The Spine Journal 16 (2016) 722–727

Clinical Study

CSPG4 as a prognostic biomarker in chordoma

Andrew J. Schoenfeld, MD, MSc[a,*], Xinhui Wang, MD, PhD[b], Yangyang Wang, MD[b], Francis J. Hornicek, MD, PhD[c], G. Petur Nielsen, MD[b], Zhenfeng Duan, MD, PhD[c], Soldano Ferrone, MD, PhD[d], Joseph H. Schwab, MD, MS[c]

[a]*Department of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, MA 02115, USA*
[b]*Department of Surgery, Massachusetts General Hospital, Harvard Medical School, 55 Fruit St, Boston, MA 02114, USA*
[c]*Department of Orthopaedic Surgery, Massachusetts General Hospital, Harvard Medical School, 55 Fruit St, Boston, MA 02114, USA*
[d]*Departments of Surgery and Orthopaedic Surgery, Massachusetts General Hospital, Harvard Medical School, 55 Fruit St, Boston, MA 02114, USA*

Received 11 August 2015; revised 27 October 2015; accepted 30 November 2015

Abstract

BACKGROUND: There are currently no generally accepted biomarkers used in the clinical treatment of chordoma tumors. CSPG4 has been associated with disease severity in other tumors.
PURPOSE: This study aimed to characterize the frequency of CSPG4 expression in chordoma tumors and to correlate it with disease severity and clinical outcome.
STUDY DESIGN: A retrospective review of clinical outcomes and immunohistochemical staining using tissue micro-array was carried out.
PATIENT SAMPLE: The sample comprised 86 patients treated for chordoma at a single center (1985–2007).
OUTCOME MEASURES: Survival and incidence of metastases were the outcome measures.
METHODS: Pathologic specimens of chordoma tumors were evaluated for the expression of CSPG4 by immunohistochemical staining with mAbs. Chi-square testing and Cox proportional hazard regression analysis were used to evaluate the impact of CSPG4 expression on survival and incidence of metastases, while controlling for patient age, sex, and surgical margins.
RESULTS: Average patient age at the time of presentation was 59.8 years (standard deviation [SD] 13.7). Average follow-up was 6.5 years (SD 4.8). Twenty (23%) patients developed metastatic disease. At the time of final follow-up, 57 patients (66%) had died. Chordoma tumors from 62 patients (72%) stained positive for CSPG4. CSPG4 expression more than doubled the risk of death (hazard ratio [HR] 2.3; 95% CI 1.04, 5.17). CSPG4 positive tumors were also associated with an increased risk of metastatic disease (31% for CSPG4 positive tumors vs. 0% in CSPG4 negative, p=.02).
CONCLUSIONS: Results presented here support the consideration of using CSPG4 as a biomarker establishing the prognosis for chordoma tumors. A positive CSPG4 stain may be associated with an increased risk of metastasis and mortality from disease.

Keywords: Biomarkers; Chordoma; CSPG4; Metastases; Outcomes; Survival

Introduction

Chordoma is the second most common primary malignant tumor of the spine and occurs in 0.08 per 100,000 patients [1–3]. Afflicting men most often, the tumor is characterized by indolent growth and local invasion [3–6]. Metastases have been reported in 5%–44% of cases [7–13]. Current recommendations for the treatment of chordoma advocate en bloc

FDA device/drug status: Not applicable.

Author disclosures: ***AJS:*** Grant: Robert Wood Johnson Foundation (F; Paid directly to institution/employer); Royalties: Wolters Kluwer Health (A); Consulting: Arbormetrix (B); Deputy Editor: The Spine Journal (A), outside the submitted work. ***XW:*** Nothing to disclose. ***YW:*** Nothing to disclose. ***FJH:*** Nothing to disclose. ***GPN:*** Nothing to disclose. ***ZD:*** Nothing to disclose. ***SF:*** Nothing to disclose. ***JHS:*** Grant: K12 MeRIT (F); Medical Research Investigator Training Program Sponsored by the Harvard Catalyst, Harvard Medical School (K12 MeRIT; Medical Research Investigator Training Program 2008–2010), pertaining to the submitted work.

The disclosure key can be found on the Table of Contents and at www.TheSpineJournalOnline.com.

* Corresponding author. Department of Orthopaedic Surgery, Brigham and Women's Hospital, Harvard Medical School, 75 Francis St, Boston, MA 02115, USA.

E-mail address: ajschoen@neomed.edu (A.J. Schoenfeld)

http://dx.doi.org/10.1016/j.spinee.2015.11.059
1529-9430/

excision with a wide margin whenever possible [1,3,12–14]. Nonetheless, obtaining a wide margin can be quite challenging, and local recurrence rates over 70% have been reported [7,15]. Moreover, in the event of recurrence, the tumor is more likely to be aggressive in nature and to metastasize [10,13].

At present no generally accepted prognostic biomarkers are routinely used in clinical practice to evaluate patients with chordoma. In recent studies we have shown that chordoma tumors express CSPG4 [16], a membrane-bound proteoglycan that is highly expressed on malignant cells in several types of cancer [16–21]. This tumor antigen participates in tumor progression through activation of multiple oncogenic pathways. Specifically, it plays a role in proliferation, survival, migration, and metastatic spread of tumor cells in vitro and in animal model systems [22,23]. The clinical significance of these experimental results is suggested by the association which has been found between CSPG4 expression and survival in several malignant diseases [18,24,25], as well as by the association of CSPG4 expression with metastasis [18].

These findings prompted us to investigate the frequency of CSPG4 expression in primary chordoma tumors and its association with the clinical course of the disease. To address this question, we immunohistochemically stained 86 chordoma tumors with CSPG4-specific monoclonal antibodies and correlated the immunohistochemical results with the clinical disease course including the development of metastases and patient survival.

EVIDENCE & METHODS

Context

The authors present results of a retrospective analysis of clinical outcomes and immunohistochemical staining for CSPG4 in 86 patients with chordomas from a single institution over a 22-year time period.

Contribution

The authors maintain that tumors positive for CSPG4 expression were associated with more than double the risk of mortality and an increased risk of metastasis.

Implications

The present findings are clearly of interest in the treatment of a rare tumor and add to the growing body of literature delineating the importance of biomarkers as prognostic factors for outcomes in the treatment of oncologic diseases. While this is a relatively large cohort of chordoma patients, the small overall number of subjects limits the useful variables in the regression analysis. The retrospective nature of the study and relatively short follow-up time for chordomas introduces selection bias and may underestimate the rate of metastases. The discussion regarding the role of CSPG4 in modulating chordoma biology and potentially being a therapeutic target remains speculative. Until further work is completed, however, the information presented here may prove useful for patient counseling and clinical management.

—The Editors

Materials and methods

Tumor specimens

Following approval from our institutional Investigational Review Board, the Massachusetts General Hospital cancer registry and orthopedic oncology databases were used to identify all patients with conventional chordoma treated at the Massachusetts General Hospital from 1985 to 2007. Data were subsequently stratified, and only those patients with archival tissue from resected specimens available through the Department of Pathology were included for review.

Data obtained for each patient identified through the registry included age, sex, dates of surgery, type of surgery, radiation treatments, presence of 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.

Antibodies

All the mouse hybridomas used to produce mAbs in our laboratory were developed by us. The mAb D2.8.5-C4B8 was generated from a mouse immunized with a recombinant CSPG4 fragment. The CSPG4 specificity of this mAb was shown by its active binding to human cells which express CSPG4 and by the SDS-PAGE profile of the antigen immunoprecipitated from human melanoma cells. The mAb D2.8.5-C4B8 recognizes a protein epitope in the second extracellular domain of CSPG4 in formalin-fixed paraffin-embedded (FFPE) tissue sections. The anti-idiotypic mAb MK2-23 [26] recognizes an idiotope in the antigen-combining site of the CSPG4-specific mAb 763.74 was used as an isotype control. All mAbs are IgG1. The mAbs were purified from mouse ascitic fluid by sequential ammonium sulfate and caprylic acid precipitation. The activity and the purity of these mAbs were monitored by binding assays and by SDS-PAGE.

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 representative hematoxylin and eosin slides from each case were reviewed. Based on microscopic analysis, areas felt to be good representations of the histopathology were marked on the slide and the corresponding paraffin block. The paraffin blocks and corresponding hematoxylin and eosin slides were then used to make a tissue array.