Programmed Cell Death Ligand 1 Expression in Chordoma

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Introduction: Chordoma is a very rare cancer that presumably originates from notochord, and accounts for 1-4% of primary malignant bone tumors and 20% of primary spine tumors. The standard treatment for these tumors is en-bloc resection; however, the critical anatomic location (spread along critical bony and neural structures) and the commonly large tumor size make clinical management of these patients difficult. Because chordoma is resistant to conventional chemo- and radiotherapy, the recurrence of chordoma is a common event. Therefore, development of novel therapeutic strategies is critical for the management of chordoma. There is growing interest in the oncology community in the immunoregulatory receptor PD-1 and the corresponding B7 family of ligands, including programmed cell death ligand 1 (PD-L1) as a potential mechanism of tumor immune tolerance and escape. In the inflammatory microenvironment, stimuli such as IFN-γ may upregulate PD-L1 expression in peripheral tissues and immune cells to suppress the immune response. Interestingly, many different malignancies can co-opt this checkpoint system by upregulating PD-L1 constitutively or in response to inflammation. However, the expression of PD-L1 in chordoma remains unknown. In this study, we determined PD-L1 expression in both chordoma cell lines and chordoma tissues. We also evaluated the association between PD-L1 expression and clinical behavior by immunohistochemical (IHC) staining of chordoma tissue microarray (TMA).

Methods: Cell lines, cell culture
UCH1 and UCH2 are established human chordoma cell lines and were kindly provided by Dr. Silke Bruderlein (University Hospitals of Ulm, Germany). Another human chordoma cell line, CH22, was established in our laboratory as previous reported. These cell lines were cultured in DMEM medium, supplemented with 10% inactivated fetal bovine serum (FBS).

Cell lines treated with IFN-γ
Chordoma cells were treated with recombinant human interferon IFN-γ (Pierce Biotechnology, Rockford, IL), as previously described. Briefly, $3 \times 10^5$ cells were incubated at 37°C for 48 hours in medium supplemented with 100 U/mL, and then washed with excess culture medium.

Human chordoma tumor tissues
Nine of the chordoma tissue samples (Tissue1-Tissue9) were obtained from the Massachusetts General Hospital Sarcoma Tissue Bank and were used in accordance with the policies of the institutional review board of the hospital. All diagnoses were confirmed histologically.

Western blotting
Western blot analysis was conducted to detect PD-L1 and brachyury protein expression in chordoma cell lines and chordoma tissues. Protein lysates were harvested from osteosarcoma cells by using 1×RIPA Lysis Buffer and the concentrations were determined using Protein Assay Reagent. The primary
antibodies for PD-L1 (1:1000 dilution), brachyury (1:1000 dilution) and actin (1:2000 dilution) were purchased from Abcam, Santa Cruz and Sigma-Aldrich respectively.

**Chordoma tissue microarray (TMA) and immunohistochemistry**

Chordoma tissue microarray was constructed from the Massachusetts General Hospital Sarcoma Tissue Bank, which contains 84 chordoma paraffin blocks. PD-L1 expression was determined by IHC with the HRP-DAB System Cell and Tissue Staining Kit. TMA was probed with primary rabbit PD-L1 antibody at 4 °C overnight (1:50 dilution, in 1% bovine serum albumin PBS). The staining intensity pattern was scored as follows: 0+, no staining; 1+, weak staining; 2+, moderate staining; and 3+, strong staining.

**Results:** All three chordoma cell lines showed PD-L1 protein expression. Higher PD-L1 protein expression was induced with IFN-γ treatment in chordoma cell lines UCH1 and UCH2. PD-L1 protein was also expressed in chordoma tumor tissues. The level of PD-L1 expression was significantly higher in non-survival patients than survival patients group.

**Discussion:** In this study we show for the first time that PD-L1 protein is expressed in chordoma cell lines and tissue tissues. IFN-γ can upregulate PD-L1 expression in UCH1 and UCH2 cell lines. Nearly 90% of chordoma tissues express PD-L1 at different levels.

**Significance:** This study demonstrated that PD-L1 protein is expressed in chordoma cell lines and chordoma tissues. Given that multiple agents targeting PD-L1 are in clinical development, the results from this study may lead to a promising PD-L1 based novel immunotherapy strategy for chordoma clinical trials.

![Figure 1. PD-L1 expression in chordoma cell lines and chordoma tissues.](image-url)
Figure 2. Association of PD-L1 expression with clinical outcome in chordoma. A. Distribution of PD-L1 staining scores among the survival and non-survival. B. Representative images of different IHC staining intensities of PD-L1 were shown in chordoma tissues.