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Axed MUC4 (MUC4/X) aggravates pancreatic malignant phenotype by activating integrin-β1/FAK/ERK pathway

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ABSTRACT

Alternative splicing is evolving as an eminent player of oncogenic signaling for tumor development and progression. Mucin 4 (MUC4), a type I membrane-bound mucin, is differentially expressed in pancreatic cancer (PC) and plays a critical role in its progression and metastasis. However, the molecular implications of MUC4 splice variants during disease pathogenesis remain obscure. The present study delineates the pathological and molecular significance of a unique splice variant of MUC4, MUC4/X, which lacks the largest exon 2, along with exon 3. Exon 2 encodes for the highly glycosylated tandem repeat (TR) domain of MUC4 and its absence creates MUC4/X, which is devoid of TR. Expression analysis from PC clinical samples revealed significant upregulation of MUC4/X in PC tissues with most differential expression in poorly differentiated tumors. *In vitro* studies suggest that overexpression of MUC4/X in wild-type-MUC4 (WT-MUC4) null PC cell lines markedly enhanced PC cell proliferation, invasion, and adhesion to extracellular matrix (ECM) proteins. Furthermore, MUC4/X overexpression leads to an increase in the tumorigenic potential of PC cells in orthotopic transplantation studies. In line with these findings, doxycycline-induced expression of MUC4/X in an endogenous WT-MUC4 expressing PC cell line (Capan-1) also displayed enhanced cell proliferation, invasion, and adhesion to ECM, compared to WT-MUC4 alone, emphasizing its direct involvement in the aggressive behavior of PC cells. Investigation into the molecular mechanism suggested that MUC4/X facilitated PC tumorigenesis *via* integrin-β1/FAK/ERK signaling pathway. Overall, these findings revealed the novel role of MUC4/X in promoting and sustaining the oncogenic features of PC.

1. Introduction

Pancreatic cancer (PC) is the third leading cause of cancer-related deaths in the USA and is predicted to become second by 2030 [1,2]. Due to high complexity and degree of heterogeneity, the molecular mechanisms for progression and early metastasis remain obscure. Alternative splicing is one of the mechanisms that contributes to the complexity and effectiveness of disease progression; therefore, it stands to reason that cancer cells adopt alternative splicing to sustain themselves within a hostile environment. Recently, alternative splicing has gained immense attention in cancer research due to its strong association with tumor development as well as an attractive anticancer therapeutic target(s) [3]. For example, splice variant of the CD44 adhesion molecule has been implicated in the metastatic spread of various human tumor cells and has been correlated with poor prognosis [4]. Another example of a well studied oncogenic splice variant in PC, is the paired related homeodomain transcription factor (PrrX1). Two alternative isoforms of PrrX1 (PrrX1a, and PrrX1b) have shown distinct but complementary roles in PC oncogenesis [5]. Prrx1b regulates epithelial to mesenchymal transition (EMT) which promotes invasion, while Prrx1a regulates mesenchymal to epithelial transition (MET) by tumor redifferentiation which enhances metastatic colonization [5]. All these previous studies suggest that splice variants have distinct and pronounced functions at different stages of tumor progression, and therefore further exploration is merited for delineating their mechanistic and therapeutic significance in a highly lethal malignancy like PC.

The type 1 transmembrane mucin MUC4, is one of the most differentially overexpressed genes in PC, with undetectable expression in normal pancreas and *de novo* expression in early precursor lesions [6]. With this differential expression in PC, MUC4 has been implicated as a

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