Immunohistochemical Analysis of Chordoma Targeting B7H3 and HMW-MAA

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ABSTRACT

BACKGROUND CONTEXT:

Chordoma is the second most common primary malignant tumor of the spine. Chordomas are often considered primarily a problem of local control, however metastasis has been reported in up to 30% of patients. In the setting of unresectable primary, recurrent, or metastatic tumors the current armamentarium of systemic adjuvant therapy for this condition is very limited. Recent research, however, has identified potential targets for immunotherapy, including the tumor associated antigens High Molecular Weight Melanoma Associated Antigen (HMW-MAA) and B7H3.

PURPOSE

The goal of this investigation was to correlate expression of B7H3 and HMW-MAA in chordoma tumors with disease severity and clinical outcome.

STUDY DESIGN/ SETTING

Laboratory investigation/ University-Affiliated Medical Center

PATIENT SAMPLE

Archival tissue from 70 chordoma tumors treated at the Massachusetts General Hospital (MGH) from 1985-2007.

OUTCOME MEASURES

The expression of B7H3 and HMW-MAA in chordoma, as well as clinical outcome, mortality and recurrence rates. The hypothesis was that expression of B7H3 and HMW-MAA in chordoma could be correlated to disease severity and the projected clinical course.

METHODS

Three Tissue MicroArrays (TMA) were constructed using an automated arrayer to include 70 conventional chordoma tumors obtained from archives at our institution. Triplicate cores (0.6 mm in diameter) from each sample were created and expression of HMW-MAA and B7H3 was evaluated by immunohistochemistry. Staining was evaluated independently by two researchers and scored using published systems. A retrospective chart review was performed for each chordoma specimen to determine demographic data, disease course, disease status at final follow-up and mortality. Clinical outcomes were then correlated to the expression of HMW-MAA and B7H3 within the chordoma lesions.

RESULTS

Chordoma tumors from 70 patients were included in this study. Average age at the time of presentation was 57.4 years (31-88 years). Average follow-up was 5.5 years (3.6 months-21 years). Fifty patients developed recurrences and 10 had metastatic disease. Twenty-six patients (37%) had died of disease at the time of final follow-up. Eighty-three percent of chordoma tumors stained positive for B7H3 while 58.6% stained positive for HMW-MAA. A HMW-MAA positive stain was associated with

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increased recurrence (62.8%), higher rate of metastases (90%) and a greater risk for dying of disease (76.9%).

CONCLUSIONS

Results indicate that expression of HMW-MAA may be predictive of more aggressive disease and shorter survival. HMW-MAA and especially B7H3, in light of its prevalence in the chordoma tumors studied here, may serve as potential targets for adjuvant immunotherapy.

INTRODUCTION

Chordoma is the second most common primary malignant tumor of the spine and occurs in 0.08 per 100,000 patients.[1] Afflicting men most often, the tumor is characterized by indolent growth and local invasion.[2-4] Metastases have been reported in 5-44% of cases.[5-8] Current recommendations for the treatment of chordoma advocate en bloc excision of the tumor whenever possible.[1,2,4,9-12] Nonetheless, en bloc resection, with the goal of achieving clear margins, can be especially challenging and, even in light of successful surgery the 5-year disease –free survival remains only 8.7-71.7%.[5-8,13] Moreover, in the event of recurrence the tumor is more likely to be aggressive in nature and to metastasize.[6,12]

While there is evidence that proton radiation can be an effective adjuvant, conventional photon radiation has not been shown to be an effective agent of local control.[8,12] Additionally, the tumor is largely resistant to conventional chemotherapy.[10,12,14] Therefore, in the setting of unresectable primary, recurrent, or metastatic, tumors the current armamentarium of adjuvant therapy for this condition is very limited. Furthermore, there are currently no identifiable biomarkers capable of predicting disease severity. Recent research, however, has identified potential targets for immunotherapy, including the High Molecular Weight Melanoma Associated Antigen (HMW-MAA) and B7H3. These factors may also have an association with disease severity and outcome.[15,16]

HMW-MAA is a membrane-bound proteoglycan that is expressed in some cancer cells, including melanoma, chordoma, and chondrosarcoma.[17,18] B7H3 is a tumor associated antigen known as an immunoregulatory protein.[13] Prior research has demonstrated that SiRNAs directed against B7H3 can inhibit cancer cell adhesion, migration and invasion.[13] The possibility of utilizing antibodies against HMW-MAA in melanoma has also been suggested by the work of Mittelman et al.[19] and Luo and co-workers.[17] Similar applications may have potential as adjuvant treatment for unresectable, or metastatic, chordoma.[18] The goal of this investigation was to correlate expression of B7H3 and HMW-MAA in chordoma tumors with disease severity and clinical outcome.

MATERIALS AND METHODS

Following approval from our institutional Investigational Review Board (IRB), the Massachusetts General Hospital cancer registry and orthopaedic oncology databases were utilized to identify all patients with chordoma treated at the Massachusetts General Hospital from 1985-2007. Data was subsequently stratified, and only those patients with archival tissue available through the Department of Pathology were included for review. Chordomas with a conventional morphology were included in the study, while dedifferentiated and chondroid chordoma subtypes were excluded. Furthermore, patients who were lost with less than one-year clinical follow-up were also excluded.

Data obtained for each patient identified through the registry included age, gender, date of birth, tumor location(s), dates of surgery, type of surgery, radiation treatments, presence of local recurrence and/or metastases, date of death if applicable, and disease status at final follow-up. Medical records were abstracted by investigators not involved in the pathologic, or immunohistochemical, analysis of specimens. Discrepancies in electronic medical records were resolved via manual review of patients' hospital and office charts. Results were stored on a personal computer in a password protected document.

Tissue Processing

All archival tissue had been obtained at the time of biopsy, or surgical excision, as a normal course of the patients' treatment. The chordoma specimens had been processed in a standard manner and stained with hematoxylin and eosin as per Department of Pathology protocol. Archival blocks and the representative hematoxylin and eosin slide from each case were reviewed microscopically by a pathologist involved in this investigation (GPN). Based on microscopic analysis, areas felt to be good representations of the histopathology were marked on the slide. The corresponding region on the paraffin block was also marked. The paraffin blocks and corresponding hematoxylin and eosin slides were then used to make a tissue array.

The tissue blocks were sent to the core facility at our institution and tissue micro-arrays (TMA) were constructed using an automated arrayer (ATA-27, Beecher Instruments, Sun Prarie, WI). Triplicate cores (0.6 mm in diameter) from each sample were created, with two sets of cores made for each chordoma specimen. One triplicate slide was incubated for 3 hrs at 37°C in a closed humid chamber with HMW-MAA-specific mAb, while the other was incubated with antibodies to B7H3. Six tissue specimens of notochord, archived in the Department of Pathology, were stained in a similar fashion and used as controls.

Chordoma HMW-MAA sections were graded separately by three investigators (JHS, SF, GPN) based on a modified protocol described by Kageshita et al.[15] Possible tissue grades were: (-) if no stain was detected, (+/-) if staining was faint and in less than 50% of the cells viewed per high power field, (+) if staining was present in more than 50% of the cells viewed per high power field, and (+/+) if the stain was strong and present in greater than 50% of the cells viewed per high power field(Fig. 1). For the purposes of analysis, results of stained sections were grouped into negative (no stain/faint) and positive (homogenous/strong). Immunohistochemical staining for B7H3 was evaluated by the same researchers, using the protocol described by Zang and colleagues.[20] B7H3 grades were: negative if staining was weak or absent, moderate in the event of staining in more than 50% of the cells viewed per high power field, and

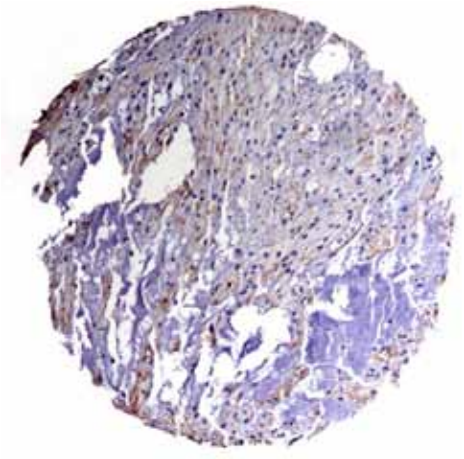

Figure 1
A section of chordoma tumor imaged at 40x magnification with a strongly positive HMW-MAA stain.

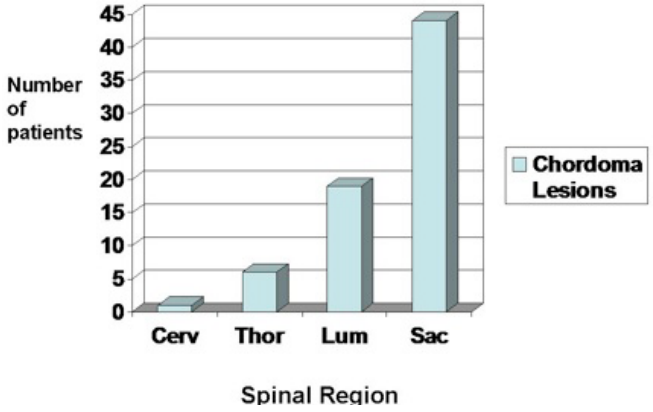

Table 1 – Distribution of Chordoma Tumors by Location

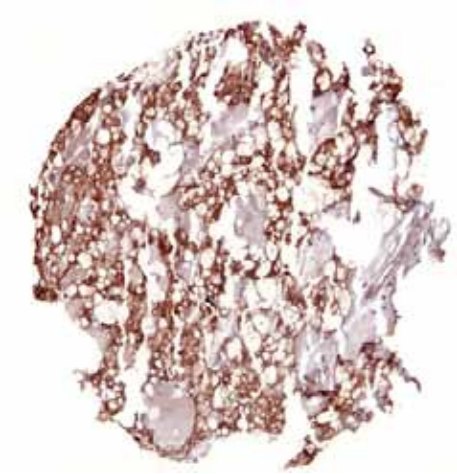

Figure 2
A section of chordoma tumor imaged at 40x magnification with a strongly positive B7H3 stain.

strong if staining was strong and present in more than 50% of the cells viewed per high power field (Fig. 2). In the subsequent analysis, staining results for B7H3 were stratified as negative (negative) or positive (moderate/strong).

Analysis of Clinicopathologic Parameters

Clinical results and immunohistochemical grades were linked utilizing anonymous, study specific, identifiers. Patients were initially divided into two groups based on whether they were positive or negative for HMW-MAA and B7H3. Mortality rate, survival data, type of surgery, surgical margins, disease status at final follow-up, and the presence of local recurrence or metastases were compared between groups. If patients had biopsies available from primary, recurrent, and/or metastatic lesions, the immunohistochemical results were compared between lesions in the same patient.

The follow-up period was calculated from the date of initial surgery to the most recent follow-up visit, or the date of death. Living patients were censored for "no evidence of disease" (NED) or "alive with disease" (AWD) at final follow-up. Patients who had died were censored for "dead of disease" (DOD) if they had died from complications of their chordoma tumor. Those who were documented to be disease free at the time of final follow-up, and passed away from other causes, were placed in the NED category.

RESULTS

In the time period 1985-2007, 89 patients treated for chordoma had archival tissue stored in the Department of Pathology at Massachusetts General Hospital. Nineteen patients were excluded due to the diagnosis of non-conventional subtype of chordoma, or insufficient follow-up, leaving 70 patients for inclusion in this study. The population included 51 males and 19 females. Average age at the time of presentation was 57.4 years (range 31-88). The majority of tumors involved the sacrum (n=44, 62.8%), while 26 involved the mobile spine (Table 1). Forty-three patients received all of their treatment at Massachusetts General Hospital, while 27 were initially treated elsewhere and only referred to our institution after developing a recurrence. Forty-four patients were treated with anterior-posterior surgeries and the remainder received posterior only surgery. No patients were treated with stand-alone anterior surgery. Forty patients received complete resection of their tumors while 30 patients had incomplete, or intralesional resections. Fifty-nine patients received radiation therapy as part of their treatment and 39 were specifically treated with proton beam radiation.

Mean follow-up for the entire cohort was 5.5 years (range 3.6 months-20.8 years). There were 50 (71.4%) patients who developed local recurrence and 10 (14.3%) had metastatic disease. Seven patients had more than one biopsy available from primary, recurrent and/or metastatic lesions available for comparison. At the time of final follow-up, 34 patients (48.5%) had died, and 36 (51.4%) were alive; 24 with NED and 12 AWD. Of those who died, 26 (76.5%) were DOD, while 8 (23.5%) had died of other causes and were NED at the time of final follow-up.

In the entire cohort, chordoma tumors from 41 patients (58.6%) stained positive for HMW-MAA. Staining for HMW-MAA was found to be more closely associated with an increased risk for recurrence and metastasis. For example, 61.4% of patients with local recurrence had chordoma tumors positive for HMW-MAA and 76.9% of patients DOD had positive stains. Additionally, chordoma lesions in 9 of the 10 patients with metastatic disease stained positive for HMW-MAA. A positive stain for HMW-MAA was associated with a 63.4% mortality. No

substantial differences in staining could be determined for multiple lesions within the same patient. All notochord controls were negative for HMW-MAA.

Chordoma tumors from 47 patients stained strongly positive for B7H3, while 11 lesions exhibited moderate staining. Only 12 patients had chordoma tumors which were negative for B7H3. Five of these patients were alive with NED at the time of final follow-up. Seven patients had died, 3 with NED at final follow-up, and 4 who were DOD. One patient with a B7H3 negative tumor had metastatic disease. Once again, no difference in staining could be determined between primary lesions and recurrent, or metastatic lesions, in the same patient. Five of 6 notochord controls were negative for B7H3.

DISCUSSION

Chordoma most commonly arises in the skull base and sacrum. Previous reports have documented a high rate of recurrence, surgical complications and metastases.[2,5-8,13,21] The goal of this investigation was to evaluate the prevalence of B7H3 and HMW-MAA in chordoma tumors and correlate their expression with disease severity and clinical outcome.

Comparatively few researchers have acknowledged the importance of understanding the histopathology and immunohistochemistry of chordoma. Only in recent years, with significant advances in biochemical techniques, immunohistochemistry, and microscopic analyses, have researchers been able to investigate the histopathology of chordoma in depth. For example, Schwab and colleagues recently published their results regarding gene profiles in chordoma tumors.[18] In their investigation, chordoma was found to over-express numerous extracellular matrix genes including aggrecan, types II and X collagen and fibronectin. High molecular weight-melanoma associated antigen (HMW-MAA) was also found to be over-expressed in chordoma and western blotting analysis demonstrated that chordoma HMW-MAA maintained a structure analogous to the HMW-MAA molecule secreted by melanoma cells. This was the first research of its kind to posit the potential for immunotherapy in the treatment of chordoma.

Prior research has demonstrated the potential for HMW-MAA and B7H3 targeted antibodies in the treatment of certain tumors,[13,17,19] but similar investigations have not been conducted in regard to chordoma. The goal of the current study was to document the percentage of chordoma tumors that express HMW-MAA and B7H3 and to correlate the expression of these markers with disease course and clinical outcome.

Results presented here indicate that the presence of HMW-MAA may predict a more aggressive disease course, an increased risk of metastases and decreased long-term survival. A positive stain for HMW-MAA was associated with a 63.4% mortality, 76.9% of patients DOD had tumors positive for HMW-MAA and 9 of 10 patients with metastatic disease were positive for HMW-MAA.

The near ubiquity of B7H3 in the chordoma tumors (82.9% moderate or strong positivity) prevented significant associations with the end-points under investigation. Nonetheless, of the 12 patients who stained negative for B7H3, only 4 were DOD at the time of final-followup. Morevover, only 1 of the 10 patients with metastatic disease had chordoma lesions negative for B7H3. While the numbers in this investigation are too small to draw firm conclusions, it is possible that a negative stain for B7H3 may indicate a more benign form of disease.

The presence of HMW-MAA and B7H3 in 58.6% and 82.9% of chordoma tumors respectively, presents the possibility that these proteins may be viable targets for adjuvant immunotherapy. The work of Luo et al.[17], Mittelman et al.[19,22] and Chen et al.[13] have documented the clinical application of antibiodies directed against HMW-MAA in melanoma and B7H3 in melanoma and breast cancer. Antibodies to these tumor associated proteins have been found to inhibit disease course and improve outcome in animal models as well as in clinical trials. While the role of adjuvant immunotherpay cannot be confirmed by the results presented here, the important impact that immunotherapy may have in terms of limiting recurrence and metastases in patients already at risk for more aggressive disease can be suggested.

This investigation was conducted using pathologic specimens from 70 patients treated for chordoma over the course of two decades. In terms of patient age, recurrence, metastatic rate, and overall survival, the cohort presented here is similar to those presented in previous reports. For example, local recurrence in this study was documented in 71.4% of cases. Such a figure lies within the range of the 19-78% recurrence rate documented in published studies.[5,6,8,12,13] Additionally, the rate of metastasis in this population was 14.3%. While this rate is somewhat lower than that encountered in several other studies, this finding still lies within the range contained in the literature (average 26.3%, range 5-44%).[12] Based on these findings, it is possible that the prevalence of HMW-MAA and B7H3 documented here may be applicable to chodroma lesions in the general population.

CONCLUSIONS

Results presented here would appear to indicate that HMW-MAA and B7H3 are biomarkers that can predict disease course and outcome. A positive HMW-MAA stain may be associated with an increased risk of metastasis and mortality from disease. Conversely, a negative B7H3 stain may be indicative of a more benign course. Additionally, the presence of HMW-MAA and B7H3 in the majority of chordoma lesions makes them viable targets for adjuvant immunotherapy. More research, of a prospective clinical, as well as basic science nature, must be performed before the role of these proteins in predicting disease course can be fully quantified.

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