Lymphocytic Infiltration and Immune Escape Mechanisms in Human Chordoma

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Disclosures:

Introduction: Tumor-infiltrating lymphocytes have been associated with more favorable prognoses in a number of malignancies, including colorectal cancer, lung cancer, breast cancer, ovarian cancer, and melanoma. In recent years, particular focus has centered around how characterizing this immune response to tumors can be used to develop novel immunotherapeutic strategies. However, the general consensus in human chordoma, a rare primary malignancy of the axial skeleton originating from notochordal remnants, has been that tumor infiltration by lymphocytes is absent. To our knowledge, no group has reported significant findings of tumor-infiltrating lymphocytes in human chordoma. Contrary to the current paradigm, we observed lymphocyte infiltration in surgically excised chordoma specimens. The long-range goal of this research program is to determine whether the lymphocytic infiltration we have unexpectedly found in chordoma tumors reflects a patient’s immune response against his/her tumor and whether this response has a clinical significance. To this end, this study has assessed the frequency and distribution of lymphocytic infiltration in 62 human chordoma tumors. Additionally, to characterize the functional properties of these lymphocytes, we have tested our hypothesis that these intratumoral lymphocytes reflect the host’s immune response to the tumor. The resulting selective pressure imposed on the tumor facilitates the outgrowth of tumor cells subpopulations which have developed escape mechanisms. We have used the expression of human leukocyte antigen (HLA) class I antigen processing machinery (APM) components as a biomarker since this machinery plays a crucial role in the generation of the HLA class I-tumor antigen peptide complex. The latter complex mediates the recognition of tumor cells by cognate T cells. Therefore, abnormalities in the APM provide tumor cells with a mechanism to circumvent the host immune response.

Methods: Patients with chordoma treated at the Massachusetts General Hospital (MGH) between 1989-2009 were identified and selected from the Orthopaedic Oncology database with approval from the MGH institutional review board (IRB). Paraffin-embedded human chordoma tissue slides from 62 patients were reviewed in tandem with our senior bone pathologist. For each patient, a minimum of 2 slides and a maximum of 19 slides were analyzed. Ten samples were stained immunohistochemically with a unique panel of monoclonal antibodies, generated in our laboratory, which recognize HLA class I APM components and HLA class II.

Results: Lymphocytic infiltration was found in 52 (84%) of the tumors removed from 62 patients (Fig. 1). The presence or absence of lymphocytes was homogeneous in 45 (73%) tumors; 35 were positive and 10 were negative. In the remaining 17 (27%) tumors, the presence of lymphocytes was heterogeneous, with areas of lymphocyte infiltrate associated with areas with no detectable lymphocytes. The lymphocyte infiltrates were distributed in two distinct patterns; either primarily in the fibrous septae between the lobules of chordoma cells or surrounding chordoma cells within tumor lobules (Fig. 2). Immunohistochemical staining of chordoma tumors with HLA class I APM component-specific monoclonal antibodies detected decreased expression of all of the components analyzed, with the exception of calnexin. The frequency of these abnormalities ranged from 30%-90% (Fig. 3). In addition, HLA class II antigens were aberrantly expressed in 50% of the tumors tested.

Discussion: The detection of lymphocytic infiltrate in 84% of surgically excised chordoma specimens challenges the paradigm that lymphocytic infiltrates are rare in this malignancy. In addition, the detection of loss of HLA class I APM component expression in chordoma is compatible with the possibility that the infiltrating lymphocytes reflect a patient’s immune response to his/her tumor. The resulting selective pressure facilitates the outgrowth of tumor cells, which have developed escape mechanisms from host immune recognition and destruction. Our data suggest that there may be a heterogeneous loss of HLA APM components in chordoma. Our results provide a strong rationale to develop and implement strategies which enhance the patient’s immune response to his/her own tumor. Along these lines, our future studies include further characterizing the frequency of HLA class I APM defects in human chordoma samples, examining tumor-infiltrating lymphocyte expression of programmed cell death protein 1 (PD-1) and chordoma cell expression of the protein’s ligand PD-L1, and corroborating this study’s findings in an in vitro model using a chordoma cell line. This study’s clinical impact could be paramount in terms of adding an important prognostic indicator for this rare disease, and also better understanding the mechanism by which disease progression occurs in human chordoma. Additionally, phenotyping chordoma tumors could contribute to identifying patients who may benefit from currently available immunotherapeutic strategies, such as vaccination with tumor antigens and administration of anti-PD-1 antibody.

Significance: This study, to our knowledge, is the first to describe lymphocytic infiltration and loss of HLA class I APM components in chordoma tumors. Our findings support the possibility that patients develop an immune response against their own tumor; therefore, they may benefit from novel immunotherapeutic strategies, such as vaccination with tumor antigens.
and/or administration of antibodies recognizing immunoregulatory molecules (i.e. PD-1), to enhance this immune response.

Acknowledgments:

References:

**Lymphocytic Infiltration in Chordoma Patients**

- 16%
- 84%

- Patients with lymphocytic infiltration, n = 52
- Patients without lymphocytic infiltration, n = 10

**Distribution of lymphocytic infiltration in chordoma tumors**

**Lymphocyte Infiltration**

- Fibrous Septum
- Chordoma Lobule
- No Lymphocyte Infiltration

**Frequency of downregulation of HLA class I APM components in 10 chordoma tumors**

<table>
<thead>
<tr>
<th>HLA Class I APM Component</th>
<th>Percent of Chordoma Tumors with Decreased Expression</th>
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<tbody>
<tr>
<td>TAP1</td>
<td>100%</td>
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<tr>
<td>Beta-2-microglobulin</td>
<td>80%</td>
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<tr>
<td>Tapasin</td>
<td>60%</td>
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<tr>
<td>CIITA</td>
<td>40%</td>
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<tr>
<td>Calretinin</td>
<td>20%</td>
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