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Bile acids-mediated overexpression of MUC4 via FAK-dependent c-Jun activation in pancreatic cancer

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ABSTRACT

The majority of pancreatic cancer (PC) patients are clinically presented with obstructive jaundice with elevated levels of circulatory bilirubin and alkaline phosphatases. In the current study, we examined the implications of bile acids (BA), an important component of bile, on the pathophysiology of PC and investigated their mechanistic association in tumor-promoting functions. Integration of results from PC patient samples and autochthonous mouse models showed an elevated levels of BA ($p < 0.05$) in serum samples compared to healthy controls. Similarly, an elevated BA levels was observed in pancreatic juice derived from PC patients ($p < 0.05$) than non-pancreatic non-healthy (NPNH) controls, further establishing the clinical association of BA with the pathogenesis of PC. The tumor-promoting functions of BA were established by observed transcriptional upregulation of oncogenic MUC4 expression. Luciferase reporter assay revealed distal MUC4 promoter as the primary responsive site to BA. *In silico* analysis recognized two c-Jun binding sites at MUC4 distal promoter, which was biochemically established using ChIP assay. Interestingly, BA treatment led to an increased transcription and activation of c-Jun in a FAK-dependent manner. Additionally, BA receptor, namely FXR, which is also upregulated at transcriptional level in PC patient samples, was demonstrated as an upstream molecule in BA-mediated FAK activation, plausibly by regulating Src activation. Altogether,

Abbreviations: PC, pancreatic cancer; PDAC, pancreatic ductal adenocarcinoma; BA, bile acids; DCA, deoxycholic acid; CDCA, chenodeoxycholic acid; FXR, farnesoid-x-receptor; FAK, focal adhesion kinase; MAPK, mitogen activated protein kinase; JNK, c-Jun N-terminal kinase; PI3K, phosphoinositide 3-kinase; ChIP, chromatin immunoprecipitation.

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these results demonstrate that elevated levels of BA increase the tumorigenic potential of PC cells by inducing FXR/FAK/c-Jun axis to upregulate MUC4 expression, which is overexpressed in pancreatic tumors and is known to be associated with progression and metastasis of PC.

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1. Introduction

In 2014, about 45,000 new cases of pancreatic cancer (PC) were diagnosed in the United States, of which pancreatic ductal adenocarcinoma (PDAC) represents the major histological type (Siegel et al., 2014). The majority of tumors (about 75%) arise at the head of the pancreas (Wisinski et al., 2007). Anatomically, the pancreatic duct is placed close to the common bile duct, and unite at the point known as the ampulla of Vater, and secrete their contents into the duodenum, the proximal site of the intestine (Bardeesy and DePinho, 2002). Approximately, 70% of PC patients develop extrahepatic cholestasis due to blockage of the common bile duct by increasing tumor size and results in multiple organ failure and early death (Nakamura et al., 2002). Due to bile duct obstruction, extrahepatic cholestasis exhibits obstructive jaundice, hyperbilirubinemia and elevated circulatory levels of bile acids (BA).

BA are amphiphilic molecules and are the main component of bile along with cholesterol, phospholipids, and bilirubin (Baptissart et al., 2013). By utilizing a series of enzymatic modifications, BA are synthesized in the liver using cholesterol as a precursor. BA are further modified by bacterial species present in the colon to form secondary BA (Baptissart et al., 2013). Dietary fat is a stimulus for BA secretion into the intestine, which is required for the proper digestion of fatty foods (Baptissart et al., 2013). Though bile-reflux has been associated with esophageal and gastric cancers, its association with PC pathogenesis has not been investigated (Sifrim, 2013; Tsoukali and Sifrim, 2013). A recently performed meta-analysis has revealed an increased risk of PDAC in patients with the cholecystectomy history (Lin et al., 2012). It has been proposed that the increased levels of cholecystokinin, which is known to stimulate the growth of human PC cell lines, promote pancreatic carcinogenesis in hamsters (Howatson and Carter, 1985).

BA have been shown to participate in tumor progression using multiple mechanisms including, alteration in the expression of oncogenic mucins (Mariette et al., 2004; Piessen et al., 2007). Interestingly, PC is characterized by aberrant mucins expression (Kaur et al., 2013c; Joshi et al., 2014; Joshi et al., 2015). Among various mucins expressed in PC, MUC4 is one of the top-differentially expressed protein compared to normal pancreas (Andrianifahanana et al., 2001; Iacobuzio-Donahue et al., 2003). We and others have established the oncogenic role of MUC4 in PC, and inhibition of MUC4 expression led to reduced PC cell proliferation, migration, and chemoresistance (Moniaux et al., 2007; Chaturvedi et al., 2007; Lakshmanan et al., 2015). In the present study, we have evaluated the role of BA in the regulation of MUC4 expression in PC. The findings from the current study, for the first time, have demonstrated that BA levels are significantly high in the serum and pancreatic juice samples obtained from PC patients. Using defined spontaneous mouse model of PC, we found that BA levels increased with the severity of PC, which we mechanistically linked with BA-mediated expression of oncogenic MUC4 through FAK-dependent activation of c-Jun. Further studies demonstrated the role of FXR as the upstream molecule in this FAK/c-Jun/MUC4 axis.

2. Materials and methods

2.1. Cell culture and reagents

All human PC cell lines were obtained from ATCC, except T3M4 and CD18/HPAF. CD18/HPAF is a metastatic clone derived from the HPAF cell line (Mullins et al., 1991), whereas T3M4 cell line is derived from lymph node metastasis of pancreatic adenocarcinoma (Okabe et al., 1983). Human ductal pancreatic epithelial (HDPE) cells were a generous gift of Dr. Thiru Arumugam (MD Anderson, Houston, Texas) and cultured in keratinocyte serum-free (KSF) medium supplemented with epidermal growth factor and bovine pituitary extract. All PC cell lines were cultured in DMEM (supplemented with 10% heat-inactivated FBS, penicillin, and streptomycin (100 μg/ml)) at 37 °C with 5% CO_2 and were tested mycoplasma-free before conducting the experiments. Deoxycholic (DCA) and chenodeoxycholic acid (CDCA) were dissolved in sterile ethanol. For inhibition studies, wortmannin (phosphoinositide 3-kinase (PI3K) inhibitor, 1 μM, Cell Signaling Technology), SP100625 (JNK inhibitor; 35 μM, Merck Millipore), FAK inhibitor 14 (FAK inhibitor, 15 μM, Cayman's chemical), U1026 (MAPK inhibitor, 10 μM, Promega) and actinomycin-D (2 μg/ml, Sigma–Aldrich) were given 1 h prior to BA treatment. To transiently knockdown FXR, commercially available FXR siRNA (Santa Cruz Biotechnologies (SCB); Dallas, TX, USA) were used. For transfection purposes, lipofectamine 2000 (Life Technologies; Carlsbad, CA, USA) was used, according to the manufacturer's protocol.

2.2. Procurement of human and murine PDAC samples

All human PDAC samples used in the present study were de-identified and a written informed consent was received from all recruited patients before enrollment at respective institutions. The collection of secreted pancreatic juice upon secretin induction from PC patients was approved by the Mayo Clinic Institutional Review Board (IRB#07-0000099) and the detailed information has been provided in our previous publication (Kaur et al., 2013a). Plasma samples were collected using an