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Original contribution

Expression and prognostic role of MMP2, MMP9, MMP13, and MMP14 matrix metalloproteinases in sinonasal and oral malignant melanomas

Svetlana Kondratiev MD[a], Douglas R. Gnepp MD[b], Evgeny Yakirevich MD, PhD[b], Edmond Sabo MD[b], Donald J. Annino MD[c], Elie Rebeiz MD[d], Nora V. Laver MD[a,*]

[a]*Department of Pathology, Tufts New England Medical Center, Box 802, Boston, MA 02111, USA*
[b]*Department of Pathology, Rhode Island Hospital, Providence, RI 02903, USA*
[c]*Department of Otolaryngology, Brigham and Women's Hospital, Boston, MA 02115, USA*
[d]*Department of Otolaryngology, Tufts New England Medical Center, Boston, MA 02111, USA*

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Summary Sinonasal and oral malignant melanomas are rare malignancies accounting for less than 2% of all melanomas. Matrix metalloproteinases (MMPs) are proteolytic enzymes required for extracellular matrix degradation in a variety of physiological and pathologic processes including wound healing, embryogenesis, tumor invasion, and metastases. We studied the correlation between expression of MMPs, nucleolar diameter of melanoma cells, different clinical and histologic parameters, and patient's outcome. Seventeen cases of sinonasal and oral malignant melanoma were studied. The expression of MMP2, MMP9, MMP13, and MMP14 was assessed immunohistochemically on paraffinized sections and measured by computer morphometry as well as silver-stained nucleolar diameter. A significant correlation was found between MMP2 and MMP14 expression and patient's outcome. Greater overall survival was seen in patients with average MMP2 expression less than 8000 $\mu m^2/\times 20$ high-power field ($P = .016$). In patients with negative MMP14 staining, survival rate by the end of the follow-up was 38% compared with patients with positive MMP14 staining where survival rate was 0 ($P = .03$). A correlation with age at onset was also found; patients younger than 66 years had better overall survival rates than patients aged 66 years or older ($P = .03$). The maximal nucleolar diameter (MaxND) was another parameter that significantly correlated with clinical outcome. Patients with MaxND of 8 μm or larger showed a significant worse prognosis compared with the group with MaxND less than 8 μm ($P = .0009$). Our pilot study demonstrates that MMP2, MMP14, MMP9, and MaxND might be used as prognostic markers in patients with sinonasal and oral malignant melanoma.

* Corresponding author.
E-mail address: nlaver@tufts-nemc.org (N. V. Laver).

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1. Introduction

Primary mucosal malignant melanomas (pMMM) of the upper aerodigestive tract including the sinonasal and oral locations are rare malignancies representing less than 2% of all malignant melanomas. PMMM may develop in any upper respiratory tract site, but are most commonly seen in the nasal cavity and paranasal sinuses; the nasopharynx, oral cavity, larynx, and middle ear are less common sites of origin. Irrespective of their initial location, pMMM as a group represent aggressive and highly lethal tumors with 5-year survival rates in the range of 6% to 17% [1]. Radical surgical excision is the treatment of choice. Adjuvant radiotherapy and chemotherapy are of questionable value in the management of pMMM [2]. Local recurrences (40%-85%) and distant metastasis (30%-70%), rather than regional lymph node metastasis (10%-30%), are responsible for most treatment failures and deaths [3]. There are no well-documented histopathologic features that correlate with tumor aggressiveness and patient prognosis. The mean longest nucleoli diameter measured on silver-stained sections in uveal melanoma cases was determined to be a prognostic factor for survival [4], but no such studies have been performed on sinonasal and oral malignant melanomas (SOMM).

Matrix metalloproteinases (MMPs) are a large group of secreted proteinases that are involved in normal physiological and pathologic processes such as embryogenesis, wound healing, angiogenesis, tissue remodeling, tumor invasion, and metastasis [5]. Currently, at least 26 human MMPs have been identified [6]. Type IV collagenases, MMP2 and MMP9, are the largest members of this gene family and are believed to play an important role in skin and uveal melanoma progression [7-10]. MMP13 (or collagenase-3) has been reported in aggressive and invasive cancers, such as squamous cell carcinoma of the head and neck and malignant melanoma [11-13]. MMP14 has been detected in the tumor cells and in the stromal cells adjacent to the invading tumor in a variety of malignant neoplasms such as breast cancer and malignant melanomas [10,14]. The expression and impact of MMPs on prognosis in SOMM has not been addressed. The primary objective of this pilot study was to determine the expression of MMP2, MMP9, MMP13, and MMP14 in SOMM and to evaluate whether their presence correlates with patient outcome along with assessment of nucleolar size as possible new prognostic factors.

2. Materials and methods

2.1. Patient selection

Seventeen formalin-fixed, paraffin-embedded tissue samples from patients with SOMM were obtained from the archives of the Tufts New England Medical Center, Rhode Island, and Miriam Hospitals diagnosed between 1991 and 2004. Institutional review board approval was obtained for this study. The location of SOMM included 6 cases from the nasal cavity, 3 from the paranasal sinuses, 3 from the sinonasal site, and 5 from the oral cavity. None of the patients had undergone radiation, chemotherapy, or immunotherapy before surgery. Cell type, necrosis, mitotic figures, vascular pattern, and inflammation were reviewed histopathologically on routine hematoxylin and eosin–stained slides. Clinical data records, including age, sex, treatment, and follow-up, were available in all cases.

2.2. Immunohistochemical staining

Immunohistochemical staining for mouse monoclonal anti-MMP2 (R&D System, Inc, Minneapolis, MN), anti-MMP9 (Chemicon International, Inc, Temecula, CA), anti-MMP13, and anti-MMP14 (Oncogene, San Diego, CA) was performed. Formalin-fixed, paraffin-embedded tissue blocks were cut at 5-μm intervals, deparaffinized, and dehydrated. Immunohistochemical staining was carried out with the Ventana Benchmark automated staining system with the Enhanced V-Red detection kit (Ventana Medical System, Tucson, AZ). Appropriate positive and negative controls were used. The binding was visualized as a red pigment.

Immunohistochemical staining for nucleoli was performed using the colloidal silver nitrate stain for nucleolar organizing regions (AgNOR). Unstained slides were heated in a 60°C oven for 20 minutes to promote adhesion; the sections were deparaffinized and bleached. One step of AgNOR4 staining was performed as described in previous publications [4].

2.3. Quantification of MMP expression

The level of MMP expression was measured quantitatively by computer morphometry using a computerized image analysis system composed of a Q-imaging Micropublisher 5.0 color digital camera (Sony, Tokyo, Japan) installed on an Olympus VANOX-T light microscope and attached to an IBM-compatible personal computer (Pentium V, PowerSpec). Histologic images were captured, digitized, and displayed on a high-resolution color 22-in monitor. Histomorphometric measurements were performed with the Olympus Micro Suite software (Olympus America, Inc, Center Valley, PA). Five microscopic fields (×20), representing the most intensive MMP staining, were selected for each case. The results were expressed as average area of MMP staining (Ave) and maximal area of MMP staining (Max) per 5 microscopic fields. The same technique was used on an assessment of nucleoli colloidal silver nitrate staining. Sixteen of 17 cases were available for nucleoli size measurement; 1 case did not have enough tissue for staining. Five representative microscopic fields (×40) including at least 50 nucleoli per case were chosen for evaluation of maximal and mean nucleoli diameter. Areas of necrosis were avoided.