a concentration gradient from the intracellular space into the extracellular space.

Conventionally, ribosomal proteins are thought to be confined in their function to intracellular protein synthesis. Many recent reports have drawn attention to “extraribosomal functions” of ribosomal proteins. Moreover, these extraribosomal functions have been observed in relation to oncogenesis in various models. The zinc finger motif of MPS-1, and other ribosomal proteins, allow binding to nucleic acids, which interferes with transcription and translation. This is a relatively new area of investigation, and all of the biologic functions of these proteins are not completely reported. Some functions known at present include: (1) DNA repair, (2) gene suppression, (3) cell-cycle control (G1 arrest), or (4) control of oncogenesis. For example, the ribosomal protein S27a, is ubiquitinilated and overexpressed in human colon cancer. Like MPS-1, it is involved in cell-cycle control and DNA replication.

MATRIX METALLOPROTEINASE

The first MMP activity discovered was a collagenase in the tail of a tadpole undergoing metamorphosis. Currently 24 different matrixins have been discovered, and 23 of these are seen in humans (Table 1), again, an example of phylogenetic conservation.

Researchers initially believed that the major role of MMPs in cancer revolved around their promotion of metastasis, the breakdown of physical barriers like the extracellular matrix (ECM) and basement membrane, thus promoting invasion. However, it is now also believed that MMPs are key regulators of both primary and metastatic tumor growth. The ECM is not just a passive scaffold for cells; it also acts as a reservoir for many growth factors and antigen presenting. An article by Visse and Nagase illustrates the wide variety of biological activities generated by MMP-mediated cleavage. Clinicians are finding that the measurement of MMPs may have clinical applications. Correlations have been found between MMP expression and various indicators of a poor prognosis in virtually all types of cancer, and in some instances, increased MMP levels represent an independent predictor of shortened disease-

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Abbreviations: MMP, matrix metalloproteinases; MT, membrane type; x, xenopus; c, chicken.
free and overall survival. Specifically, MMP expression has proven to correlate with outcomes in melanoma, breast, lung, and gastric cancers. In addition to their role in diagnosis and prognosis, MMPs are also being applied to the development of new anticancer therapeutics.

Several references describe the relationship of MMP expression with measurable clinical outcomes such as lymph node metastasis, tumor invasion, patient survival, prognosis, diagnosis, and treatment10 (Table 2). We have divided our review of this topic into these same categories.

**LYMPH NODE METASTASIS**

Because of the rich supply of lymphatics in the head and neck and the aggressive nature of squamous cell carcinoma, these tumors are characterized by a high rate of regional metastasis. Lymph node metastasis in HNSCC has long been felt to be the strongest predictor for poor prognosis in patients with this disease. Unfortunately, it has been difficult to predict which patients with HNSCC will develop metastases prospectively. Measures such as tumor size, tumor thickness, and stage are used to predict risk for lymph node metastasis. These are known to be imperfect. Markers for metastasis would assist in knowing which tumors might behave more aggressively, perhaps even antecedent to metastasis.

Studies using cancer cell lines have shown promotion of metastasis by excessive expression of MMPs, while inhibitors of MMPs were found to inhibit metastasis. Measurement of MMP expressions could be a useful way to help predict lymph node metastasis.

Kusakawa et al began suggesting that MMPs may predict lymph node metastases. In a study of 46 patients with oral squamous cell cancer who had undergone neck dissections, the authors measured and compared immunohistochemical levels of MMP-2 expression in the tumors of patients with neck disease versus the levels of MMP-2 expression in the tumors of patients without neck disease. In 20 of 26 patients (76.9%) with lymph node metastases, MMP-2 was strongly expressed, whereas the production of MMP-2 in tissue was detected in only 5 of 20 patients (25%) who had no lymph node metastases (p < .001). Others have also found that MMP-2 in particular is related to lymph node metastasis. Kawata et al investigated MMP-2 concentrations in 21 cases with head and neck carcinomas and 6 cases with normal mucosa. MMP-2 concentrations did not differ between normal mucosa and tumor tissue without lymph node metastases. MMP-2 concentrations in tumor tissue with lymph node metastases were higher than that in cases without lymph node metastasis (p < .05). In 50 patients with laryngeal carcinoma, Krecicki et al determined a correlation existed between MMP-2 expression and lymph node metastasis, yet these findings were not statistically significant (p = .07). Taken in aggregate, MMP-2 appears to be a predictor of metastasis in HNSCC.

Membrane type (MT)1-MMP, which is a known activator of MMP-2, is 1 matrixin that may be predictive of metastasis. Imanishi’s performed a multivariate logistic regression analysis which confirms that MT1-MMP significantly correlates with lymph node metastasis independent of any other variable (MT1 mRNA expression, p = .0081; MT1 immunostaining, p = .0193). The expression of other MT-MMPs studied, MT2- and MT3-MMP, were almost negligible. In addition to their work with MMP-2, Kusukawa et al have also shown that MMP-3 expression can be correlated with lymph node metastasis though their study was small and not statistically significant.

In a recent meta-analysis of the significance of MMPs for lymph node disease in patients with HNSCC, Wiegand et al have called for the standardization of staining procedures and evaluation protocols to allow for valid comparisons of results published by different study groups. They argue that the literature on this subject is difficult to interpret because it is difficult to compare. They found the literature difficult to compare because much of it is missing data on precise clinical and pathologic lymph node status. Furthermore,
much of the literature lacks analysis of MMP expression versus negative controls. Despite dissatisfaction with the heterogeneity of studies, the authors were able to reach certain conclusions through their meta-analysis. Expression of MMP-2, -3, -14 may infer an increased likelihood of developing a lymph node metastasis; the value of expression of MMP-1, -9 for predicting lymph node metastasis remains unclear.

In conclusion, many reports now exist that suggest that MMPs may help predict lymph node metastasis. Because clinical staging and imaging remain relatively poor, predictors of early lymph node metastasis in HNSCC, MMPs theoretically could improve precision of prediction of metastasis, prognosis, and deciding the utility of elective neck dissection.17

**TUMOR INVASION**

MMPs enhance tumor cell invasion. Specifically, Rosenthal et al25 demonstrated that HNSCC tumor cells transfected with expression vectors for MT1-MMP and MMP-2 genes were found to invade in the absence of growth factor stimulation. These results suggest that the MT1-MMP/MMP-2 protease system participates in squamous cell carcinoma (SCC) invasion of collagenous matrices.25 These and other in vitro studies26–29 have laid the groundwork for subsequent clinical research demonstrating the high expression of certain MMPs in locally aggressive HNSCC tumors.

MMP-13, or collagenase-3, degrades the native helix of fibrillar collagens and degrades other parts of the ECM (basement membrane, type IV collagen, and fibronectin).30 Cazorla et al31 have suggested that the expression of MMP-13 not only correlates with the grade of a HNSCC tumor, but also is predominantly expressed in locally advanced carcinomas. Not surprisingly, these authors also found that other enzymes such as membrane-type 1 MMP (MT1-MMP) and gelatinase A (MMP-2) correlated with MMP-13 expression.

Other MMPs have also been implicated in tumor invasiveness. Kurahara et al32 have shown a decrease in the staining of ECMs in cases with an increased expression of MMP-1, -2, and -9. MMP-3 expression was positively correlated with tumor size, depth of tumor invasion, and diffuse invasive mode according to Kusukawa et al.23 In their work, MMP-3-containing tumors were shown to invade adjacent normal tissues more aggressively, including lymphatics and blood vessels. They proposed, therefore, that the immunohistochemical examination of MMP-3 expression in biopsy specimens could provide information useful in predicting the malignant potential of early SCC of the oral cavity.33

Though not directly related to clinical outcomes, some scientists have compared MMP expression to other useful cancer predictors. This study documents the existence of a correlation between MMP-9 expression, activity of the inducible nitric oxide synthase pathway, p53 status, and angiogenesis in patients with HNSCC. This raises the possibility that the p53 mutation, which is frequently seen in HNSCC, may result in increased angiogenesis and invasiveness related to increased nitric oxide and MMP production by tumor cells, ultimately contributing to tumor progression and therefore a poorer prognosis and survival.6

**SURVIVAL AND PROGNOSIS**

Though the best predictor of prognosis in patients with HNSCC has historically been TNM staging, outcome may vary greatly within each stage group. Because of the unpredictable nature of these tumors, a molecular marker for more aggressive tumors would not only help with more accurate prognosis, but may also help determine the need for more aggressive therapy in particular patients. Recently many studies have shown that certain MMPs, as measured either by tissue immunohistochemistry or in serum, may have implications regarding prognosis.

In 2004, Ruokolainen et al33 published the first study with a long-term follow-up showing that a MMP in head and neck carcinoma is associated with shortened relapse-free and cause-specific survival. In this study, the authors measured tissue MMP-9 by immunohistochemistry. In a subsequent study, the same authors demonstrated that serum levels of MMP-9 carry implications of poor prognosis. Specifically, pretreatment serum levels of MMP-9 were quantitatively measured by enzyme linked immunosorbent assay (ELISA) in 67 patients seen with a primary HNSCC. The 5-year cause-specific survival rate was 40% in a patient group with high serum MMP-9, and 69% for patients with a low MMP-9 level \((p = .027)\). The cumulative relapse-free survival rate was 36% in patients seen with a high serum MMP-9 and 66% in those with a low MMP-9 level \((p = .074)\).34

In a separate work by these same authors, tissue inhibitor matrix protein (TIMP)-1 has been
demonstrated to have the same effects on cause-specific survival and relapse-free survival. The 5-year cause-specific survival was significantly lower in patients seen with a high serum TIMP-1 level than in those with a low level of TIMP-1 (38% vs 64%, p = .034). They also had an unfavorable 5-year relapse-free survival rate (37% vs 56%, p = .035).37

Yoshizaki et al36 reported that MMP-2 is also a predictor of poor prognosis for patients with HNSCC. In fact, they conclude that not only MMP-2 itself, but also MT1-MMP, a known activator of MMP-2, and TIMP-2, a supposed inhibitor of MMP-2, all contribute to tumor metastasis and correlate with poor prognosis. In a later review article, the authors describe the interactions between TIMP-2, MT1-MMP, and MMP-2 and the clinical implications that these different proteins may have.37

Finally, correlations have been made between MMP levels and tumor chemosensitivity. Blons et al38 identified that a relation exists between MMP-3 and response to chemotherapy. Indeed, patients with a particular MMP-3 genotype responded more frequently (86%) to treatment as compared with patients with alternative genotypes (65% and 55%, p = .04). This showed that MMP-3 genotyping might predict chemosensitivity in HNSCC patients.

As an indication that not all MMP studies yield positive results, Netterville and coworkers39 published their results from a study of 21 patients with HNSCC in which they were unable to correlate expression of 1 or a combination of MMPs with tumor behavior or clinical staging. There certainly remains considerable controversy as to the value of these proteins with regard to clinical oncology.40–43 MMPs are considered putative markers for HNSCC as measured by either tissue immunohistochemistry or in the serum.33,34 Further clinical trials will hopefully identify which MMPs, and in what setting, are clinically useful.

**DIAGNOSIS AND SURVEILLANCE**

Diagnosis of the first HNSCC is usually prompted by symptoms or a mass and seldom would a biomarker be critical in making the initial diagnosis. Having a value for a reliable biomarker at this point in the patient’s medical history would serve as a positive baseline from which to compare results during and after treatment completion and during the cancer surveillance period. MPS may offer the clinician these and other diagnostic amenities.

There are many examples of biomarkers that have been shown to have a high fidelity to certain diseases or types of malignancy. The best of these markers have been incorporated into care guidelines and routine clinical practice. Excellent examples include prostate specific antigen, thyroglobulin, and calcitonin, among others. Many markers have been reported to be associated with HNSCC, the most frequent being cited are p53, epidermal growth factor receptor (EGFR), transforming growth factor α, and cyclin D among others.44,45 These markers, however, have yet to emerge as single markers, or even in a panel of markers, used by head and neck practitioners to manage HNSCC. Most of these markers are still undergoing translational investigations and may yet impact upon clinical practice. When a reliable single or panel of HNSCC biomarkers become available and proven for routine clinical use, potential applications might include: general population screening, high-risk (alcohol and tobacco abusers) screening, diagnostic testing, measurement of therapeutic effect, risk assessment, prognostication, proxies for more expensive diagnostic modalities, and/or surveillance.46

Matrix metalloproteins have been studied as predictive biomarkers for HNSCC. Serum levels of MMP-9,34 MMP-8,47,48 MMPs-3 and 948 have been shown to correlate to HNSCC with respect to prognosis, stage, and cancer versus control, respectively. Yorioka showed increased MMP-2 and 9 in the medial of oral HNSCC tumor explants grown in culture.49 Reidel reported increased MMP-9 but not MMP-2 in HNSCC versus control serum samples.50 Kawata reported no correlation between serum levels of MMP-2 and 9 and lymph node metastases.17 Ruokolainen demonstrated an immunohistochemical association, which did not translate into a serologic correlation of HNSCC clinical behavior (as defined by nodal and distant metastases) for MMP-2, TIMP-2, and MMP-2:TIMP-2 complex.51

The physiology of MPS-1 expression and our initial experience with this protein in HNSCC has led us to conclude that MPS-1 and MPS-1-like metalloproteins may be useful markers in the effort to screen for and analyze the extent of HNSCC. Our experience with MPS in this regard will serve to review the potential use of biomarkers for HNSCC in general and specifically highlight how a specific metalloprotein may fill this role.

**Metallopanstimulin Radioimmunoassay.** Although labor intensive and largely abandoned for ELISA tests, radioimmunoassay (RIAs) were used to
develop many biomarkers. The RIA heat treated metallopanstimulin (MPS-H) levels from a HNSCC group were compared to a control group of healthy volunteers and to a control group of smokers who volunteered for screening for head and neck cancer (Figure 1). Mean MPS-H was 10.2 ng/mL for the healthy control group and 12.8 ng/mL for the smoking control group. Mean MPS-H for the HNSCC group was 41.5 ng/mL. This was significantly higher than both control groups ($p < .0001$). Those successfully treated and clinically free of HNSCC had consistently lower MPS-H levels over the first year than patients living with clinically proven head and neck cancer. The receiver operating characteristic (ROC) curve for the MPS-H levels (Figure 2), a reflection of its ability to detect cancer, shows that the area under the ROC curve is 0.73 (95% CI: 0.71–0.75; $p = .001$). We have observed numerous instances where elevated MPS-H levels in patients seen with head and neck neoplasms dropped to normal levels following successful therapy. We have also noted examples of persistent elevations or increases in MPS-H levels in patients with failure to respond to therapy or recurrence of tumor, respectively. RIA observations have confirmed by other antibody based technologies. Further testing on larger populations will be required to obtain normative MPS levels which could serve as a standard for diagnosis of with or without disease status.

Metallopanstimulin and Advanced Proteomics. Biomarker development has been advanced greatly with advanced proteomic tools such as mass spectroscopy. These tools represent an advance in new biomarker discovery, precision measurement, and increased sensitivity to nano- and picogram quantities of putative markers. Counterintuitively, proteomic advanced instrumentation, which remains economically prohibitive for routine clinical use, has also provided a disincentive for conventional antibody based marker research utilizing established laboratory platforms. This is perhaps a result of anticipation that mass spectroscopy will supplant ELISA for clinical use.

A peak with an average mass of 10,068 Da was consistently identified in many of the serum samples by surface enhanced laser desorption ionization (SELDI, ciphergen, www.ciphergen.com). Figure 3 represents combined spectra of representative samples from each group (normal controls, HNSCC cases, and smoker controls). Common detection of the 10,068-Da peak in the HNSCC group compared with its relative absence in the normal controls and in the smoker control group is clearly shown.

Compared with the MPS-1 pooled serum samples (positive controls), the 10,068-Da peak in HNSCC sera was, by mass, consistent with the same peak in the pooled sera. Furthermore, the MPS-1 pooled sera peak at 10,068 Da was graded in relative intensity proportional to the relative level of MPS-1 present detected by RIA (Figure 3). Further provisional identification of this known peak with an average mass of 10,068 Da was consistently identified in many of the serum samples by surface enhanced laser desorption ionization (SELDI, ciphergen, www.ciphergen.com). Figure 3 represents combined spectra of representative samples from each group (normal controls, HNSCC cases, and smoker controls). Common detection of the 10,068-Da peak in the HNSCC group compared with its relative absence in the normal controls and in the smoker control group is clearly shown.

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tumor marker was performed using a SELDI based immunoassay. The MPS-1 peak seen in sera using the IMAC3 ProteinChip was detected at the same mass using the anti-MPS-1 IgG bound to an immunochip. No 10,068-Da peak was detected when normal rabbit IgG replaced the anti-MPS-1 IgG. These data provide further indirect evidence to indicate that the peak seen at 10,068 Da is MPS-1. To our knowledge, this was the first report of identification of a known tumor marker in HNSCC using SELDI technology.

Metallopanstimulin as a Proxy for Positron Emission Tomography? Positron emission tomography (PET) scan has revolutionized HNSCC diagnosis and surveillance. It is an expensive modality that requires fasting and 4 hours of patient time. It is an attractive proposition to examine biomarkers...
FDG-PET and clinical assessment

The biggest shortcoming of the MPS versus PET or clinical assessment is the possibility that the patients may have had an early recurrence detectable by MPS-H but not yet by FDG-PET. The 68 FDG-PET positive cases that showed low MPS-H levels suggest that the tumors were unable to produce high levels of MPS-H, perhaps due to cellular changes within the tumor from previous chemotherapy or radiation. Or these were perhaps false positive PET scans.

Table 3 reports results tests of agreement between MPS-H, FDG-PET, and clinical assessment. As seen in Table 3, assuming MPS-H ≥ 35 as the threshold for a positive test yielded a \( \kappa \)-coefficient of .395, which suggests “slight” correlation after accounting for chance agreement. However, McNemar’s test suggested that there was systematic disagreement between FDG-PET and MPS-H (p < .0001). This could be from the high number of false-negative MPS-H results. To mitigate this effect, we also studied a strategy that viewed MPS-H levels between 20 and 35 as inconclusive. This strategy resulted in a \( \kappa \)-coefficient of .318, suggesting “fair” agreement between FDG-PET and MPS, but did not reduce the proportion of false negative MPS-H results. MPS-H was more highly correlated with findings from routine office examinations. As seen in Table 3, the \( \kappa \) coefficients were .347 for the 35 ng/mL threshold and .404 for the 20/35 ng/mL threshold, suggesting fair agreement between MPS-H and serial clinical exams.

McNemar’s test suggested also significant systematic disagreement between MPS-H and results from physical examinations (p < .0001).

Since these data are not robust, it is not suggested that MPS is ready to be a surrogate for PET scanning. This work, however, raises intriguing possibilities of biomarkers substitution for other conventional testing in the future.

**FUTURE IDEAS: METALLOPROTEIN INHIBITORS**

Because scientific evaluation of the structure and function of MMPs has shown their critical role in...
the processes of tumor invasion and metastasis, the obvious next step has been to attempt to discover how to block these pathways of invasion and metastasis. Attempts have been made to apply MMP inhibitors (MMP-I) to chronic obstructive pulmonary disease (COPD), rheumatic diseases, anticancer therapeutics, and myocardial remodeling to name only a few.61–64 Below, we review some of the initial studies attempting to inhibit MMP action in HNSCC.

In a study involving SCC lines overexpressing epidermal growth factor, O-Charoenrat et al64 found that the MMP inhibitor marimastat (BB-2516) could prevent tumor progression not only by inhibiting invasion and angiogenesis, but also by its ability to inhibit autocrine signaling. The authors recommended clinical trials to test this hypothesis. In a second study involving marimastat, nude mice implanted with an oral squamous cell carcinoma line were given increasing doses of this nonspecific MMP inhibitor. Cervical lymph node status was then microscopically examined and activation of MMP-2 in the primary oral tumor was examined by gelatin zymography. Both cervical lymph node metastasis and activation of MMP-2 were significantly suppressed in the group receiving the highest dose of BB-2516. Moreover, the group of mice receiving the highest dose had a significantly better survival than the group receiving the lowest dose of drug. Certainly these animal studies suggest a positive role for marimastat in the inhibition of MMP-2 activation and prevention of cervical lymph node metastasis in oral squamous cell carcinoma.65 MMP-I are also being tested against many other tumors (prostate, lung, pancreas, breast).66–69 Unfortunately, most clinical trials of MMP-I have yielded disappointing results, perhaps due to inappropriate study design or imprecise clinical data collection. Because MMPs are ubiquitous proteins, new drugs must be highly selective for particular protein targets. Positive results have been seen in gastric cancer with marimastat and in Kaposi’s sarcoma with metastat.70

Orally administered metallomatrix inhibitor-166, a newer MMP-I specific for MMP-2 and -9, has shown reduced in vivo tumor growth of HNSCC through inhibition of angiogenesis and induction of apoptosis accompanied by the reduction of MMP production and activity.71 Huang et al72 examined the impact of molecular blockade of the EGFR on the invasive and metastatic capacity of HNSCC using in vitro and in vivo model systems. Additionally, this inhibition was characterized by down-regulation in the expression of matrix metalloproteinase-9.

Detailed structural and functional analyses of MMPs have led to the development of numerous potent synthetic inhibitors of matrixins. Some are in clinical trials to treat patients with cancer, arthritis, periodontal disease, and corneal ulceration.7 There are currently no clinical trials administering matrix metalloproteinase inhibitors for the treatment of HNSCC; however, such trials might begin in the near future. Other novel uses for MMP-based technologies consist of the molecular analysis of surgical margins73 and the development of MMP genes as tumor markers and tumor vaccine/gene therapy candidates.74

Like MMPs, MPS has inhibitors. This class of compounds, derivatives of nicotinic acid, have activity among many cell lines in vitro.75 Fusaric acid (FA), a byproduct of nicotinic acid metabolism in vivo and fusarum fungal degradation of cereal crops, has demonstrated activity against HNSCC.76 FA has cytostatic activity against HNSCC in vitro when administered as a single agent.76 When FA is given in combination with traditional chemotherapy agents in vitro, its effect is synergistic and cytotoxic against HNSCC.77 Similar results have been demonstrated in vivo.78 Additionally, FA has in vivo activity when administered orally.78 Additional studies are ongoing to study enterally administered FA in combination with parenteral taxetere or EGFR blockade in a nude mose model.

CONCLUSION
Metalloproteins are a promising class of molecules for understanding the behavior and diagnosing head and neck squamous malignancy. Ongoing and future research promises to discover improved applications of this class of proteins in both research and clinical applications.

In many studies, MMP expression has not been well correlated with stage, and yet historically, tumor staging has been used to predict likelihood of metastasis and patient prognosis. It should also be recognized that the staging system is imperfect and groups many heterogenous tumors in a limited number of categories.

The following MMPs seem strongly predictive of lymph node metastasis: MMP-2, MMP-3, and MMP-14 (MT1-MMP). MMP-1,-2,-3,-9,-13 and MT1-MMP can be considered biomarkers for locally aggressive HNSCC. Pretreatment serum
levels of both MMP-9 and TIMP-1 could serve as a prognostic factor in HNSCC.\textsuperscript{33–35} MMP-2, MT1-MMP, and TIMP-2 each independently may predict poor prognosis\textsuperscript{36,37,43} by immunohistochemistry. Finally, MMP-3 genotyping could help predict chemosensitivity in HNSCC patients. Potential uses of MMPs include: prediction of lymph node metastases, tumor invasion, response to therapy, and patient prognosis. Furthermore, matrix metalloproteinase inhibitors are being developed as future anticancer therapeutics.

MPS has been studied in a population of HNSCC patients and appears to be a promising marker for presence, absence, or recurrence of the disease. MPS has also been studied as a potential target for novel therapies as it is felt to be an effector molecule in the antiapoptotic behavior of HNSCC and not just a marker of the disease.\textsuperscript{72}

This review suggests that metalloproteins may allow for the development of a reliable screening test for the early detection, surveillance, and diagnosis of HNSCC, as well as the potential identification of targets for novel directed therapies.\textsuperscript{4,44,45}

REFERENCES

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