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Original contribution

# B7-H4 is a prognostic biomarker for poor survival in patients with pancreatic cancer☆,☆☆

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**Summary** B7-H4 belongs to the immune costimulatory B7 family and is thought to negatively regulate T-cell–mediated immunity, and may contribute an important role in tumor immune evasion. Although the expression of B7-H4 has been observed in human pancreatic cancer, the prognostic significance of this expression is poorly understood. This present study explored the prognostic value of B7-H4 in pancreatic cancer. Patients with pancreatic cancer and healthy controls were recruited at the Second Affiliated Hospital to Zhejiang University from January 2011 to December 2014. Expression of B7-H4 was assessed by immunohistochemistry. Immunohistochemical analysis indicated that B7-H4 was expressed in 100% (188/188) of the pancreatic cancer tumor tissue samples, while only in 68% (17/25) of normal pancreatic tissue samples. Furthermore, the expression levels of B7-H4 in pancreatic cancer patients were significantly higher than in controls ($P < .01$). A significant difference in B7-H4 expression was observed between patients with late tumor-node-metastasis (TNM) stage (III and IV) and early TNM stage (I and II) ($P < .01$). The expression of B7-H4 was associated with distant metastasis ($P < .01$) and differentiation ($P < .01$). In addition, B7-H4 expression ($P < .01$), distant metastasis ($P < .01$), TNM stage ($P < .01$), differentiation ($P < .01$) and chemotherapy treatment ($P < .05$) were indicators of poor overall survival time. Multivariate survival analysis indicated that B7-H4 expression, distant metastasis, and chemotherapy treatment ($P < .05$) were

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independent prognostic indicators of poor overall survival. In conclusion, B7-H4 is highly expressed in pancreatic cancer, and is an independent predictor of poor prognosis in patients with pancreatic cancer. B7-H4 may represent an immunotherapeutic target in pancreatic cancer.


## 1. Introduction

Pancreatic cancer has one of the highest mortality rates of all cancers, with an overall 5-year survival rate of <6% [1]. The prognosis for pancreatic cancer is poor because of the difficulties in diagnosis and aggressive nature of the disease [2]. Pancreatic cancer is characterized by rapid growth and metastasis in the early stages, and patients are often not diagnosed until the disease has reached an advanced stage [3]. There is a critical need to identify biomarkers of early disease, as well as to define biomarkers with prognostic significance for progression and metastasis of pancreatic cancer; these biomarkers may be discovered by evaluation of tissues from patients who have undergone resection of pancreatic cancer [4]. The B7 family members are hypothesized to play roles in tumor immunity, and may in turn impact the prognosis of patients with pancreatic cancer [5].

B7-H4 that is a member of B7 protein family is a negative co-stimulatory molecule which inhibits T cell proliferation, interleukin-2 production, cell-cycle progression, and is negative correlated with T cell infiltration in tumor [6,7]. B7-H4 is expressed in various tumors, including non–small cell lung cancer [8], breast cancer [9], ovarian cancer [10], and renal cell carcinoma [11]. B7-H4 was highly expressed in cancerous and non-cancerous cells in patients with and without pancreatic cancer [12]. It was reported that tumor-associated Tregs can trigger macrophages to secrete IL-6, then STAT3 activated by IL-6 binds to the promoter of B7-H4 gene and in turn enhances B7-H4 expression in both tumor cells and APCs or other microenvironment-supporting cells [13-15]. B7-H4 induced apoptosis in tumor cells [16]. Aberrant expression of B7-H4 was found to associate with poorer overall survival [17]. The previous studies indicated that B7-H4 might be a useful biomarker for tumor diagnosis and prognosis [18,19]. Some studies have reported B7-H4 expression in pancreatic cancer [11,16,20], and the expression level was found to be associated with adverse pathological features [21]; however, the association of B7-H4 protein expression with histological grade or disease stage of pancreatic cancer has not been definitively assessed.

Our present study evaluated the expression of B7-H4 in pancreatic cancer using immunohistochemical stain analysis to determine whether B7-H4 is an independent prognostic indicator for overall survival (OS) in patients with pancreatic cancer. These findings may provide an experimental basis for exploring the potential of B7-H4 as an immunotherapeutic target in pancreatic cancer.

## 2. Materials and methods

### 2.1. Patients and tissue specimens

Tissue samples from 188 patients pathologically diagnosed with pancreatic cancer between January 2011 and December 2014 at The Second Affiliated Hospital, Zhejiang University School of Medicine were analyzed. Clinical parameters included patient demographics, tumor differentiation, tumor stage, and treatment modality. Furthermore, 25 patients with benign pancreatic disease, such as pancreatitis and cysts, were enrolled as a control group, and were accrued in the same period from January 2011 to December 2014. All tissues were fixed in 10% buffered formalin and embedded in paraffin. All archival hematoxylin and eosin (H&E)–stained sections were reviewed by two pathologists. The present study was approved by the Ethics Committee of The Second Affiliated Hospital, Zhejiang University School of Medicine.

### 2.2. Immunohistochemistry staining

Immunohistochemistry staining was performed using the two-step EnVision™ method (Dako, Glostrup, Denmark). Briefly, paraffin-embedded tissues were cut into 5 μm serial sections, transferred onto adhesive slides, and dried at 65°C for 2 hours. The sections were deparaffinized with xylene and rehydrated through graded alcohols. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide solution for 30 minutes at room temperature and antigen retrieval was performed at 100°C for 30 minutes in a citrate buffer (10 mmol/L; pH 6.0). After washing three times with phosphate-buffered saline (PBS) for 5 minutes each, sections were incubated with 10% normal goat serum to block nonspecific binding. Sections were then incubated with a rabbit anti-human B7-H4 monoclonal antibody (1:400 dilution; clone number EP1165; Abcam) at 4°C overnight, followed by immunodetection using the DAKO EnVision detection system (K5007). Slides were counterstained with Mayer's hematoxylin, dehydrated in graded alcohol, and mounted with neutral resin. Negative control staining was performed by replacing the primary antibody with PBS. Human tonsil tissue was used as a positive control sample for B7-H4 expression.

### 2.3. Evaluation of B7-H4 staining

The sections were examined and scored by two pathologists without knowledge of the patients' clinical records. Five