Chordoma: Immunohistochemical Analysis of Brachyury

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ABSTRACT

AIM: Chordomas are rare, slow growing but locally aggressive malignancies of the axial skeleton. Skull base chordomas, due to their intricate anatomical localization, pose significant challenges to managing physicians. In classical and chondroid chordomas, the disease course cannot be reliably determined using only morphological criteria. Brachyury (T Gene) was shown to play a central role in chordoma pathogenesis and several studies also showed that this gene also carries potential as a prognostic biomarker. This study aims to correlate Brachyury expression with the clinical course in surgically treated skull base chordomas.

MATERIAL and METHODS: Chordoma tumor samples from 14 patients with skull base chordomas, diagnosed using histopathological and immunohistochemistry criteria (epithelial membrane antigen (EMA), S100, pan cytokeratin (panCK)) were retrospectively analyzed for Brachyury expression using immunohistochemistry. Brachyury expression was graded using a 4 point semi-quantitative scoring system. Focal (grade II) and diffuse staining (grade III) were considered as overexpression. Patient recurrence-free survival and total survival were compared between Brachyury overexpressing and non-overexpressing groups using Kaplan-Meier survival analysis.

RESULTS: Among the stained tumor samples, 85.7% were positive for brachyury expression. In both groups, there was one sample that was negative. We did not observe any significant difference among the groups for staining, grade and percentage of brachyury positive cells.

CONCLUSION: Brachyury expression in tumor samples is not a sensitive indicator of prognosis in chordomas.

KEYWORDS: Chordoma, Brachyury, Microsurgery, Immunohistochemistry

INTRODUCTION

Chordomas are rare, slow growing but aggressive bone malignancies of the axial skeleton that account for 1 to 4% of all bone tumors (1). They originate from the remnants of embryonic notochord and may occur anywhere along the skeletal neuroaxis (2). Chordomas are commonly observed in the clivus (32%), the sacrum and the coccyx (29%), yet rare cases have been reported in the legs, feet and ribs (1,3). Due to their invasive and destructive growth characteristics and their metastatic potential, chordomas are considered as malignant tumors.

Chordomas are resistant to chemotherapy and relatively resistant to radiotherapy, rendering surgical resection the main treatment option. However, due to the tumor location that makes it difficult to achieve negative surgical margins, surgical treatment is a major challenge. More than 40% of patient experience local recurrence (4,5). Metastasis is also observed during the follow-up period in 10 to 50% of all patients (5).

Histologically, 3 types of chordomas are described. Conventional chordomas, which are characterized by the absence of cartilaginous or mesenchymal components, are the most common form. Chondroid chordomas, which display
chordomatous features, are the second most frequent form accounting for 5 to 15% of all chordomas. The undifferentiated type accounts for 2 to 8% of chordomas (2). Typically, undifferentiated chordomas are considered to be more aggressive and likely to metastasize whereas chordoid chordomas are considered less aggressive. Despite the fact that this classification is widely accepted and cited, its prognostic value is under debate and remains to be fully elucidated (6,7). Novel and better markers that can predict the clinical course of the condition in individual patients are required.

Prognosis in some cases of chordoma is relatively good even after simple limited resection, while in other cases relentless growth despite aggressive surgery and multimodal adjuvant treatment protocols is observed. Determinants of this difference in behavior is not known but are assumed to depend on the intrinsic biology of chordomas. There are at least 2 subsets of patients with distinct clinical prognosis: some with a benign course and another group with an aggressive and rapidly progressive course over 3–5 years.

The brachyury gene is associated with chordomas and it is among the most common proteins observed during analyses of chordomas. Brachyury is encoded by the T gene, located at 6q27 (8). Single nucleotide polymorphisms (SNP) and duplication of brachyury is a risk factor for chordoma. Almost all patients with sporadic chordomas have a form of SNP which is thought to render them susceptible to chordoma development (9). In rare familial cases of chordoma, an extra copy of the brachyury gene has also been identified (10). Chordomas tend to express the brachyury protein. Brachyury expression has been reported in 75 to 100% of chordomas (8). As such, brachyury expression has become a potential marker that can be used to differentiate between aggressive and relatively less aggressive forms of chordomas.

In this study, we examined the brachyury expression in samples taken from chordoma patients with aggressive and benign course to evaluate the prognostic value of immunohistochemical analysis of brachyury expression.

### MATERIAL and METHODS

#### Patients and Surgery

Between 2006 and 2016, 17 chordoma patients underwent microsurgical chordoma resection at the Neurosurgery Department of Acibadem University School of Medicine, Istanbul, Turkey. Three patients were not included in the trial since their follow-up periods had not reached 2 years so far. Total excision was radiologically confirmed with postoperative 24-hour magnetic resonance (MR) imaging examination and 14 (5 recurrent and 9 non-recurring) were selected for immunostaining (Figure 1A-F). Eight (57%) patients were operated by the transtemporal approach, 4 (29%) patients by the pterional approach, 1 (7%) patient by the transoral approach and 1 (7%) patient by the suboccipital median approach. Five (36%) patients had tumor recurrences in their follow-up period.

#### Immunohistochemistry

The analyses were performed at the Neuro-oncology and Pathology laboratories of Acibadem University School of Medicine. In each case, multiple serial sections were cut from

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![Figure 1: Preoperative MR images of patient 4 with chordoma, A) sagittal section with iv gadolinium, B) axial section with iv gadolinium and C) coronal section with iv gadolinium. Postoperative MR images of patient number 4 with view of total removal of chordoma (D,E,F).](image-url)
the paraffin-embedded tissue by using a microtome and were prepared for immunohistochemical analysis for the brachyury gene. Tissue sections were cut and rehydrated in an alcohol gradient before analysis with the standard streptavidin-biotin technique. Endogenous peroxidase activity was blocked by incubation in 3% H$_2$O$_2$ (0.6 mL of H$_2$O$_2$), 2.7 mL of methanol and 2.7 mL of distilled H$_2$O. Slides were blocked with 5% normal goat serum, brachyury antibody (Santa Cruz Biotechnology Inc, Santa Cruz, California, USA) at 4°C. Then each one was incubated for 30 minutes at 25°C with secondary biotinylated goat immunoglobulin G (Vector Laboratories, Inc, Burlingame, California, USA). The final step was incubation with streptavidin-peroxidase complex, using the Vectastain ABC Elite kit (Vector Laboratories, Inc). Chromogenic reactions were completed with 3.3 diaminobenzidine, yielding a positive brown stain (Vector Laboratories, Inc). Each slide was counterstained with hematoxylin and eosin (H&E) and then mounted and examined under the light microscope (Figures 2, 3).

Levels of expression were classified using a 4-point scoring system:

Grade 0: No expression

Grade 1: Moderate expression

Grade 2: Marked expression with focal distribution

Grade 3: Marked expression with diffuse distribution

Negative control sections were obtained by omission of the primary antibodies. Human alveolar macrophages were used as positive control.

Statistical Analysis

The groups with different prognosis (Benign vs. Aggressive) were compared for brachyury expression. Student’s t-test was performed using SPSS 12.0 software (SPSS, Inc., Chicago, IL, USA). p<0.05 was considered to indicate a statistically significant difference.

**RESULTS**

Tumor samples from 14 patients with confirmed total tumor removal were separated into 2 groups depending on the recurrence status within the 2 years of follow-up. Among the samples, one from each group was negative for brachyury immunohistochemical staining and the rest (85.7%) were positive. The mean values in staining grade within the Aggressive and Benign groups were both 1.8. There was no statistically significant difference among groups in staining grades (p=0.9715). In the aggressive and benign groups, the mean percentage of cells that were positive for brachyury was 46% and 48.89% respectively, with no statistically significant difference (p=0.8559) (Table I).

**DISCUSSION**

Chordomas are non-encapsulated, extra-axial tumors that are locally invasive within the bones of the axial skeleton. On macroscopic examination the tumor has a smooth or lobulated surface. The interior of the tumor is soft and may contain cyst formation, calcifications, focal hemorrhages and cartilage. Three histological variants have been described: Classic (International classification for disease-oncology [ICD]: M-9370=3), chondroid (ICD: M-9371=3) and undifferentiated chordomas (ICD: M-9372=3). Under the light microscope, classic chordoma presents as cells with clear and granular appearance with an eosinophilic cytoplasm that stains positive with the periodic-acid Schiff (PAS) stain (11). So-called “physaliferous cells” with eccentric, hyperchromatic nuclei and reticulated cytoplasm (due to intracellular accumulation of glycosaminoglycans) are typically observed (Figure 2). In classic chordoma, necrosis, hypervascularity, mitoses are absent or very rare.

Chondroid chordoma is a variant of chordomas described in 1973 by Heffelfinger et al. (7). Chondroid chordomas contain islands of stellate cells resembling chondrocytes spread through areas of typical chordoma. Chondroid
cells may be scarce and spread or dominate the tissue. Similar to classic chordoma, anaplastic features are lacking (7,12). Undifferentiated chordomas have been observed and described in very few cases. They mostly have a sacrococcygeal location and are in the pediatric population. Histologically this variant exhibits atypical features resembling spindle cell sarcoma or round cell tumors. The tumor consists of small epithelioid cells with clear to eosinophilic cytoplasm and irregular nuclei. Unlike other variants, higher mitotic activity and necrosis are present (13,14).

Prognosis reveals there are at least two types of chordomas, one with a benign course and another group that is prone to recurrence with an aggressive and rapidly progressive course over 3–5 years. The natural history and differences in prognosis among different forms of chordomas have been a topic of research and histological forms have been investigated for their relation to the prognosis. In their influential paper where they first described chordoid chordomas, Heffelfinger et al. reported lower rates of recurrence and longer survival in this variant (7). This finding has been later questioned by various studies and lead to an ongoing debate about the prognostic value of the phenotype (6,7,12). Undifferentiated chordomas are almost exclusively associated with aggressive behavior but there are only rare cases with few reports. Despite well documented differences, classical histological classification of chordomas does not offer much in terms of predicting prognosis. More research for identifying and establishing novel prognostic markers are warranted.

Brachyury is a member of the T-box gene family which consists of more than 20 members that share a unique DNA binding domain. It encodes a transcription factor that plays a key role in the notochord formation. SNP and duplication of Brachyury is a risk factor for chordoma. Almost all patients with sporadic chordomas have a form of SNP which is thought to render them susceptible to chordoma development (9). Detailed genetic analysis in chordoma patients revealed an SNP (rs2305089) that lies in exon 4, which encodes part of the DNA-binding domain of brachyury that has been shown to alter the DNA-binding properties of this transcription factor (9). In rare familial cases of chordomas, extra copies of the brachyury gene are identified (10). Through the analysis of familial cases a 6q haplotype that contains regions of 6q27 duplicated to various extents has been found to confer major susceptibility to chordomas (20).

Table I: Clinical Data of Patients with Chordoma

<table>
<thead>
<tr>
<th>Patient No</th>
<th>Gender</th>
<th>Symptom</th>
<th>Age (Years)</th>
<th>Tumor volume (cm³)</th>
<th>Staining Score</th>
<th>Percent Stained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aggressive Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>F</td>
<td>Headache</td>
<td>28</td>
<td>1.5</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>3rd nerve palsy</td>
<td>21</td>
<td>111.4</td>
<td>3</td>
<td>70%</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>Right Amaurosis</td>
<td>89</td>
<td>35.0</td>
<td>3</td>
<td>80%</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>Headache</td>
<td>19</td>
<td>96.3</td>
<td>1</td>
<td>20%</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>3rd nerve palsy</td>
<td>66</td>
<td>70.0</td>
<td>2</td>
<td>60%</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>44.6</td>
<td>62.8</td>
<td>1.8</td>
<td>46%</td>
</tr>
<tr>
<td>Benign Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>Headache</td>
<td>58</td>
<td>48.0</td>
<td>2</td>
<td>60%</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>Headache</td>
<td>33</td>
<td>56.0</td>
<td>2</td>
<td>55%</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>Diplopia</td>
<td>28</td>
<td>49.0</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>3rd nerve palsy</td>
<td>67</td>
<td>10.0</td>
<td>3</td>
<td>85%</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>Diplopia</td>
<td>65</td>
<td>10.0</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>Headache</td>
<td>18</td>
<td>99.0</td>
<td>1</td>
<td>30%</td>
</tr>
<tr>
<td>12</td>
<td>F</td>
<td>Diplopia</td>
<td>36</td>
<td>48.0</td>
<td>2</td>
<td>50%</td>
</tr>
<tr>
<td>13</td>
<td>F</td>
<td>Gait ataxia</td>
<td>31</td>
<td>22.5</td>
<td>1</td>
<td>40%</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>Headache</td>
<td>42</td>
<td>31.5</td>
<td>3</td>
<td>70%</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td></td>
<td>38.4</td>
<td>41.6</td>
<td>1.8</td>
<td>48.89%</td>
</tr>
</tbody>
</table>

M: Male, F: Female.
Findings from various studies indicate that brachyury expression may be an indicator of prognosis in several cancer types (10). Brachyury was found to play a role in the epithelial to mesenchymal transition of human carcinoma cell lines. Additionally, it promotes metastasis in human tumor xenografts (15). In colorectal and primary lung carcinoma, brachyury expression was associated with the prognosis (16,17).

In this study, we examined the brachyury expression in samples taken from chordoma patients with an aggressive and benign course to evaluate the prognostic value of immunohistochemical analysis of brachyury expression (Figure 3). Samples from fourteen patients that had complete tumor removal were divided into two groups (aggressive and benign) depending on the recurrence status in 2 years follow-up. Levels of expression were classified using a 4-point scoring system along with the counts of cells expressing brachyury. Among the 14 samples, 12 were positive for brachyury (85.7%), which is in the same range as previous studies (18,19). Both aggressive and benign groups had one negative sample (14.3%). Brachyury positive cell counts revealed that brachyury positive tumors also contained brachyury negative cells. There was no significant difference among groups for expression scores or number of brachyury positive cells. These finding are in contradiction to the study by Kitamura et al. where the authors reported that brachyury expression is a predictor of prognosis in skull base chordomas (10). However, two recent studies failed to find any evidence for the prognostic value of brachyury expression in chordomas (19,21).

**CONCLUSION**

Our study supports the notion that brachyury expression in tumor samples is not a sensitive indicator of prognosis in chordoma.

**REFERENCES**