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Research Article

Conditionally immortalized human pancreatic stellate cell lines demonstrate enhanced proliferation and migration in response to IGF-I

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ABSTRACT

Pancreatic stellate cells (PSCs) play a key role in the dense desmoplastic stroma associated with pancreatic ductal adenocarcinoma. Studies on human PSCs have been minimal due to difficulty in maintaining primary PSC in culture. We have generated the first conditionally immortalized human non-tumor (NPSC) and tumor-derived (TPSC) pancreatic stellate cells via transformation with the temperature-sensitive SV40 large T antigen and human telomerase (hTERT). These cells proliferate at 33°C. After transfer to 37°C, the SV40LT is switched off and the cells regain their primary PSC phenotype and growth characteristics. NPSC contained cytoplasmic vitamin A-storing lipid droplets, while both NPSC and TPSC expressed the characteristic markers αSMA, vimentin, desmin and GFAP. Proteome array analysis revealed that of the 55 evaluated proteins, 27 (49%) were upregulated ≥3-fold in TPSC compared to NPSC, including uPA, pentraxin-3, endoglin and endothelin-1. Two insulin-like growth factor binding proteins (IGFBPs) were inversely expressed. Although discordant IGFBP-2 and IGFBP-3 levels, IGF-I was found to stimulate proliferation of both NPSC and TPSC. Both basal and IGF-I stimulated motility was significantly enhanced in TPSC compared to NPSC. In conclusion, these cells provide a unique resource that will facilitate further study of the active stroma compartment associated with pancreatic cancer.

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Abbreviations: PSC, pancreatic stellate cell; NPSC, non-tumor derived pancreatic stellate cell; TPSC, tumor-derived pancreatic stellate cell; GFAP, glial fibrillary acidic protein; αSMA, alpha smooth muscle actin; ECM, extra cellular matrix; IGF, insulin-like growth factor; IGFBP, insulin-like growth factor binding protein; ITS, insulin-transferrin-selenium

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Introduction

Pancreatic ductal adenocarcinoma is a highly malignant tumor with the highest death rate among all cancers. As this cancer shows no symptoms in its early stages it has a low probability of detection, and the majority (>85%) of pancreatic cancer patients already have locally advanced disease or metastases at time of initial diagnosis making them unsuitable for surgical treatment [1]. In addition, pancreatic cancer is resistant to chemotherapy and currently available treatments provide only marginal survival benefits. The characteristic dense stroma surrounding the tumor cells is believed to promote tumor survival and resistance to treatment by creating a physical barrier and block drug distribution [2,3]. Although being a central pathological feature of pancreatic cancer, the cellular and molecular interactions underlying the stroma reaction remain incompletely understood.

Pancreatic stellate cells (PSCs) are myofibroblast-like cells and the key fibrogenic cell type of the pancreas. They are believed to be the source of the stromal reaction associated with pancreatic cancer [4]. PSCs are normally found in an inactive, quiescent state in the healthy pancreas, where they maintain normal pancreatic architecture. Quiescent PSCs can be identified by the presence of cytoplasmic retinoid (vitamin A)-storing lipid droplets and expression of intermediate filaments and mesenchymal markers such as vimentin, desmin and glial fibrillary acidic protein (GFAP) [5,6]. During local inflammation or pancreatic cancer, the PSCs transform from a quiescent to an active state, leading to loss of cytoplasmic vitamin A-storing lipid droplets, increased expression of alpha smooth muscle actin (αSMA) and induction of a number of factors such as PDGF, TGF-β, IL-6 and others that contribute to the aggressive growth of pancreatic cancer [7]. Activated PSCs have also been suggested to play an important role in development of pancreatic cancer metastases by facilitating migration, extracellular matrix (ECM) deposition and remodeling.

Until recently, studies on human PSCs have been minimal due to limited availability of fresh resected pancreatic specimens, as well as difficulties in isolating and culturing these cells in the laboratory environment. Once isolated, the primary PSCs undergo senescence within a limited number of passages, making it difficult to obtain sufficient cell quantities for functional studies [8]. To overcome the limited availability, other laboratories have previously developed immortalized cell lines from human PSCs that unlike the primary cells, divide at a fast rate in the laboratory [9,8]. These cell lines rapidly generate enough cells to study. However, their fast growth rate and continuous proliferation do not accurately represent the primary cell phenotype.

The aim of the present study was to develop exclusively unique and highly relevant cell lines from human normal non-tumor (NPSC) and tumor-derived (TPSC) pancreatic stellate cells. For this purpose, we have generated the first conditionally immortalized human pancreatic stellate cell lines by infection with the temperature-sensitive *SV40LT* gene and telomerase. This technology has been successfully applied previously in cultured human mammary fibroblasts, podocytes, glomerular endothelial and mesangial cells [10–13]. These cells rapidly divide and grow at the "permissive" temperature (33°C). After transfer to the "non-permissive" temperature (37°C), the *SV40LT* gene is switched off and the cells regain their primary PSC phenotype and growth rate. Using these cell lines we sought to identify NPSC and TPSC phenotype characteristics at the protein level and to elucidate their functional responses to insulin-like growth factor-I (IGF-I) on growth and migratory capabilities.

Materials and methods

Primary culture of pancreatic stellate cells

Non-neoplastic and neoplastic pancreatic specimens were obtained from a patient with moderately differentiated pancreatic ductal adenocarcinoma and new onset type-2-diabetes undergoing primary surgical resection. This study was approved by the regional ethical review board in Lund, Sweden. Isolated pancreatic stellate cells from adjacent safe margin specimen were considered to be normal. Pancreatic stellate cells were isolated by the outgrowth method as described previously [6] and cultured in 25-cm^2 flasks in DMEM/Hams Nutrient Mix F12 (Invitrogen, Paisley, UK) medium with added antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Invitrogen), 1% Amphotericin-B (Sigma-Aldrich, St. Louis, Mo, USA), 2 mM L-glutamine (Sigma-Aldrich) and 10% FBS (Invitrogen) in a humidified 5% CO_2 atmosphere at 37°C. Pancreatic stellate cell outgrowths appeared at 7–14 d, and allowed to reach 80% confluence before the cells were passaged and transfected with the tsSV40LT gene and hTERT constructs.

Conditional immortalization – retroviral constructs and virus infection

A packaging cell line producing a bicistronic construct containing a temperature-sensitive simian virus-40 large T-antigen (tsSV40LT) and human telomerase (hTERT) as described previously was used [10]. Briefly, cultures of primary human non-tumor or tumor-derived PSCs (at passage 3) were infected with retrovirus-containing supernatants from the packaging cell line (tsTTERT C126). Midconfluent cells in log-phase growth were exposed to freshly thawed filtered (0.45 μm) supernatant mixed 7:3 with growth medium and 10 μg/ml polybrene (Sigma-Aldrich). After 24 h, cultures were replaced with normal growth medium and the cells were allowed to reach confluence at 37°C. The culture medium was then supplemented with 0.5 mg/ml G418 (Life Technologies BRL, Paisley, UK) until selection was completed (14 days). Selection and continuous culture were carried out at 33°C. After 2 weeks clones of healthy selected cells began to appear. Surviving cells were allowed to approach confluence in standard medium. Subcloning by limited dilution was achieved by seeding cells in 96-well plates at a density of ~1 cell/well. Approximately 10 clones of each cell line were further characterized based on the greatest similarity to the primary cells and subjected to a second round of subcloning and characterization. Conditionally immortalized PSCs were growth in RPMI 1640 medium supplemented with antibiotics (100 U/ml penicillin and 100 μg/ml streptomycin; Invitrogen), ITS (Sigma-Aldrich) and 10% FBS (GIBCO). Cells were grown to 80% confluence before thermo-switching to 37°C. At both temperatures, cells were fed with fresh medium three times per week.

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