Membrane type -1 matrix metalloproteinase and CD-44 expression in oral cavity carcinomas with micrometastases

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Introduction

The standard of care today for most oral cavity squamous cell carcinomas (SCCA) includes elective neck dissection (END) due to the risk of occult metastasis to regional lymph node basins. Identifying molecular markers in the primary tumor that predict the presence of micrometastatic disease, would avoid unnecessary neck dissections in a number of patients with oral cavity SCCA.

The matrix metalloproteinases (MMP's) are a family of endopeptidases that play a major role in extracellular matrix (ECM) degradation related to cancer cell invasion and metastasis. Membrane type -1 matrix metalloproteinase (MT1-MMP), a 582 amino acid protein with a transmembrane domain, is an important member of this family. It is known to play a central role in ECM degradation through its inherent collagenase activity and by activation of other MMP's. It also cleaves CD44 from the surface of cells, resulting in increased cell migration. For this reason, expression and tissue localization of MT1-MMP and CD44 in early metastatic SCCA is an active area of investigation.

Lymph node metastasis is the only biomarker currently used to guide the selection of neck dissection in patients with clinically node-negative oral cavity tumors. Current methods include analysis of immunohistochemical staining associated with micrometastasis. By studying MT1-MMP and CD-44 expression in oral cavity carcinomas with evidence of micrometastasis, we hope to be able to find a molecular marker that correlates with early lymph node metastasis.

Methods and Materials

Samples were collected in a prospective fashion from patients who underwent sentinel lymph node biopsy for oral cavity squamous cell carcinoma, with or without extracapsular lymphadenectomy, at the University of Miami between 1998 and 2006 (N = 30). Patient demographics and tumor location and stage were recorded. Specimens (N=30) were fixed in 10% formalin and then embedded in paraffin. Blocks were sectioned at 5μ and attached to positively charged charged slides. The slides were placed in a section drying oven for 30 minutes at 580°C, de-paraffinized in xylene and rehydrated through graded alcohols. Next, the slides were briefly washed in de-ionized water (DDI) and placed in phosphate buffered saline (PBS). The slides were then incubated in a 3% solution of H2O2 in PBS to block the staining of endogenous peroxidase. For CD-44, high temperature epitope retrieval was accomplished by exposing the slides to Antigen Dismasking Solution (Vector Laboratories, Burlingame, CA) in a pressure cooker. Pronase digestion was used to expose the Antigen site for MT1-MMP. Two sections from each primary tumor were then sequentially incubated with horse normal serum (Vector Laboratories, Burlingame, CA) and either monoclonal antibody MT1-MMP (R&D Systems, Minneapolis, MN; clone 5H2) or monoclonal antibody anti-sol CD44 (Bender MedSystems Inc., Burlington, Arizona; clone HCD-44-1) followed by incubated with anti-antibody site. The sequences were then counterstained with May-Grünwald 1 for 1 minute, blued for 5 minutes in running water, then dehydrated through graded alcohols, cleared in xylene and coverslipped. Omission of the primary antibody served as the negative control. Known positive and reactive cells in the skin served as positive controls.

Immunohistochemical reactivity of the specimens for MT1-MMP and CD44 was characterized by an experienced pathologist blinded to SLNB status. Staining patterns were classified as follows for MT1-MMP: 0 = no staining, 1 = peripheral staining, 2 = mixed staining, and 3 = universal staining. The statistical significance of differences between groups I and II for each type of staining pattern was calculated using the Fisher exact method. P-values <0.05 were considered to indicate statistical significance.

Results

There were two groups identified based on results of sentinel lymph node biopsy: patients with negative sentinel lymph node biopsy who represented true clinically and pathologically negative necks (NO; group I), and those with confirmed lymph node metastasis (N+; group II). Group I represented 19/30 (63%) cases and group II the remaining 11/30 (37%) cases. The American Joint Committee on Cancer (AJCC) staging of primaries and locations by subsite are depicted below (Fig 3.4).

For CD-44, there were 29 cases that underwent successful immunohistochemical staining. In group I (negative SLNB), there were 3/19 (16%) cases with type 1 or peripheral staining, 8/19 (42%) cases with type 2 or mixed staining and 5/19 cases with type 3 or universal staining. In group II (positive SLNB), staining patterns were as follows: 1 = 2/10 (20%), 2 = 5/10 (50%), 3 = 3/10 (30%). Differences between groups for each staining pattern were calculated by the chi square and were statistically significant; P = 0.65, 0.38 and 0.51 for types 1, 2 and 3 respectively.

For MT1-MMP, there were 21 cases (group I, N = 15; group II, N = 6) that did not reach statistical significance (p=0.13). We detect a statistical difference.

There was no statistically significant difference in staining patterns for CD44 between the patients with (group I) and without (group I) micrometastases. This may suggest that CD44 expression does not directly correlate with early tumor metastasis. Alternatively, the low sample size in group II (N=10) may not have provided sufficient power to detect a statistical difference.

For MT1-MMP, there were 21 cases (group I, N = 15; group II, N = 6) that did not reach statistical significance (p=0.13). We detect a statistical difference. There was a striking difference in MT1-MMP staining between groups I (20%) and II (67%), that did not reach statistical significance (p=0.13). We only included specimens with a strong internal control to differentiate, true staining from possible background staining with our anti-MT1-MMP antibody. This reduced the power of our study (group II, N = 6). It is possible that by increasing the number of specimens, we may detect a significant difference. Quantitative RT-PCR measurements of MT1-MMP levels in these tissues may further differentiate early node positive and node negative specimens.

Lymphoscintigraphy followed by sentinel lymph node biopsy is a sensitive means to detect early tumor spread. By studying this unique subset of patients we hope to identify a reliable molecular marker for micrometastasis, which may ultimately change the way we manage the neck for early oral cavity cancer.

Conclusions

There was no statistically significant difference in staining patterns for CD44 between patients with (group I) and without (group I) micrometastases. This may suggest that CD44 expression does not directly correlate with early tumor metastasis. Alternatively, the low sample size in group II (N=10) may not have provided sufficient power to detect a statistical difference.

Discussion

CD44: Peripheral staining pattern

CD44: Mixed staining pattern

CD44: Universal staining pattern

References