Overexpression of MPS Antigens by Squamous Cell Carcinomas of the Head and Neck: Immunohistochemical and Serological Correlation with FDG Positron Emission Tomography

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Abstract. Survival from advanced primary or recurrent Squamous Cell Carcinoma (SCC) of the head and neck (H&N) is poor. More accurate detection of primary tumors and recurrence may provide ways to improve survival. No standard serum tumor marker is routinely used for surveillance of SCC-H&N. In this paper, we evaluated the performance characteristics of the MPS-H tumor marker test for the quantitative measurement of “MPS-H” heat-generated immunoreactive proteins and assessed the clinical utility of this marker in the detection and monitoring of SCC-H&N. In approximately 92% of the subjects having no evidence of SCC-H&N, the MPS-H levels were lower than 15 ng/mL. In 76% of patients having SCC-H&N at various stages (T1-T4), the MPS-H level was > 15 ng/mL (range: 20-200 ng/mL). In addition, we found a statistically significant correlation between PET positive cases and high MPS-H serum levels in SCC-H&N patients with recurrent disease. These results suggest that MPS-H may provide an initial screening test that would allow for selective PET imaging in these patients. Furthermore, we found that there was greater expression of MPS-I in tumors of higher histological grades. Thus, in tumors with more histological aggressiveness there is more MPS-I, indicating the potential usefulness of this marker in prognosis for SCC-H&N. Considering the immunohistochemical, serological, and FDG-PET data presented here, and the compelling need to expedite the early diagnosis of primary and recurrent epithelial malignancies of the head and neck, we are further evaluating the system of MPS antigens in a large patient population as a tool for the early serologic and histologic diagnosis of SCC-H&N.

Effective therapy for epithelial malignancies of the head and neck is dependent on early diagnosis and intervention (1). Regardless of advances in conventional, organ-sparing, or novel therapies, it will always be singularly effective to diagnose and treat these neoplasms when the tumor burden is the lowest at the primary site and its lymphatic involvement is the least. Despite the obvious advantage of earlier diagnosis of head and neck malignancies, no “early warning” screening tests have proven to be effective to detect these tumors. Currently, the majority of cases of SCC of the head and neck are detected at a time when the tumor has extended beyond the perimeter of the organ of origin, making it incurable (1,2).

Tumor markers have enhanced the understanding of the molecular biology and natural history of many cancers. In addition they allow the evaluation of therapeutic efficacy. For certain types of tumors, the use of tumor markers has changed clinical practice specially to monitor effectiveness of therapy. In addition, the data suggest that tumor marker tests may be useful in the early diagnosis of cancer. More accurate detection of primary SCC of the head and neck and its recurrence may provide ways to improve survival. However, no standard serum tumor marker test is routinely used for surveillance of SCC of the head and neck, indicating the need to explore new markers for diagnosis of this highly aggressive neoplasm.

The experimental tumor marker test of the present paper is based on the study of a gene, initially denoted Metallopanstimulin (MPS-I), which encodes a multifunctional S27 ribosomal protein involved in various cellular functions such as DNA repair and recognition of abnormal mRNAs (3-13). The biological properties of MPS-I coincide well with the...
contention that the MPS-I protein is a good marker for cell proliferation and active oncogenic processes (3,4). Numerous experiments with human tissue culture cells and human pathological tissue specimens demonstrated that the MPS-I mRNA and its encoded protein are expressed in normal cells to a much lesser degree than in premalignant or malignant tumor cells, and they are present at very low levels in senescent cells compared to young healthy cells (3,4,6-9). It has been shown that the MPS-I DNA sequence and the protein can be used in diagnostic methods such as detection of malignant cells associated with numerous tumors (3,7,8). Recently, we have reported an experimental method for determining the presence of certain types of abnormal proliferative conditions and/or active oncogenic processes in patients by RIA measurement of proteins common to various forms of cancer denoted MPS-N and MPS-N like proteins in human serum (5,6,9).

This paper is focused on the detection of SCC of the head and neck by an experimental tumor marker test derived from the studies delineated above with MPS-I (5,6,9). This empirical tumor marker test, denoted heretofore as the "MPS-H" test, is based on the detection of specific heat-resistant molecules with sequence homology to the MPS-I protein, which interact with specific anti-MPS-N antibodies. The use of this empirical immunoassay system, allows the detection of numerous active oncogenic processes with high efficiency (5,6,9).

In this paper, we present experimental evidence that the MPS-H test can be used for detection, monitoring and management of SCC of the head and neck. In addition, we present biological information obtained by immunohistochemistry of MPS-I in squamous cell carcinomas and the use of MPS-H serum marker as an initial screening test for selective FDG-PET imaging in patients having recurrent SCC of the head and neck.

Materials and Methods

Patients. The diagnosis was based on detailed computerized clinical history, containing laboratory information, radiologic information, histopathologic information, stage, treatment, and conditions (Table I); tumor registry information was also available. The number of patients used for this study is indicated in each section. The age range for all normal subjects and patients studied was 20 to 84 years.

Analysis of blood samples. SCC head and neck tumor specimens consisted of serum samples obtained by written consent (reviewed by the IRB) from patients treated at the St. Louis University Medical Center. The use of residual blood samples for RIA (MPS-H test) and immunohistochemistry of MPS-I was approved by the Human Studies Subcommittee, DVA Medical Center, as "Research Exempt from IRB Review". Numerous other samples were control samples purchased from reference laboratories or donated by apparently healthy subjects.

MPS-H serum assay. Technical details of the preparation of reagents for MPS-N antigen determinations, RIA procedure, and patient sample preparation are published elsewhere (5,6). Each patient sample was run in duplicate as were all blanks, controls, and standards. All high MPS-H serum samples were diluted. Quality control was done following the
NCCLS recommendations (5). The target proteins, for example MPS-N and MPS-N-like proteins were activated or released from the precursor or carrier proteins by heat-denaturing the serum under controlled conditions. It should be noted here, that the immunoreactive substances detected by the MPS-H test do not reflect the true levels of authentic immunoreactive MPS-1/2/7 ribosomal protein in the circulation under non-denaturing conditions.

**Tissue specimens.** The primary tumor and regional lymph nodes were examined from patients who underwent surgical resection of their head and neck squamous cell carcinoma at the St. Louis University Medical Center. All tissues were fixed in buffered formalin and embedded in paraffin. Sequential 5 micron slices were cut and mounted for analysis of all margins and lymph nodes. Standard stains consisted of hematoxylin and eosin.

**Immunohistochemical procedures for MPS-1.** For studies to determine whether MPS-1 was expressed in SCC, we used methodology described in detail elsewhere (8). Briefly, Immunohistochemistry assays to detect the localization of the MPS-1 protein were performed on routinely processed formalin-fixed paraffin embedded tissues. The method used to detect the localization of the MPS-1 antigen using anti-MPS-N terminus antibodies was the Biotin-Streptavidin Amplified system (StrAviGenTM, Biogenex, San Ramon, CA) (8). In this system, the second antibody link is biotinylated and streptavidin is conjugated to alkaline phosphatase which generates a chromogenic reaction with the appropriate reagents. Each tissue section analyzed for the presence of the MPS-N antigen was analyzed in parallel with a contiguous section from the same tissue in which the primary anti-peptide-N antibody was omitted from the preparation as a control. Further details on this procedure are reported in Reference 4.

**FDG-PET imaging, PET data analysis, and correlation of PET with serum MPS-H levels.** FDG-PET imaging was performed on an ECAT 951/31 PET scanner (Siemens Medical Systems, Inc., Hoffman Estates, IL) that is on site. The F-18 fluoride ions were transferred to an automated system for synthesis of [18F]2-deoxy-2-fluoro-D-glucose (FDG) by the Hamacher method (2). FDG was tested for sterility, pyrogenicity, and radiochemical purity on each production run.

Transmission scans using a germanium-68 ring source were performed at 10 minutes per position on all patients prior to injection of FDG. Emission images of two bed position to include the area from the inferior orbit to the upper lung fields were obtained 50 minutes after intravenous injection of 370 MBq of FDG. Thereafter nonattenuation corrected images from the inferior orbit to the iliac crest were performed. These could be generally performed in six bed positions.

Transmission images were reconstructed using filtered back-projection smoothed with a Hann window of 7.0 mm width. Emission images were reconstructed using filtered back-projection with a Hann window of 5.0 mm width. Emission data were corrected for scatter, random events and deadtime losses using the manufacturer’s software.

Visual analysis of the FDG-PET data was performed to evaluate the ability of FDG-PET to identify malignancy. Nuclear physicians, experienced in interpreting FDG-PET images, and blinded from all clinical, serum MPS-H and immunohistochemical data for MPS-N, reviewed the FDG-PET data. The physicians interpreted the studies as indicating the presence of malignancy or not, and the extent of the disease. The sensitivity and specificity of these results for disease presence were calculated relative to standard pathologic findings and also compared to serum MPS-H results.

Serum was collected from patients within one week of the FDG-PET scan and MPS-H levels were determined as described above. FDG-PET interpretations and MPS-H level determinations were performed in a blinded, independent fashion.

**Results**

**Immunohistochemistry of MPS-1 in SCC.** Immunohistochemical studies were conducted in four tissue samples to examine the expression of MPS-1 protein in normal epithelium and malignant SCC lesions. Protein antigen, detected with anti MPS-N antibodies was found in both normal and malignant lesions but the staining patterns were different. Normal epithelial cells stained in an orderly pattern, the staining range was from weak to moderately intense, and very few cells present in the stroma were positive for MPS-1 (data not shown). In contrast, SCC specimens showed intense non-uniform staining in the nests of cancer cells while the stromal cells were not significantly stained (Figure 1A, B). While peripheral areas in the nests of malignant cells were strongly positive, central areas were less stained (Figure 1A,B). All lymph nodes studied here showed at least one SCC focus which expressed MPS-1 antigen. SCC metastatic to lymph nodes showed less staining in comparison to the primary tumor in the limited sampling studied (data not shown).

**Serum MPS-H levels in normal subjects and in patients with SCC of the head and neck.** The normal reference range for the MPS-H test presented here was established from data previously presented (5,6). The mean value for MPS-H in the normal group was 10.4 ± 3 SD with the upper limit of normal established as 19.4 (mean plus 3 SD). There is no significant difference in MPS-H levels between healthy male and female subjects (5). The results of studies with healthy subjects indicate an MPS-H reference range of non-detectable to 15 ng/mL, which encompass 94% of the healthy population. Six percent of the healthy subjects in this group had MPS-H levels in the range of 16-20 ng/mL. Figure 2 shows the levels of MPS-H in patients with SCC. These studies demonstrated that from 30 patients, a significant majority (76% or 23/30) of the patients with SCC were detected, when a cutoff value of >15 ng/mL MPS-H was used. Thus, in patients suspected of having SCC of head and neck, MPS-H elevations greater than 15 ng/mL may indicate the existence of an active oncogenic process.

**Correlation of FDG-PET results with MPS-H levels.** Seventeen patients having a total of 54 scans had FDG-PET to determined the correlation between MPS-H tumor marker data, and the presence or absence of SCC of the head and neck (Table II). Table II shows that in 8 cases elevated MPS-H levels correlated with positive PET scans (45%; 8/17); in 2 cases both MPS-H and PET scan were negative; in 4 cases PET scan was positive and MPS-H was low; and in 2 cases MPS-H was elevated and PET was negative. The average
MPS staining patterns in squamous cell carcinomas. (A) 45 year old patient diagnosed as having a moderately differentiated, keratinizing epidermoid carcinoma of the larynx, stage T4N2M0; (B) 55 year old patient diagnosed as having moderately differentiated, keratinizing epidermoid carcinoma of the mouth invading the mandibular bone, stage T4N0M0. Note that both tissue samples from separate patients are positive for MPS-I while the stroma is not stained.

MPS-H level for PET positive cases was 53 (±68 SD) and for PET negative cases was 27 (SD ±26) ("p" = 0.02).

Figure 3 shows an example of correlation of FDG-PET and serum MPS-H in a patient with SCC. The 18 FDG-PET scan demonstrates bilateral cervical metastatic disease in an advanced laryngeal carcinoma. The nodal metastasis escaped detection by CT and physical examination. The MPS-H levels were 35 ng/mL. Pathological diagnosis at the time of surgery demonstrated bilateral cervical SCC lymph node metastasis.
Correlation between FDG-PET scan and serum MPS-H level. This figure illustrates the visualization of squamous cell carcinoma of the neck by Positron Emission Tomography (PET). The 18F-FDG-PET scan demonstrates bilateral cervical metastatic disease (indicated by arrows) in an advanced laryngeal carcinoma. The nodal metastases escaped detection by CT and physical examination. The MPS-H level at the time of PET scan was 35 ng/mL. Pathological diagnosis at the time of surgery demonstrated SCC, confirming the origin of the metastases, and documenting that this patient stage was T4N2M0.

Palpable (1). As tumor stage is more advanced, the morbidity of resection worsens due to an increased loss of tissue volume and involvement of vital structures (1). Although surgical advances in free tissue transfers ameliorate some of the morbidities of resection, form and function are still compromised sufficiently to have a life-long impact on the patient (1). Failure in the treatment of SCC of head and neck results from inadequacy of resection, occult microscopic disease which persists despite therapy, or delay in diagnosis which results in advanced stage disease at presentation for treatment. SCC of head and neck patients not only require regular follow-up due to their risk for recurrence but are at increased risk for development of a second regional primary. Since the exposure to tobacco and/or alcohol or underlying genetic defects involve all mucosal surfaces of the aerodigestive tract, these patients require long-term surveillance (1). The assumption in this instance is that surveillance results in earlier diagnosis and consequent increased efficacy of the therapy employed. Therefore, any diagnostic tools which could enhance the sensitivity of the current surveillance measures should positively impact upon the prospects for salvage or second therapy. We believe that our data indicates that the MPS-H test may be useful for these purposes.
Histologic examination by hematoxin and eosin, of the tumor, surgical margins, and cervical nodes is the current means of determining the pathologic extent of disease. These data are then used to determine adequacy of resection and the need for adjuvant therapy. MPS-1 tissue staining may be helpful in increasing the ability of the pathologist to identify small foci of malignancy in biopsies from suspect locations for occult primaries which might otherwise go unnoticed. Localization of the unknown primary will help focus surgical or radiotherapy treatment and potentially reduce morbidity. Moreover, the extent of nodal metastases may be better elucidated with MPS-1 immunostaining thus allowing for improved staging data and more directed radiotherapy to the regional lymphatics.

Surveillance in the post treatment condition for SCC of the head and neck patients has traditionally centered around regular physical examination. Although routine imaging such as CT scan has been useful in the diagnosis of recurrent disease, by the time the recurrence is large enough to image, it often is extensive or involves critical structures (2). Again, like initial tumor diagnosis, recurrences are best treated when diagnosed early. The serum MPS-H serological test which is routinely used in our laboratory is an empirical means to detect early and with great frequency the presence of an oncogenic process. This test may be a useful addition to standard procedures utilized for the detection of SCC of the head and neck. Of all malignancies in which MPS-H has been studied to date, epithelial malignancies of head and neck possess no alternative tumor markers which have thus far been effective for diagnosis or surveillance. More accurate detection of recurrent SCC of the head and neck by using the MPS-H test may provide a new way to improve survival.

FDG-PET is the most accurate way to assess tumor recurrence of the head and neck but is quite expensive (2). No standard serum tumor marker is routinely used for head and neck cancer surveillance therefore limiting alternatives to frequent FDG-PET imaging. MPS-H has shown promise in detecting malignancy by serological diagnosis in other tumor types, with high accuracy. In the present prospective study we compared FDG-PET imaging to MPS-H levels in head and neck cancer patients. PET scan was performed both before and after therapy for head and neck cancer. FDG-PET interpretation and MPS-H level determination were performed independently and blinded from the results of the other test. A statistically significant correlation was noted between PET positive cases and high MPS-H serum levels in head and neck cancer patients. It is pertinent to note here that the results of MPS-H and PET correlated in 10 out of 17 cases (Table II). In two cases MPS-H was elevated but no cancer was found by PET, suggesting that the patients had early H&N cancer recurrence detectable by MPS-H but not yet by PET. The four PET positive cases which show low MPS-H levels suggest that the tumors were unable to produce high levels of MPS-H, perhaps due to previous chemotherapy. We suggest that MPS-H may provide an initial screening test that would allow for selective PET imaging in these patients. Further study with larger patient groups is ongoing to assess the optimal cutoff levels of MPS-H to determine with high efficiency the likelihood of PET positivity.

Considering the limited data presented in this paper, which is in agreement with previous results with other tumor types (5-9), and the compelling need to expedite the early diagnosis of primary and recurrent epithelial malignancies of the head and neck, we are further evaluating the MPS-1 and MPS-H tests as tools for histologic and serologic diagnosis, respectively, in a much larger number of patients. Since there is a good, although not infallible correlation between detection of MPS-H in the sera and FDG-PET positivity for SCC, the results suggest the potential of the MPS-H test for becoming a screening test for SCC of the head and neck, followed by selective confirmatory FDG-PET imaging.

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References


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