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ARTICLE

nm23-H1 Expression in Squamous Cell Carcinoma of the Head and Neck

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Archival material from 47 patients with primary squamous cell carcinoma of the head and neck (SCCHN) was studied immunohistochemically for the presence of nm23-H1 protein. Our data indicate that nm23-H1 protein expression is a common event in SCCHN and that there is a trend toward correlation of increased expression of nm23-H1 with increasing tumor size ($p = 0.072$). The results also show that when adjusting for age and cause of death, there tended to be an inverse relationship between overall survival and the expression of nm23-H1 gene in the primary tumor ($p = 0.088$). (Pathology Oncology Research Vol 2, No1–2, 34–36, 1996)

Key words: head and neck cancer, nm23-H1 gene expression, immmunohistochemistry, prognosis

Introduction

Squamous cell carcinoma of the head and neck (SCCHN) is a growing health problem. Despite recent advances in therapeutic modalities, there has been little improvement in prognosis over the last two decades. Although prevention through elimination of risk factors and early detection remain the key factors in controlling these cancers, it is thought that identification of prognostic markers would provide valuable direction in choosing the appropriate treatment modality/ies and improving survival.

Since Steeg and Leone[17] first demonstrated an inverse relationship between nm23 gene expression and tumor metastatic potential, a variety of human malignant tumors have been studied relative to this. A correlation has been shown between reduced nm23-H1 expression and the development of lymph node metastasis, a reduced disease-free survival and overall survival in breast carcinomas.[1,16] Alternately, an elevated level of nm23-H1 expression has been shown to be related to a lower frequency of metastasis with good prognosis in human breast cancer.[8] Reduced expression of nm23-H1 gene has also been associated with development of distant metastases in colorectal, stomach and hepatocellular carcinoma.[13,14,18] Also, the allelic deletion and/or mutation of nm23-H1 has been shown to correlate with the metastatic progression in colorectal carcinoma.[2,3,19] A reduction in the disease-free interval of malignant melanoma has been associated with reduced nm23-H1 expression.[6]

Conversely, some reports have shown that amplification of nm23-H1 contributes to carcinogenesis and tumor progression,[5,7,10,12] while a lack of prognostic value of nm23-H1 expression has been shown in pulmonary adenocarcinoma and in oligodendrogliomas.[9,15]

The purpose of this project was to study the expression of the nm23-H1 protein in primary SCCHN to determine if a correlation exists relative to tumor size, stage, development of lymph node metastases cause of death or overall survival. To the best of our knowledge this is the first report of nm23-H1 expression in SCCHN.

Materials and Methods

Archival material from 47 patients treated for SCCHN between 1982 and 1987 at the University of Cincinnati College of Medicine, Department of Otolaryngology-Head and Neck Surgery, and the Veterans Administration Hospital, Cincinnati, Ohio, were studied. All patients included in this study had an initial single primary lesion and none had undergone any preoperative treatment.

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Tumors, tissues

Tissue was fixed in 10% neutral buffered formalin. Four micron thick tissue sections were mounted onto polychloropren-treated slides which, following deparaffinization in xylene, were rehydrated and fixed in methanol. Slides were cleared with PBS and incubated in 0.02% trypsin for 15 minutes at 37°C. Non-specific binding was blocked by applying normal rabbit serum. The peroxidase/anti-peroxidase method was used as a detection system with 3, 3'-diaminobenzidine tetrahydrochloride as a chromogen, and the slides were counter stained with hematoxylin. The nm23-H1 protein was immunolocalized using the mouse monoclonal antibody (nm301 A mAb, Molecular Oncology, Inc., Gaithersburg, MD) that reacts with human nm23-H1 protein, which is localized in the cytoplasm. According to the manufacturer the antibody is specific for nm23-H1 and does not cross react with either cell derived or recombinant nm23-H2 in Western blot assays.[4] The negative controls were reacted with non-specific mouse IgG (5 mg/ml) instead of the anti-nm23 antibody.

Evaluation of slides

The slides were evaluated by an oral and maxillofacial pathologist (JSM) to determine the fraction of expression of nm23-H1 protein in each tumor. Nm23-H1 expression was graded and assessed according to the intensity of the staining (none = 0; weak = 1; strong = 2) and the estimated percentage of cells stained (< 5% = 0; 5-33% = 1; 33-66% = 2; > 66% = 3). A value was then given to each specimen as the sum of the intensity and percentage of cells stained. The tumor specimens were grouped into three categories: negative staining (0), weak staining (2,3), strong staining (4,5). All tissue samples with an estimated 5% of nm23-H1 reactive cells were recorded as positive.

Statistics

The data for the patients was entered into the computer and checked for errors. Summary statistics and frequency tables were constructed in the usual manner. The level of staining and the expression of nm23-H1 was analyzed for association with the other data by stepwise logistics regression and stepwise survival analyses.

Results

Thirty eight of the 47 squamous cell carcinomas (SCC) studied stained positively for nm23-H1 protein with the majority of these (63%) staining weakly. Intense staining was noted in only 14 (30%) of the 47 cases.

There were 17 females and 30 males in this study. There was a significant difference in correlation between nm23-H1 expression and sex with males tending to show greater nm23-H1 expression than females.

The data was evaluated to determine if there was a correlation between the expression of nm23-H1 and a variety of parameters, most notably tumor size, stage, the presence or absence of lymph node metastasis, cause of death and overall survival. No correlation was noted between nm23-H1 expression and stage of disease, the presence or absence of lymph node metastasis at the time of surgery, or the cause of death. When staining was compared with tumor size, there was a trend that as tumor size increased, staining intensity increased at a suggestive significant value of p = 0.072 (*Table 1*). Also, when adjusting for age and cause of death and when comparing those tumors which either stained negatively or weakly for nm23-H1 with those demonstrating an intense level of staining, the level of staining and hence the expression of nm23-H1 approached significance in predicting over all survival at a level p = 0.088 (*Fig. 1*).

Table 1. **The number of carcinomas for each combination of stain intensity and tumor size**

Intensity of staining	*T1,T2*	*T3,T4*	*Total (%)*
0	6	3	9 (19)
2,3	11	13	24 (51)
4,5	4	10	14 (30)
Total	21 (45)	26 (55)	47 (100)

Discussion

A review of the literature revealed no reports of nm23-H1 gene expression in SCCHN, including the esophagus, with the only upper aerodigestive tract cancers studied being lung cancers. As opposed to breast, colorectal, stomach, and hepatocellular carcinoma in which reduced ex-

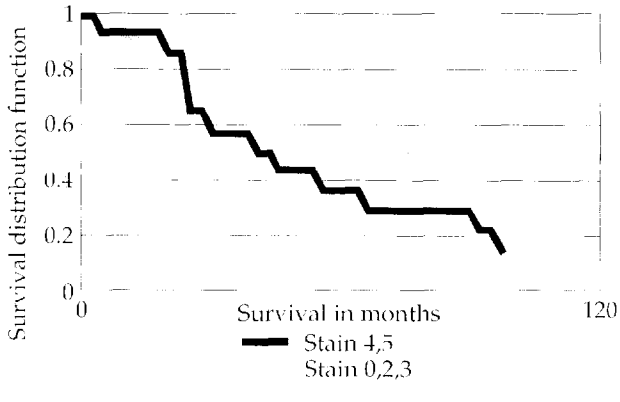

Figure 1. *Survival curve for SCCHN based on the presence or absence of nm23-H1 protein (p = 0.088)*

pression of nm23-H1 gene had been associated with the development of lymph node metastasis and reduced disease free survival,[1,3,13,14,16,18] increased nm23-H1 gene expression in squamous cell carcinoma of the lung has been shown to correlate with advanced stages of disease and grade of differentiation of the SCC.[10] Our data shows that nm23 expression is a common event in SCCHN and similar to Huwer et al[10] suggests a correlation of increased expression of nm23-H1 with increasing tumor size. Our results also show that when adjusting for age and cause of death there tended to be an inverse relationship between overall survival and the expression of nm23-H1 in the primary tumor. Taking Huwer's findings into consideration, this suggests that increased expression of nm23-H1 in cancers of the upper aerodigestive tract may have different prognostic implications than elsewhere in the body.

In conclusion, our data indicate that there is a trend toward correlation of increased expression of nm23-H1 with increasing tumor size. The results also show that when adjusting for age and cause of death, there tended to be an inverse relationship between overall survival and the expression of nm23-H1 in the primary tumor.

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