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MUC4 potentiates invasion and metastasis of pancreatic cancer cells through stabilization of fibroblast growth factor receptor 1

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MUC4 is a type-1 transmembrane mucin differentially expressed in multiple cancers and has previously been shown to potentiate progression and metastasis of pancreatic cancer. In this study, we investigated the molecular mechanisms associated with the MUC4-induced invasion and metastasis in pancreatic cancer. Stable silencing of MUC4 in multiple pancreatic cancer cells resulted in the downregulation of N-cadherin and its interacting partner fibroblast growth factor receptor 1 (FGFR1) through downregulation of partly by pFAK, pMKK7, pJNK and pc-Jun pathway and partly through PI-3K/Akt pathway. The downregulation of FGFR1 in turn led to downregulation of pAkt, pERK1/2, pNF-κB, pIkBα, uPA, MMP-9, vimentin, N-cadherin, Twist, Slug and Zeb1 and upregulation of E-cadherin, Occludin, Cytokeratin-18 and Caspase-9 in MUC4 knockdown BXPC3 and Capan1 cells compared with scramble vector transfected cells. Further, downregulation of FGFR1 was associated with a significant change in morphology and reorganization of the actin-cytoskeleton, leading to a significant decrease in motility ($P < 0.00001$) and invasion ($P < 0.0001$) *in vitro* and decreased tumorigenicity and incidence of metastasis *in vivo* upon orthotopic implantation in the athymic mice. Taken together, the results of the present study suggest that MUC4 promotes invasion and metastasis by FGFR1 stabilization through the N-cadherin upregulation.

Introduction

Despite a welcome decline in mortality rate over the past decade, pancreatic cancer (PC) still remains the 10th most commonly diagnosed cancer and the 4th leading cause of cancer-related death in the USA (1,2). The median survival of PC patients is about 4.1 months with the overall 5-year survival rate being less than 5% (2–4). The clinical manifestations of PC usually occur at a late stage, at which time the disease has already spread to local and distant organs (in 85% of patients) (5). To acquire such invasive abilities, epithelial cancer cells undergo several phenotypic changes, similar to those seen during embryonic development. This process is termed epithelial to mesenchymal transition (EMT). Despite growing knowledge about the events underlying PC development, translation of this information into effective therapies and treatments are limited. Besides, precise molecular mechanisms by which PC cells progress from a non-invasive to a highly metastatic stage are largely unclear. Hence, in the present study, efforts are being made to identify the molecular events that underlie the metastatic ability of this lethal disease.

Abbreviations: DMEM, Dulbecco's modified Eagle's medium; EMT, epithelial to mesenchymal transition; FAK, focal adhesion kinase; FGFR1, fibroblast growth factor receptor 1; MMP-9, matrix metalloproteinase-9; PC, pancreatic cancer.

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Previous reports have shown that around 90% of cancer-related deaths are mainly due to metastasis, not due to primary tumors (6). The process of invasion and metastasis in PC is still inadequately understood. Normally, invasion and metastasis occurs in sequential steps, which involves detachment of cancer cells from the primary tumor and invasion into the surrounding healthy tissues followed by intravasation, extravasation and finally colonization at distant sites. However, in recent years, an enormous amount of data has suggested that cancer cells utilize the same mechanisms as healthy embryonic cells (i.e. grastulation by the process of changing from an epithelial to a mesenchymal-like phenotype) called EMT. This is a phenomenon whereby malignant cells contribute to invasion, metastatic dissemination and acquisition of therapeutic resistance (7,8). The process of EMT involves the disruption of cell–cell and cell-extracellular matrix interactions, loss of cell polarity, reorganization of the actin cytoskeleton, acquisition of a mesenchymal phenotype with reduced intercellular interactions and increased migratory capacity. This is associated with a significant increase in the expression of mesenchymal markers such as vimentin and vitronectin-75 (9), downregulation of epithelial markers such as E-cadherin and cytokeratin-18 (10) and upregulation of transcription factors associated with the EMT process such as Twist, Snail and Slug (11), leading to invasion and metastasis.

MUC4 is a large membrane-anchored glycoprotein that is aberrantly expressed in many cancers (12–18). Its expression is undetectable in the normal pancreas but increases progressively in pancreatic intraepithelial neoplasia (19,20) and is strongly expressed in PC (20–23). We have previously shown that MUC4 induces cellular transformation of NIH 3T3 fibroblast cells, potentiates PC cell growth and metastasis and contributes to gemcitabine resistance (24–27). Subsequently, we have also reported that MUC4, via its interaction with the epidermal growth factor receptor family member human epidermal growth factor receptor-2, induces downstream signaling that favors proliferation, motility, invasion and promotes cell survival in PC and other malignancies (25,28). Further, human epidermal growth factor receptor-2 also activates focal adhesion kinase (FAK), a key protein involved in PC metastasis and invasion (25,28), highlighting its role as a promoter of aggressiveness in PC cells. However, its precise involvement in the metastasis and invasion of PC through a process of EMT has not been explored. In the current study, we have explored the signaling mechanism by which MUC4 potentiates invasion and metastasis, partly through regulating the EMT process and stabilizing fibroblast growth factor receptor 1 (FGFR1), which may improve our understanding of the events involved in the progression and metastasis of PC and may aid in the identification of novel targets for better management of PC.

Materials and methods

Antibodies

The anti-MUC4 mouse monoclonal antibody (8G7) used in this study was developed by our laboratory (29). The antibodies, cleaved caspase-9 (Asp330), phosphorylated/total pAkt (Serine 473)/tAkt, pMKK7 (Ser271/Thr275)/tMKK7, pJNK(Thr183/Tyr185)/tJNK, pc-JUN(Ser63)/tc-JUN and phospho-ERK1/2 / tERK1/2, phospho–FAK (pFAK-Tyr 925, Tyr 576/577)/tFAK, pHER2 (Tyrosine 1248)/tHER2, NFκB, and pIκB/IκB were obtained from cell signaling (Danvers, MA, USA). The antibodies against MMP-9 and N and E-cadherin were gifts from Dr. Keith R. Johnson (University of Nebraska Medical Center). The β-actin antibody was obtained from Sigma-Aldrich (St. Louis, MO). The secondary antibodies, anti-mouse and anti-rabbit IgGs conjugated to horseradish peroxidase, were obtained from GE Healthcare Biosciences, (Uppsala, Sweden). The fluorescein isothiocyanate–conjugated anti-mouse secondary antibody was obtained from Invitrogen (California, USA).