Journal of Clinical Neuroscience 18 (2011) 96–99

Contents lists available at ScienceDirect

Journal of Clinical Neuroscience

journal homepage: www.elsevier.com/locate/jocn

Neuropathology Report

Classic chordoma coexisting with benign notochordal cell rest demonstrating different immunohistological expression patterns of brachyury and galectin-3

Jun Shen [a,b,†], Chao-Ding Li [a,†], Hui-Lin Yang [a,*], Jian Lu [a], Tian-Ming Zou [b], Dong-Lai Wang [b], Min Deng [c]

[a] Department of Orthopaedic Surgery, The First Affiliated Hospital of Soochow University, 188 Shi Zi Road, Suzhou, Jiangsu Province 215006, China
[b] Department of Orthopaedic Surgery, Suzhou Municipal Hospital, Suzhou, Jiangsu Province, China
[c] Department of Pathology, The First Affiliated Hospital of Soochow University, Suzhou, Jiangsu Province, China

ARTICLE INFO

Article history:
Received 22 February 2010
Accepted 28 March 2010

Keywords:
Brachyury
Chordoma
Galectin-3
Immunohistochemistry
Notochordal cell

ABSTRACT

Increasing numbers of studies support the hypothesis that chordoma arises from notochordal cell rests, although the mechanism awaits further research. Brachyury is the first specific molecule linking chordoma with the notochord, and galectin-3 has been widely used as a marker of notochordal cells. We conducted a histological study of the expression of these two molecules in 46 classic chordoma specimens and unexpectedly found that classic chordoma tumor cells coexisted with benign notochordal cell rests in six specimens. Brachyury and galectin-3 expression were investigated by immunohistochemistry. All specimens contained atypical chordoma tumor cells set within an abundant myxoid matrix, which strongly expressed brachyury and galectin-3. However, brachyury and galectin-3 were not expressed in the notochordal cells. Benign notochordal cells, present as notochord rests, could undergo malignant transformation to form chordoma; however, the cause and role of brachyury and galectin-3 expression in chordoma tumorigenesis requires further careful study.

1. Introduction

Chordoma is the fourth most common primary malignant neoplasm of the skeleton and one of the few that expresses epithelial cell markers. Virchow and Muller first documented the existence of notochordal vestiges around the base of the skull,[1] and since then, evidence has accumulated linking persistent notochordal remnants with chordoma (including that the site of the vestiges corresponds closely to the distribution of chordomas,[2] the morphological similarities shown by both transmitted and electron light microscopy,[3] and the shared immunophenotype[3,4]).

Benign notochordal cell tumors have been recently identified;[2,5,6] they are found in approximately 20% of autopsy cases, and their anatomical characteristics are identical to those of classic chordomas. Recently, increasing numbers of studies support the hypothesis that benign notochordal cell tumors or lesions are the precursors of chordomas.[2,5,7,8] Yamaguchi et al. first documented a classic chordoma that was histologically confirmed to arise within a pre-existing benign notochordal lesion,[7] and then found two interesting microscopic incipient chordomas coexisting with benign notochordal cell tumors in the coccyx.[6]

Brachyury, a transcription factor known to be crucial in notochord development, was localized in chordoma cells in all 53 examined primary chordomas in one study, but not in a wide variety of 323 other neoplasms or in diverse normal tissue.[4] This is the first observation linking chordomas to notochord by a specific molecule; it provides compelling evidence to support the hypothesis that chordomas are derived from the notochord and demonstrates that brachyury is a specific marker for both notochord and notochord-derived tumors.[4]

Galectin-3 is a major non-integrin laminin-binding protein, and extracellular galectin-3 is required for cell adhesion in organogenesis and tumorigenesis. Because of its notochordal localization, galectin-3 has been widely used as a marker of notochordal cells.[9] Given its particular expression in chordoma, galectin-3 seems to be a reliable immunohistochemical marker that may be a useful adjunct in distinguishing chordoma from myxoid low-grade chondrosarcoma.[10]

Now that both brachyury and galectin-3 are highly associated with chordoma, we conducted a histological study to examine their expression patterns in classic chordoma specimens. Unexpectedly, we found that notochordal rests coexisted with chordoma tumor components in six specimens, and that classic

* Corresponding author. Tel.: +86 51267780101; fax: +86 51267780999.
E-mail address: szshenjun@sina.com (H.-L. Yang).
† These authors contributed equally to this paper.

0967-5868/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.jocn.2010.03.066

chordoma tumor cells and notochordal cells demonstrated completely different expression patterns of brachyury and galectin-3.

2. Materials and methods

2.1. Tissue preparation

The chordoma tissue samples were acquired from 46 patients aged between 28 and 72 years, all of whom underwent operations in the Orthopedic Department of the First Affiliated Hospital of Soochow University between 2002 and 2008. The samples were obtained from the sacral bone (28 patients), the lumbar region (12 patients) and the thoracic vertebral column (six patients). All of the specimens were obtained during surgery and put in 10% neutral buffered formalin within 2 hours. All the tumor diagnoses was confirmed by two pathologists.

2.2. Immunohistochemistry

Serial sections from paraffin-embedded samples were dewaxed in xylene and rehydrated in a graded alcohol series (100%, 90%, 80%, and 70%), after which microwave antigen retrieval was performed. Endogenous peroxidase was subsequently blocked with 0.3% peroxide for 30 minutes. The sections were incubated with primary antibodies overnight at 4 °C: rabbit anti-brachyury, 1:100 dilution (Abcam; Cambridge, UK); rabbit anti-galectin-3, 1:100 dilution (Santa Cruz Biotechnology; Santa Cruz, CA, USA).

The following antibodies were used to confirm the tumor diagnosis: vimentin (Abcam), S-100 protein (Dako; Carpinteria, CA, USA), epithelial membrane antigen (Dako Cytomation; Glostrup, Denmark), cytokeratin (CK) (AE1/AE3) (Dako Cytomation), and CK 18 (Dako Cytomation). These sections were then exposed to a streptavidin-biotin-peroxidase complex (Vector Laboratories; Burlingame, CA, USA), and the color was developed with 3,3-diaminobenzidine hydrochloride. Mayer's hematoxylin was used for counterstaining. To determine the specificity of immunostaining, we included known positive and negative tissues as controls. None of the negative controls showed any staining.

3. Results

3.1. Hematoxylin-eosin (HE) sections

All the confirmed chordoma specimens (from 46 patients) contained a large number of cords, strands, or solid nests of atypical physaliphorous cells within an abundant myxoid matrix, often separated by fibrous septa. The specimens showed enlarged, atypical, and eccentrically located oval or round nuclei and eosinophilic cytoplasm. Some tumor cells displayed non-vacuolated and eosinophilic cytoplasm. They often showed irregularly shaped hyperchromatic nuclei and moderate to marked nuclear polymorphism (Fig. 1a). Mitotic figures were observed in some cells that were considered classic chordoma cells.[2,6]

In this study, we unexpectedly found six chordoma specimens that obviously contained two types of cells: classic chordoma tumor cells and another cell type consisting of sheets of cells with centrally or peripherally located round and bland nuclei and large intracytoplasmic vacuoles, exhibiting an adipocyte-like morphology. Some nuclei were slightly hyperchromatic and atypical. In general, little or no extracellular myxoid matrix was observed; this cell type lacked mitotic figures, and their morphology was identical to benign notochordal cells[2,6] (Fig. 1b). The border between the two types of cells was relatively sharp.

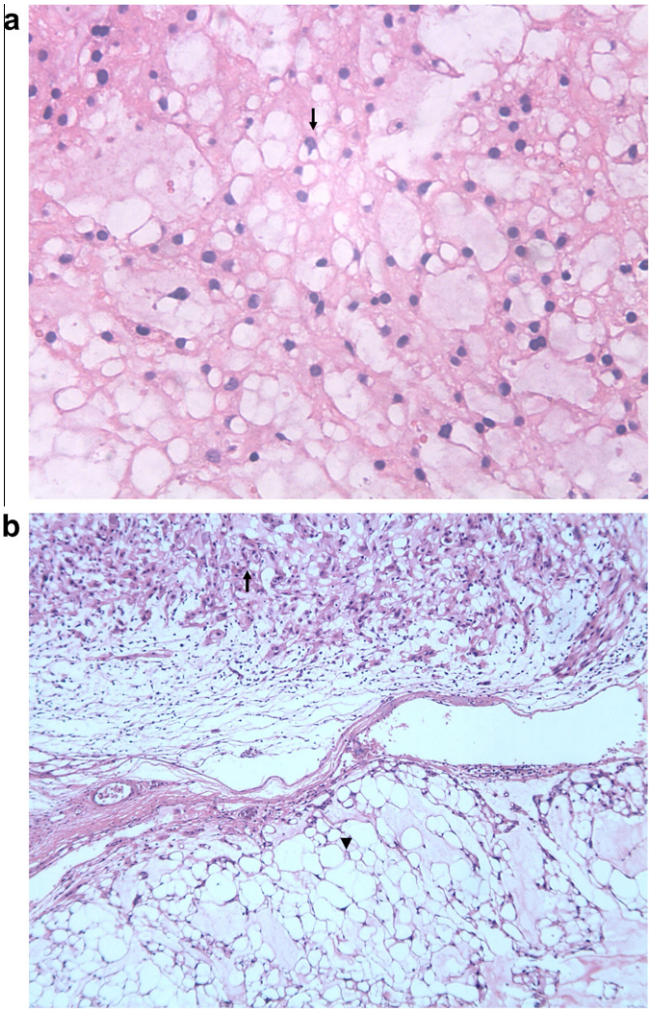

Fig. 1. Hematoxylin and eosin stained sections of a chordoma showing: (a) atypical physaliphorous cells (arrow) set within a myxoid matrix, observed in all specimens (original magnification ×400); and (b) two types of cells with a relatively sharp interface between them: classic chordoma tumor cells (arrow) and benign notochordal cells (arrowhead). This dual pattern was observed in six chordoma specimens (original magnification ×100). (This figure is available in colour at www.sciencedirect.com.)

3.2. Immunohistochemistry

3.2.1. Brachyury expression

Chordoma tumor cells from each of the 46 patients strongly expressed brachyury in the nuclei, representing more than 83% of the malignant cell population. The nuclei showed a dense or granular staining pattern. Although the staining signal of brachyury appeared mainly to be nuclear, some cells showed strong nuclear and diffuse cytoplasmic immunoreactivity; however, intracellular vacuoles were not involved. Brachyury immunopositivity was also observed in blood vessels and nerve and muscle tissues, but no cellular staining was detected in fibrous strands or extracellular mucoid areas (Fig. 2a).

Six chordoma specimens containing two types of cells that demonstrated opposite brachyury staining patterns: benign notochordal cells and tumor cells. Almost no positive signal was observed in benign notochordal cells, whereas the surrounding atypical classic chordoma tumor cells had strong nuclear expression of brachyury. Some areas had a clear border, and a sharp comparison existed between the two types of cells, separated by fibrous septa (Fig. 2b). Some other areas did not have obvious septa; and brachyury-negative notochordal cells with a benign