The role of the B7x signaling pathway in the progression of pancreatic neuroendocrine tumors

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Introduction

Cancer immunotherapy is rapidly becoming an important component of treatment for patients with a variety of tumor types. The B7 family, and their receptors the CD28 family, are major immune checkpoints that regulate T-cell function, which makes these pathways very attractive therapeutic targets. A newly characterized member of the B7/CD28 family, B7x, is expressed in a number of tumors and can modulate cancer development and progression by inhibiting T-cell function, thus making it an appealing target for immunotherapy. In the present study, we explored the expression and role of B7x in the development and progression of pancreatic neuroendocrine tumors (PNETs).

Materials and Methods

Materials:
- Human tissue specimens: 30 PNET tissues and matched normal tissue.
- Men1 KO mice: We developed a Men1 conditional KO mouse model that develops islet hyperplasia at 6 months and insulinomas at 12 months.
- N134 islet β-cell tumor cell line: A pancreatic β-cell tumor cell line derived from a tumor from a RIP-Tag transgenic animal.

Experimental methods:
- To determine B7x expression in human and murine PNETs by LCM real-time RT-PCR and IHC assays.
- To investigate the molecular mechanism of B7x immune checkpoint regulation in PNET tumorigenesis using our in vitro and in vivo models.
  - To evaluate the expression of PCNA, HIF-1α, and B7x in the tumor microenvironment of mice at different time points (3, 6, and 12 months) by an IHC assay.
  - To investigate if increased HIF-1α expression induces B7x transcriptional activation under hypoxic conditions (1% O2) by a luciferase assay.
  - To determine if the transcription factor HIF-1α binds the B7x promoter by a chromatin immunoprecipitation (ChIP)-PCR assay.

Results

B7x over-expression in human PNET samples:
I. B7x is expressed in 60.5% of human PNETs (Stage I: 54.8%, II: 57.5%, III: 75%, and IV: 100%).
II. There is a significant correlation between B7x expression with tumor size (R=0.7818, P<0.001) and tumor cell proliferation by Ki67 staining (R=0.8621, P<0.001).

Conclusions

Our results showed that B7x was expressed to a high degree in PNETs and the molecular mechanism behind the upregulation of the immune checkpoint B7x may be the expression of HIF-1α following relative hypoxia resulting from the rapid growth of tumor cells in the tumor microenvironment. These findings suggest that targeting B7x offers a promising strategy for the immunotherapy of patients suffering from PNETs.

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