# 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).

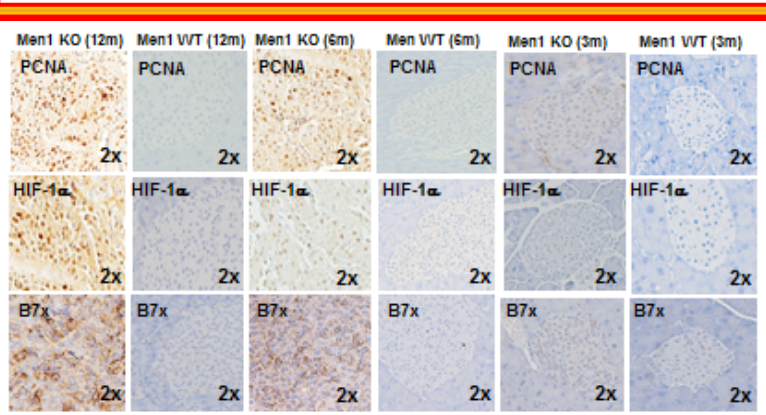


**Figure 1: HIF-1α overexpression coincides with the upregulation of B7x in the tumor microenvironment of *Men1* KO mice**. The proliferated β-cells resulting in the overexpression of HIF-1α in the tumor microenvironment of *Men1* KO mice at 12 months (insulinoma) and at 6 months (hyperplasia) are associated with the upregulation of B7x but not in islets of *Men1* KO mice at 4 months and islets of *Men1* WT mice by IHC staining.

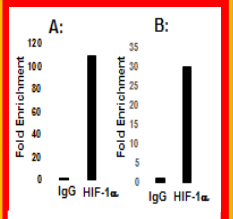


**Figure 2:** ChIP-PCR analysis of HIF-1α binding to the B7x promoter in the N134 β-cells under hypoxic conditions (A) and tumor tissues (B).


**Figure 3: HIF-1α regulates B7x expression *in vitro*.** When cultured under hypoxic conditions, N134 islet tumor β-cells that lack B7x expression were found to induce the upregulation of HIF-1α and B7x (A); however, the expression levels of HIF-1α and B7x were not induced in the islet tumor cells following HIF-1α siRNA treatment (B).

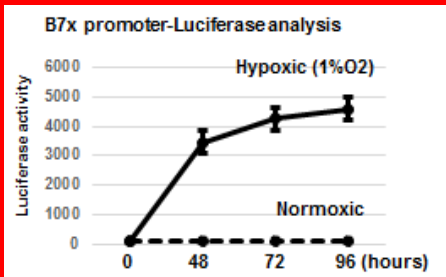


**Figure 4:** We demonstrated that B7x transcriptional activity was significantly increased under hypoxic conditions in N134 β-cells using a promoter luciferase analysis.

## 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.

### Acknowledgements

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