Pancreatic Stem Cell Literatures

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on pancreatic stem cell.
[Smith MH. **Pancreatic Stem Cell Literatures**. *Stem Cell* 2012;3(2):146-163] (ISSN 1545-4570). http://www.sciencepub.net/stem. 7

Key words: stem cell; life; gene; DNA; protein; pancreatic stem cell

Literatures

Abe, Y., T. Ito, et al. (2009). "Nonmyeloablative allogeneic hematopoietic stem cell transplantation as immunotherapy for pancreatic cancer." Pancreas **38**(7): 815-9.

OBJECTIVE: Advanced unresectable pancreatic cancer has an extremely poor prognosis despite intensive chemotherapy. As a new therapeutic modality, we investigated nonmyeloablative allogeneic hematopoietic stem cell transplantation from a related donor. METHODS: Five patients with chemotherapy-resistant pancreatic cancer received allogeneic peripheral blood stem cell transplantation after a conditioning regimen consisting of low-dose total body irradiation and fludarabine. The prophylaxis for graft-versus-host disease consisted of mycophenolate mofetil and cyclosporine. RESULTS: The median age of the 5 patients was 54 years, and the median duration from diagnosis to nonmyeloablative allogeneic hematopoietic stem cell transplantation was 10 months. Three of the 5 patients achieved complete donor chimerism of peripheral T cells, at a median time of day 42. Acute graft-versus-host disease developed in 3 patients: grade 2 in 2 patients and grade 1 in 1. Tumor reduction was observed in 2 patients: 1 patient showed disappearance of the pancreatic tumor, and the other patient showed approximately 20% reduction of the tumor. Marked elevation of tumor necrosis factor alpha was observed as the tumor regressed. CONCLUSIONS: Although advanced pancreatic cancer progresses rapidly, some graft-versus-tumor effects and pivotal role of tumor necrosis factor alpha were suggested. To obtain the durable response, patient selection and new strategies become important.

Argani, P., C. Rosty, et al. (2001). "Discovery of new markers of cancer through serial analysis of gene expression: prostate stem cell antigen is overexpressed in pancreatic adenocarcinoma." Cancer Res **61**(11): 4320-4.

Serial analysis of gene expression (SAGE) can be used to quantify gene expression in human tissues. Comparison of gene expression levels in neoplastic tissues with those seen in nonneoplastic tissues can, in turn, identify novel tumor markers. Such markers are urgently needed for highly lethal cancers like pancreatic adenocarcinoma, which typically presents at an incurable, advanced stage. The results of SAGE analyses of a large number of neoplastic and nonneoplastic tissues are now available online, facilitating the rapid identification of novel tumor markers. We searched an online SAGE database to identify genes preferentially expressed in pancreatic cancers as compared with normal tissues. SAGE libraries derived from pancreatic adenocarcinomas were compared with SAGE libraries derived from nonneoplastic tissues. Three promising tags were identified. Two of these tags corresponded to genes (lipocalin and trefoil factor 2) previously shown to be overexpressed in pancreatic carcinoma, whereas the third tag corresponded to prostate stem cell antigen (PSCA), a recently discovered gene thought to be largely restricted to prostatic basal cells and prostatic adenocarcinomas. PSCA was expressed in four of the six pancreatic cancer SAGE libraries, but not in the libraries derived from normal pancreatic ductal cells. We confirmed the overexpression of the PSCA mRNA transcript in 14 of 19 pancreatic cancer cell lines by reverse transcription-PCR, and using immunohistochemistry, we demonstrated PSCA protein overexpression in 36 of 60 (60%) primary pancreatic adenocarcinomas. In 59 of 60 cases, the

adjacent nonneoplastic pancreas did not label for PSCA. PSCA is a novel tumor marker for pancreatic carcinoma that has potential diagnostic and therapeutic implications. These results establish the validity of analyses of SAGE databases to identify novel tumor markers.

Bretzel, R. G., M. Eckhard, et al. (2004). "Pancreatic islet and stem cell transplantation: new strategies in cell therapy of diabetes mellitus." Panminerva Med **46**(1): 25-42.

Long-term studies strongly suggest that tight control of blood glucose can prevent the development and retard the progression of chronic complications of type 1 diabetes mellitus. In contrast to conventional insulin treatment, replacement of a patient's islets of Langerhans either by pancreas organ transplantation of by isolated islet transplantation is the only treatment to achieve a constant normoglycemic state and avoiding hypoglycemic episodes, a typical adverse event of multiple daily insulin injections. However, the expense of this benefit is still the need for immunosuppressive treatment of the recipient with all its potential risks. Islet cell transplantation offers the advantage of being performed as a minimally invasive procedure, in which islets can be perfused percutaneously into the liver via the portal vein. As of June 2003, 705 pancreatic islet transplants worldwide have been reported to the International Islet Transplant Registry (ITR) at our Third Medical Department, University of Giessen/Germany. Data analysis shows at 1 year after adult islet transplantation a patient survival rate of 97%, a functioning islet graft in 54% of the cases, whereas insulin independence was meanwhile achieved in 20% of the cases. However, using a novel protocol established by the Edmonton Center/Canada, the insulin independence rates have improved significantly reaching meanwhile a 50-80% level. Finally, the concept of islet cell or stem cell transplantation is most attractive since it offers many perspectives: islet cell availability could become unlimited and islet or stem cells my be transplanted without life-long immunosuppressive treatment of the recipient, just to mention 2 of them.

Cao, D., H. Ji, et al. (2005). "Expression of mesothelin, fascin, and prostate stem cell antigen in primary ovarian mucinous tumors and their utility in differentiating primary ovarian mucinous tumors from metastatic pancreatic mucinous carcinomas in the ovary." Int J Gynecol Pathol **24**(1): 67-72.

Metastatic pancreatic mucinous adenocarcinomas in the ovaries can be difficult to distinguish from primary ovarian mucinous neoplasms because the former can simulate the latter grossly and histologically and both tumor types share the same cytokeratin 7/cytokeratin 20 immunoprofile. We previously reported the utility of loss of Dpc4 expression in distinguishing metastatic pancreatic carcinomas from primary ovarian mucinous tumors. Recently several new pancreatic carcinoma markers have been identified, including mesothelin, fascin, and prostate stem cell antigen (PSCA). In this study we investigate the expression patterns of these markers in 35 primary ovarian mucinous tumors (28 atypical proliferative [borderline] tumors and 7 invasive carcinomas) and 11 metastatic pancreatic mucinous carcinomas in the ovary. Primary ovarian mucinous tumors expressed mesothelin (17%), fascin (26%), and PSCA (43%) less frequently than metastatic pancreatic adenocarcinomas (73%, 73%, and 82%, respectively). Expression of all three markers was seen only in metastatic pancreatic adenocarcinomas (45%), and coexpression of at least two markers was observed significantly more frequently in metastatic (82%) than primary ovarian mucinous tumors (17%). Our results indicate that an immunohistochemical panel including Dpc4, mesothelin, fascin, and PSCA is useful for evaluating difficult mucinous tumors in the ovary when the differential diagnosis includes metastatic pancreatic adenocarcinoma.

Chandra, V., S. G, et al. (2009). "Generation of pancreatic hormone-expressing islet-like cell aggregates from murine adipose tissue-derived stem cells." Stem Cells **27**(8): 1941-53.

The success of cell replacement therapy for diabetes depends on the availability and generation of an adequate number of islets, preferably from an autologous origin. Stem cells are now being probed for the generation of physiologically competent, insulin-producing cells. In this investigation, we explored the potential of adipose tissue-derived stem cells (ASCs) to differentiate into pancreatic hormone-expressing islet-like cell aggregates (ICAs). We initiated ASC culture from epididymal fat pads of Swiss albino mice to obtain mesenchymal cells, murine epididymal (mE)-ASCs. Subsequent single-cell cloning resulted in a homogeneous cell population with a CD29(+)CD44(+)Sca-1(+) surface antigen expression profile. We formulated a 10-day differentiation protocol to generate insulin-expressing ICAs from mE-ASCs by progressively changing the differentiation cocktail on day 1, day 3, and day 5. Our stage-specific approach successfully differentiated mesodermic mE-ASCs into definitive endoderm (cells expressing Sox17, Foxa2, GATA-4, and cytokeratin [CK]-19), then into pancreatic endoderm (cells expressing pancreatic and duodenal homeobox [PDX]-1, Ngn3, NeuroD, Pax4, and glucose transporter 2), and finally into cells expressing

pancreatic hormones (insulin, glucagon, somatostatin). Fluorescence-activated cell sorting analysis showed that day 5 ICAs contained 64.84% +/- 7.03% PDX-1(+) cells, and in day 10 mature ICAs, 48.17% +/- 3% of cells expressed C-peptide. Day 10 ICAs released C-peptide in a glucose-dependent manner, exhibiting in vitro functionality. Electron microscopy of day 10 ICAs revealed the presence of numerous secretory granules within the cell cytoplasm. Calcium alginate-encapsulated day 10 ICAs (1,000-1,200), when transplanted i.p. into streptozotocin-induced diabetic mice, restored normoglycemia within 2 weeks. The data presented here demonstrate the feasibility of using ASCs as a source of autologous stem cells to differentiate into the pancreatic lineage.

Chang, C., X. Wang, et al. (2009). "Mesenchymal stem cells adopt beta-cell fate upon diabetic pancreatic microenvironment." Pancreas **38**(3): 275-81.

OBJECTIVES: : This study observed whether mesenchymal stem cells (MSCs) adopt beta-cell fate upon diabetic microenvironment. METHODS: : We transplanted male porcine MSCs to diabetic female pigs by directly injecting into pancreas. Recipients' sera and pancreatic tissue were analyzed to assess the therapeutic effect. Islets were collected from the sections using laser-capture microdissection. The RNAs from these specimens were extracted and analyzed for insulin and pancreas duodenum homeobox 1 messenger RNA (mRNA) expression. SRY gene was detected from the specimens. RESULTS: : Compared with untreated diabetic controls, blood glucose level decreased greatly in recipients from 18 days (15.44 +/- 0.31 mmol/L vs 16.66 +/- 0.11 mmol/L) and insulin increased from 14 days (0.048 +/- 0.006 U/L vs 0.030 +/- 0.004 U/L). Hematoxylin and eosin-stained sections demonstrated increased islets in recipients and few lymphocytes present. The newly formed islets were smaller than normal islets (47.2 mum +/- 19.6 vs 119.6 +/- 27.7 mum). Reverse transcription-polymerase chain reaction showed that microdissected cells expressed insulin and pancreas duodenum homeobox 1 mRNA (79.3% +/- 16.2% of control, 65.2% +/- 14.8% of control, respectively). Immunoreactivity showed that the transplanted MSCs expressed insulin. SRY gene and insulin mRNA double-positive cells were found in microdissected cells by fluorescence in situ hybridization. CONCLUSIONS: This study shows that MSCs could adopt beta-cell fate in diabetic pancreatic microenvironment without obvious immune rejections. Stem cell transplantation in orthotope is a promising therapy for diabetes.

Cho, Y. M., J. M. Lim, et al. (2008). "Betacellulin and nicotinamide sustain PDX1 expression and induce pancreatic beta-cell differentiation in human embryonic stem cells." Biochem Biophys Res Commun **366**(1): 129-34.

The major obstacle in cell therapy of diabetes mellitus is the limited source of insulin-producing beta cells. Very recently, it was shown that a five-stage protocol recapitulating in vivo pancreatic organogenesis induced pancreatic beta cells in vitro; however, this protocol is specific to certain cell lines and shows much line-to-line variation in differentiation efficacy. Here, we modified the five-stage protocol for the human embryonic stem cell line SNUhES3 by the addition of betacellulin and nicotinamide. We reproduced in vivo pancreatic islet differentiation by directing the cells through stages that resembled in vivo pancreatic organogenesis. The addition of betacellulin and nicotinamide sustained PDX1 expression and induced beta-cell differentiation. C-peptide-a genuine marker of de novo insulin production-was identified in the differentiated cells, although the insulin mRNA content was very low. Further studies are necessary to develop more efficient and universal protocols for beta-cell differentiation.

Danner, S., J. Kajahn, et al. (2007). "Derivation of oocyte-like cells from a clonal pancreatic stem cell line." Mol Hum Reprod **13**(1): 11-20.

Adult pancreatic stem cells (PSCs) are able to differentiate spontaneously in vitro into various somatic cell types. Stem cells isolated from rat pancreas show extensive self-renewal ability and grow in highly viable long-term cultures. Additionally, these cells express typical stem cell markers such as Oct-4, nestin and SSEA-1. Although differentiation potential is slightly decreasing in long-term cultures, it is possible to keep cell lines up to passage 140. Clonal cell lines could be established from different passages and showed similar characteristics. Remarkably, one clonal cell line, generated from passage 75, showed deviant properties during further culture. Clonal cells formed aggregates, which built tissue-like structures in suspension culture. These generated 3D aggregates produced permanently new cells at the outside margin. Released cells had remarkable size, and closer examination by light microscopy analysis revealed oocyte-like morphology. A comparison of the gene expression patterns between primary cultures of passages 8 and 75, the clonal cell line and the produced oocyte-like cells (OLCs) from tissue-like structures demonstrated some differences. Expression of various germ cell markers, such as Vasa, growth differentiation marker 9 and SSEA-1, increased in the clonal cell line, and OLCs showed additionally

expression of meiosis-specific markers SCP3 and DMC1. We here present a first pilot study investigating the putative germ line potential of adult PSCs.

Dor, Y., J. Brown, et al. (2004). "Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation." Nature **429**(6987): 41-6.

How tissues generate and maintain the correct number of cells is a fundamental problem in biology. In principle, tissue turnover can occur by the differentiation of stem cells, as is well documented for blood, skin and intestine, or by the duplication of existing differentiated cells. Recent work on adult stem cells has highlighted their potential contribution to organ maintenance and repair. However, the extent to which stem cells actually participate in these processes in vivo is not clear. Here we introduce a method for genetic lineage tracing to determine the contribution of stem cells to a tissue of interest. We focus on pancreatic beta-cells, whose postnatal origins remain controversial. Our analysis shows that pre-existing beta-cells, rather than pluripotent stem cells, are the major source of new beta-cells during adult life and after pancreatectomy in mice. These results suggest that terminally differentiated beta-cells retain a significant proliferative capacity in vivo and cast doubt on the idea that adult stem cells have a significant role in beta-cell replenishment.

Eshpeter, A., J. Jiang, et al. (2008). "In vivo characterization of transplanted human embryonic stem cell-derived pancreatic endocrine islet cells." Cell Prolif **41**(6): 843-58.

OBJECTIVES: Islet-like clusters (ILCs), differentiated from human embryonic stem cells (hESCs), were characterized both before and after transplantation under the kidney capsule of streptozotocin-induced diabetic immuno-incompetent mice. MATERIALS AND METHODS: Multiple independent ILC preparations (n = 8) were characterized by immunohistochemistry, flow cytometry and cell insulin content, with six preparations transplanted into diabetic mice (n = 42), compared to controls, which were transplanted with either a human fibroblast cell line or undifferentiated hESCs (n = 28). RESULTS: Prior to transplantation, ILCs were immunoreactive for the islet hormones insulin, C-peptide and glucagon, and for the ductal epithelial marker cytokeratin-19. ILCs also had cellular insulin contents similar to or higher than human foetal islets. Expression of islet and pancreas-specific cell markers was maintained for 70 days post-transplantation. The mean survival of recipients was increased by transplanted ILCs as compared to transplanted human fibroblast cells ($P < 0.0001$), or undifferentiated hESCs ($P < 0.042$). Graft function was confirmed by secretion of human C-peptide in response to an oral bolus of glucose. CONCLUSIONS: hESC-derived ILC grafts continued to contain cells that were positive for islet endocrine hormones and were shown to be functional by their ability to secrete human C-peptide. Further enrichment and maturation of ILCs could lead to generation of a sufficient source of insulin-producing cells for transplantation into patients with type 1 diabetes.

Esposito, I., J. Kleeff, et al. (2002). "The stem cell factor-c-kit system and mast cells in human pancreatic cancer." Lab Invest **82**(11): 1481-92.

Stem cell factor (SCF) and its receptor c-kit take part in the regulation of developmental processes of mast cells, hematopoietic stem cells, and melanocytes, as well as in the growth control of human malignancies. To explore the possible role of the SCF-c-kit system and of mast cells in pancreatic cancer, the concomitant expression and distribution of the two molecules were examined in 17 normal and 26 cancerous human pancreatic tissues and in 6 cultured pancreatic cancer cell lines. Mast cell distribution was also evaluated in the same tissue samples. In addition, the effects of SCF and of the c-kit tyrosine-kinase inhibitor STI571 on the growth of the cancer cell lines and of the normal pancreatic ductal cell line TAKA-1 were assessed. SCF immunoreactivity was absent in acinar, ductal, and islet cells of the normal pancreas and faint in pancreatic cancer tissues and cell lines. In contrast, c-kit was clearly present in some normal and hyperplastic ducts of the normal pancreas, in the cancer cells of 73% of the tumor samples, and in all the cell lines tested. Mast cells, identified by tryptase and chymase immunostaining on consecutive tissue sections, showed immunoreactivity for SCF and c-kit in both normal and cancerous specimens and their number was significantly increased ($p = 0.03$) in pancreatic cancer compared with the normal pancreas. SCF showed a dose-dependent growth inhibitory effect on TAKA-1 cells ($p < 0.001$), whereas pancreatic cancer cells were resistant to the SCF-induced growth inhibition. Nonetheless, the growth of TAKA-1 cells and pancreatic cancer cells was inhibited by the c-kit tyrosine kinase inhibitor STI571. In conclusion, the SCF-c-kit system, possibly with the contribution of mast cells, may have a growth-regulating role in the normal pancreas, which is altered during malignant transformation.

Foss, C. A., J. J. Fox, et al. (2007). "Radiolabeled anti-claudin 4 and anti-prostate stem cell antigen: initial imaging in experimental models of pancreatic cancer." Mol Imaging **6**(2): 131-9.

Global expression profiling of pancreatic cancers has identified two cell surface molecules, claudin 4 and prostate stem cell antigen (PSCA), as being overexpressed in the vast majority of cases. Two antibodies, anti-claudin 4 and anti-PSCA, were radiolabeled with iodine 125 ((125)I) for imaging pancreatic cancer xenografts in mice using gamma scintigraphy and single-photon emission computed tomography-computed tomography (SPECT-CT). Immunofluorescence staining of intact and permeabilized Colo357 human pancreatic cancer cells showed strong extracellular staining by both anti-PSCA and anti-claudin 4. Biodistribution studies in claudin 4 and PSCA-expressing Colo357 and PANC-1 subcutaneous xenograft models in mice showed that [(125)I]anti-claudin 4 tumor to muscle ratio uptake was 4.3 in Colo357 at 6 days postinjection and 6.3 in PANC-1 xenografts at 4 days postinjection. Biodistribution of [(125)I]anti-PSCA showed tumor to muscle ratio uptake of 4.9 in Colo357 at 6 days postinjection. Planar gamma scintigraphic imaging in Colo357 xenograft-bearing mice showed clear tumor uptake of [(125)I]anti-claudin 4 by 24 hours postinjection and by 48 hours postinjection for [(125)I]anti-PSCA. SPECT-CT imaging with [(125)I]anti-claudin 4 and [(125)I]anti-PSCA in an L3.6PL orthotopic xenograft model showed strong tumor and spleen uptake at 5 days postinjection. Both anti-claudin 4 and anti-PSCA demonstrate promise as radiodiagnostic and possibly radiotherapeutic agents for human pancreatic cancers.

Gou, S., T. Liu, et al. (2007). "Establishment of clonal colony-forming assay for propagation of pancreatic cancer cells with stem cell properties." Pancreas **34**(4): 429-35.

OBJECTIVE: Pancreatic cancer is among the most aggressive solid malignancies. It is possible that pancreatic cancer contains cancer stem cells responsible for its malignancy. The purposes of this study were (1) to establish an assay in which a subset of pancreatic cancer cell line (PANC-1) cells with stem cell properties can propagate, and (2) to identify the cells obtained from this assay. METHODS: The PANC-1 cells were cultured in Dulbecco modified eagle medium F12 supplemented with epidermal growth factor, basic fibroblast growth factor, insulin, transferrin, selenium, and bovine serum albumin at a density of 1000 cells/mL for 10 to 14 days to form spheres. Cells of spheres were cultured in different conditions to evaluate their ability of self-renewal and differentiation. Clone formation assay and tumor formation assay were used to identify the ability of propagation in vitro and in vivo. The cells and spheres were also stained by using Hoechst 33342 dye to evaluate their capacity of excluding Hoechst dye. Real-time polymerase chain reaction was used to detect expressions of LY6E, c-Met, TACSTD1, CD34, and CD44 mRNA. RESULTS: A subpopulation of PANC-1 cells could propagate to form spheres in this assay. Cells of obtained spheres had the hallmark of excluding Hoechst 33342 dye. Cultured in serum-free medium, the dissociated single cells of primary spheres could form filial spheres again; cultured in serum-containing medium, these cells generated both the cells with the ability of excluding Hoechst 33342 dye and the cells without that ability. The propagation capacity of PANC-1 spheres was higher than that of cells cultured in serum-containing medium both in vitro and in vivo. In addition, LY6E, TACSTD1, and CD44 mRNA were overexpressed in PANC-1 spheres. CONCLUSIONS: A subpopulation of PANC-1 cells can propagate to form spheres with properties of stem cells in this assay; enough of these cells can be obtained for further study. Considering the overexpression of mRNA, it was tentatively suggested that LY6E, TACSTD1, and CD44 proteins may act as surface markers for sorting pancreatic cancer stem cells with fluorescence-activated cell sorter/magnetic-activated cell sorter.

Grubbs, E. G., Z. Abdel-Wahab, et al. (2006). "Utilizing quantitative polymerase chain reaction to evaluate prostate stem cell antigen as a tumor marker in pancreatic cancer." Ann Surg Oncol **13**(12): 1645-54.

BACKGROUND: Real-time quantitative polymerase chain reaction (qPCR) may prove to be a sensitive technique by which to evaluate potential tumor markers in pancreatic cancer. METHODS: The prostate stem cell antigen (PSCA) gene was identified as a marker highly expressed in pancreatic adenocarcinoma and not normal pancreas. RNA from pancreatic and nonpancreatic cancer cell lines as well as tissue and blood from pancreatic cancer and control patients was reverse-transcribed and PSCA quantified by qPCR. RESULTS: Individual operator experience affects the results of qPCR, with significantly different copy numbers at experiment numbers 5, 15, and 40. Five of six pancreatic cell lines had PSCA/actin ratios 10-fold greater than nonpancreatic cancer lines. Mean PSCA expression in pancreatic tumor tissue was significantly higher ($P < 0.05$, Student's t-test) than in the tissue of benign pancreatic processes. The close correlation of PSCA/actin copy number with number of tumor cells in the blood was demonstrated by regression analysis ($r = 0.768$, $P = 0.0001$). PSCA copy number was significantly higher in the blood of patients with metastatic pancreatic cancer than in that of normal patients ($P < 0.05$, Student's t-test). CONCLUSIONS: Such trends suggest that PSCA may

prove to be a valuable pancreatic cancer tumor marker. More generally, the technique of qPCR is shown to provide a sensitive method of evaluating markers in cancer patients.

Guo, T. and M. Hebrok (2009). "Stem cells to pancreatic beta-cells: new sources for diabetes cell therapy." Endocr Rev **30**(3): 214-27.

The number of patients worldwide suffering from the chronic disease diabetes mellitus is growing at an alarming rate. Insulin-secreting beta-cells in the islet of Langerhans are damaged to different extents in diabetic patients, either through an autoimmune reaction present in type 1 diabetic patients or through inherent changes within beta-cells that affect their function in patients suffering from type 2 diabetes. Cell replacement strategies via islet transplantation offer potential therapeutic options for diabetic patients. However, the discrepancy between the limited number of donor islets and the high number of patients who could benefit from such a treatment reflects the dire need for renewable sources of high-quality beta-cells. Human embryonic stem cells (hESCs) are capable of self-renewal and can differentiate into components of all three germ layers, including all pancreatic lineages. The ability to differentiate hESCs into beta-cells highlights a promising strategy to meet the shortage of beta-cells. Here, we review the different approaches that have been used to direct differentiation of hESCs into pancreatic and beta-cells. We will focus on recent progress in the understanding of signaling pathways and transcription factors during embryonic pancreas development and how this knowledge has helped to improve the methodology for high-efficiency beta-cell differentiation in vitro.

Iki, K. and P. M. Pour (2006). "Expression of Oct4, a stem cell marker, in the hamster pancreatic cancer model." Pancreatology **6**(4): 406-13.

BACKGROUND: Oct4 has been shown to present a stem cell marker that is expressed in embryonic cells and in germ cell tumors. Recently, its expression in a few human tissues and cancer cells has been reported. Because in the hamster pancreatic cancer model most tumors develop from within islets presumably from stem cells, we investigated the expression of Oct4 in this model. METHODS: Two normal pancreases and 15 pancreatic cancers induced by N-nitrosobis(2-oxypropyl)amine (BOP) were processed for immunohistochemistry using a monoclonal Oct4 antibody at a concentration of 1:500. RESULTS: In the normal pancreas, Oct4 was expressed only in islet cells in a diffuse cytoplasmic pattern. No nuclear staining was found in any cells. In 14 of the pancreatic cancers, nuclear staining was detected in many cells or in small foci. Diffuse cytoplasmic but no nuclear staining was found in one tumor and a mixed Golgi type and nuclear staining in two cases. Nuclear staining was also identified in early intrainsular ductular and in Ca in situ lesions. CONCLUSIONS: BOP reactivates the Oct4 gene and can be considered an early tumor marker in this model.

Immervoll, H., D. Hoem, et al. (2008). "Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas." BMC Cancer **8**: 48.

BACKGROUND: It has been suggested that a small population of cells with unique self-renewal properties and malignant potential exists in solid tumors. Such "cancer stem cells" have been isolated by flow cytometry, followed by xenograft studies of their tumor-initiating properties. A frequently used sorting marker in these experiments is the cell surface protein CD133 (prominin-1). The aim of this work was to examine the distribution of CD133 in pancreatic exocrine cancer. METHODS: Fifty-one cases of pancreatic ductal adenocarcinomas were clinically and histopathologically evaluated, and immunohistochemically investigated for expression of CD133, cytokeratin 19 and chromogranin A. The results were interpreted on the background of CD133 expression in normal pancreas and other normal and malignant human tissues. RESULTS: CD133 positivity could not be related to a specific embryonic layer of organ origin and was seen mainly at the apical/endoluminal surface of non-squamous, glandular epithelia and of malignant cells in ductal arrangement. Cytoplasmic CD133 staining was observed in some non-epithelial malignancies. In the pancreas, we found CD133 expressed on the apical membrane of ductal cells. In a small subset of ductal cells and in cells in centroacinar position, we also observed expression in the cytoplasm. Pancreatic ductal adenocarcinomas showed a varying degree of apical cell surface CD133 expression, and cytoplasmic staining in a few tumor cells was noted. There was no correlation between the level of CD133 expression and patient survival. CONCLUSION: Neither in the pancreas nor in the other investigated organs can CD133 membrane expression alone be a criterion for "stemness". However, there was an interesting difference in subcellular localization with a minor cell population in normal and malignant pancreatic tissue showing cytoplasmic expression. Moreover, since CD133 was expressed in shed ductal cells of pancreatic tumors and was found on the surface of tumor cells in vessels, this molecule may have a potential as clinical marker in patients suffering from pancreatic cancer.

Jimeno, A., G. Feldmann, et al. (2009). "A direct pancreatic cancer xenograft model as a platform for cancer stem cell therapeutic development." Mol Cancer Ther **8**(2): 310-4.

There is an enormous gap between the antiproliferative and in vivo antitumor efficacy of gemcitabine in cell line-based models and its clinical efficacy. This may be due to insensitiveness of the precursor, cancer stem cell (CSC) compartment to cytotoxic agents. The hedgehog pathway is associated with CSC signaling and control. We used a direct xenograft model of pancreatic cancer and a two-stage approach was used to test the hypotheses that targeting CSC could increase the efficacy of gemcitabine. Tumors from a gemcitabine-sensitive xenograft were treated with gemcitabine first, and randomized, after tumor regression to continuing treatment with gemcitabine, a hedgehog inhibitor alone or in combination with gemcitabine. We tested markers described as associated with CSC such as CD24, CD44, ALDH, nestin, and the hedgehog pathway. After induction with gemcitabine, treated tumor showed an enrichment in CSC markers such as ALDH and CD24. Subsequently, a release from gemcitabine prompted a repopulation of proliferating cells and a decrease in such markers to equilibrate from pretreatment levels. Combined treatment with gemcitabine and cyclopamine induced tumor regression and decrease in CSC markers and hedgehog signaling. Cytoplasmic CD24 and ALDH were inversely and strongly associated with growth and were expressed in a minority of cells that we propose constitute the CSC compartment. Hedgehog inhibitors as part of a dual compartment therapeutic approach were able to further reduce tumor growth and decreased both static and dynamic markers of CSC. Direct tumor xenografts are a valid platform to test multicompartment therapeutic approaches in pancreatic cancer.

Kanda, Y., Y. Komatsu, et al. (2005). "Graft-versus-tumor effect against advanced pancreatic cancer after allogeneic reduced-intensity stem cell transplantation." Transplantation **79**(7): 821-7.

BACKGROUND: The prognosis of advanced pancreatic cancer is extremely poor and therefore a novel treatment strategy is desired. The authors thus started a prospective study of allogeneic reduced-intensity hematopoietic stem cell transplantation (RIST) for patients with advanced pancreatic cancer to evaluate the feasibility and efficacy of this approach for such patients. METHODS: Only patients with pathologically proven pancreatic cancer that was locally advanced or metastatic and not amenable to curative resection were included. The conditioning regimen consisted of gemcitabine, fludarabine, and busulfan. RESULTS: In the first stage of this study, the authors treated seven patients. Treatment-related mortality before day 100 was observed in one patient. The median survival after RIST was 229 days. An objective response on computed tomographic scan was observed in two patients and another had a tumor marker response. Marked tumor shrinkage was observed in one of the remaining patients after donor lymphocyte infusion. These antitumor effects appeared after the effect of the conditioning regimen had disappeared. In addition, some of these responses were associated with an increase in the serum anticarcinoembryonic antigen antibody level. CONCLUSIONS: Pancreatic cancer appeared to be sensitive to a graft-versus-tumor effect; therefore, a larger clinical study with a refined strategy is warranted.

Kim, D., Y. Gu, et al. (2003). "In vivo functioning and transplantable mature pancreatic islet-like cell clusters differentiated from embryonic stem cell." Pancreas **27**(2): e34-41.

INTRODUCTION: Although the differentiation of embryonic stem (ES) cells to islet like clusters using differentiation method without employing gene transfer technique has been recently reported, neither endocrine granules in the cytoplasm nor in vivo function of differentiated islet like clusters has been demonstrated. AIMS: To investigate whether ES cells could be differentiated to mature islet like clusters which show in vivo function after transplantation as well as retain endocrine granules in the cytoplasm by electron microscopic observation. METHODOLOGY: In this experiment, using mouse embryonic stem (mES) cells as a model system for lineage specific differentiation, we tried to differentiate mES cells to pancreatic islet-like cell clusters (PICCs) through a series of treatments (4-step procedure). Differentiated PICCs were analyzed and characterized by various techniques, such as RT-PCR, immunohistochemistry, electron microscopic observation, in vitro static incubation test, and in vivo transplantation to diabetic animals. RESULTS: Differentiated islet-like cell clusters from ES cells using our newly developed method (four-step procedure) showed strong expression of essential specific genes to the endocrine pancreas and also specific genes to the exocrine pancreas demonstrating that these islet-like clusters were mature from the developmental biologic point of view. These differentiated cells clearly revealed many mature insulin secretory granules of pleomorphic shape in the cytoplasm as well as well-developed rough endoplasmic reticulum. In vitro study indicated that differentiated cells retain a potent insulin secretory

responsiveness to glucose stimulation. Furthermore, the islet-like cell clusters significantly decreased high blood glucose levels almost to normal levels when grafted to streptozotocin-induced diabetic mice without induction of any teratoma formation after transplantation. CONCLUSION: Our results provide evidence that ES cells could differentiate to functioning and transplantable mature pancreatic islet-like cell clusters using our newly developed differentiation method without employing gene transfer technique. This study may lead to a basis for production of indefinite sources of islets that could be applicable for future clinical trial.

Kodama, M., F. Takeshita, et al. (2008). "Pancreatic endocrine and exocrine cell ontogeny from renal capsule transplanted embryonic stem cells in streptozocin-injured mice." J Histochem Cytochem **56**(1): 33-44.

In this study, we describe pancreatic cell ontogeny in renal capsule-transplanted embryonic stem cells (ES) after injury by streptozocin (STZ), showing pancreatogenesis in situ. Seven-week-old female BALB/c nude mice were treated with either a single 175- or 200-mg/kg STZ dose, a regimen that induces substantial beta-cell damage without overt hyperglycemia, and transplanted 24 hr later with 1 x 10(5) ES. Immunohistochemistry was performed on ES tissue at 15, 21, and 28 days after transplantation using antibodies against stage- and lineage-specific pancreatic markers. After 21 days, PDX-1+ pancreatic foci first appeared in the renal capsule and expressed both amylase and endocrine hormones (insulin, glucagon, and somatostatin). These foci increased in size by day 28 because of acinar and duct cell proliferation, whereas endocrine cells remained non-dividing, and made up 2-4% of ES tumor volume. PDX-1, Nkx6.1, Ngn3, and ISL-1 protein localization patterns in pancreatic foci were comparable with embryonic pancreatogenesis. A prevalence of multihormonal endocrine cells, a characteristic of adult beta-cell regeneration, indicated a possible divergence from embryonic islet cell development. The results indicate that beta-cell damage, without overt hyperglycemia, induces a process of fetal-like pancreatogenesis in renal capsule-transplanted ES, leading to beta-cell neogenesis.

Kruse, C., J. Kajahn, et al. (2006). "Adult pancreatic stem/progenitor cells spontaneously differentiate in vitro into multiple cell lineages and form teratoma-like structures." Ann Anat **188**(6): 503-17.

Cells isolated from pancreas have a remarkable potential for self-renewal and multilineage differentiation. We here present a comprehensive characterisation of stem/progenitor cells derived from exocrine parts of the adult rat pancreas. Using purified cells from either single colonies or even single-cell clones, we specifically demonstrate: (i) the cells contain the typical stem/progenitor cell markers alkaline phophatase, SSEA-1, Oct-4, CD9, Nestin, Pax6, CD44, a-Fetoprotein and Brachyury, demonstrated by immunocytochemistry and RT-PCR; (ii) the cells have the potential to differentiate into lineages of all three germ layers in vitro; (iii) a clonal analysis revealed that even cell lines derived from a single cell have stem/progenitor cell properties such as self-renewal and spontaneous differentiation into various cell lineages; (iv) the cells have the propensity to form three-dimensional, teratoma-like structures in vitro, which contain cells of different lineages; and (v) external stimuli can activate the generation of certain cell types. For instance, cells treated with retinoic acid show an increased expression of alpha-smooth muscle actin. These results suggest that exocrine glands, such as pancreas may be a potential source of adult stem/progenitor cells, suitable for cell therapy of degenerative diseases.

Lees, J. G. and B. E. Tuch (2006). "Conversion of embryonic stem cells into pancreatic beta-cell surrogates guided by ontogeny." Regen Med **1**(3): 327-36.

Cellular therapies to treat Type 1 diabetes are being devised and the use of human embryonic stem cells (hESCs) offers a solution to the issue of supply, because hESCs can be maintained in a pluripotent state indefinitely. Furthermore, hESCs have advantages in terms of their plasticity and reduced immunogenicity. Several strategies that have so far been investigated indicate that hESCs are capable of differentiating into insulin producing beta-cell surrogates. However the efficiency of the differentiation procedures used is generally quite low and the cell populations derived are often highly heterogenous. A strategy that appears to have long term potential is to design differentiation procedures based on the ontogeny of the beta-cell. The focus of this strategy is to replicate signaling processes that are known to be involved in the maturation of a beta-cell. The earliest pancreatic progenitors found in the developing vertebrate fetus are produced via a process known as gastrulation and form part of the definitive endoderm germ layer. hESCs have recently been differentiated into definitive endoderm with high efficiency using a differentiation procedure that mimics the signaling that occurs during gastrulation and the formation of the definitive endoderm. Subsequent events during pancreas development involve a section of the definitive endoderm forming into pancreatic epithelium, which then branches into the pancreatic mesenchyme to form islet clusters of

endocrine cells. A proportion of the endocrine precursor cells within islets develop into insulin producing beta-cells. The challenge currently is to design hESC differentiation procedures that mimic the combined events of these stages of beta-cell development.

Lin, H. T., S. H. Chiou, et al. (2006). "Characterization of pancreatic stem cells derived from adult human pancreas ducts by fluorescence activated cell sorting." World J Gastroenterol **12**(28): 4529-35.

AIM: To isolate putative pancreatic stem cells (PSCs) from human adult tissues of pancreas duct using serum-free, conditioned medium. The characterization of surface phenotype of these PSCs was analyzed by flow cytometry. The potential for pancreatic lineage and the capability of beta-cell differentiation in these PSCs were evaluated as well. METHODS: By using serum-free medium supplemented with essential growth factors, we attempted to isolate the putative PSCs which has been reported to express nestin and pdx-1. The Matrigel(TM) was employed to evaluate the differential capacity of isolated cells. Dithizone staining, insulin content/secretion measurement, and immunohistochemistry staining were used to monitor the differentiation. Fluorescence activated cell sorting (FACS) was used to detect the phenotypic markers of putative PSCs. RESULTS: A monolayer of spindle-like cells was cultivated. The putative PSCs expressed pdx-1 and nestin. They were also able to differentiate into insulin-, glucagon-, and somatostatin-positive cells. The spectrum of phenotypic markers in PSCs was investigated; a similarity was revealed when using human bone marrow-derived stem cells as the comparative experiment, such as CD29, CD44, CD49, CD50, CD51, CD62E, PDGFR-alpha, CD73 (SH2), CD81, CD105(SH3). CONCLUSION: In this study, we successfully isolated PSCs from adult human pancreatic duct by using serum-free medium. These PSCs not only expressed nestin and pdx-1 but also exhibited markers attributable to mesenchymal stem cells. Although work is needed to elucidate the role of these cells, the application of these PSCs might be therapeutic strategies for diabetes mellitus.

Madsen, O. D. (2007). "Pancreas phylogeny and ontogeny in relation to a 'pancreatic stem cell'." C R Biol **330**(6-7): 534-7.

Blood glucose regulation has likely evolved during early vertebrate evolution to allow and secure the concurrent evolution of complex brains and nervous systems: an inner milieu of constant blood glucose levels through millions of years has provided an extra degree of freedom for the brain to evolve without having to think of getting energy supply. Key regulators of blood glucose, insulin, and glucagon are produced by the dominating cell types of the pancreatic islet of Langerhans: the insulin producing beta cells and the glucagon producing alpha cells. Interestingly, it appears that the beta cell pioneered the formation or the foundation of the pancreatic organ according to current phylogenetic insights. Such phylogenetic aspects of a pancreatic stem cell are at the end discussed in relation to directed differentiation of embryonic stem cells/ES cells towards therapeutic beta cells.

McCarthy, D. M., A. Maitra, et al. (2003). "Novel markers of pancreatic adenocarcinoma in fine-needle aspiration: mesothelin and prostate stem cell antigen labeling increases accuracy in cytologically borderline cases." Appl Immunohistochem Mol Morphol **11**(3): 238-43.

The interpretation of pancreas fine-needle aspiration (FNA) is extremely difficult given the cytologic overlap of neoplastic and reactive processes. Using serial analysis of gene expression, we have discovered 2 new markers of pancreatic adenocarcinoma, mesothelin and prostate stem cell antigen (PSCA), and confirmed their specificity by immunohistochemical labeling. Here we evaluate the potential contribution of immunohistochemical labeling of mesothelin and PSCA to the interpretation of pancreas FNAs. Thirty pancreas FNAs with follow-up data were reviewed. Unstained cell block sections from these aspirates labeled for mesothelin and PSCA using immunohistochemistry were compared with initial cytologic diagnoses and with follow-up diagnoses. On follow-up, 19 patients proved to have cancer, and 11 did not. Initial cytologic diagnosis of malignancy correlated with carcinoma on follow-up in 12 of 12 cases, and initial benign cytologic diagnosis correlated with benign follow-up in 8 of 9 cases (sensitivity, 92%; specificity, 100%). Six of the 9 patients with suspicious cytology were found to have a carcinoma on follow-up. PSCA labeling was present in 16 of the 19 patients who ultimately were proven to have carcinoma; PSCA labeling was absent in 10 of the 11 lesions proven to be benign (sensitivity, 84%; specificity, 91%). Mesothelin labeling was present in 13 of the 19 patients who ultimately were proven to have carcinoma; mesothelin labeling was absent in 10 of the 11 lesions proven to be benign (sensitivity, 68%; specificity, 91%). Five of the 6 cytologically suspicious cases with malignant follow-up labeled for either PSCA or mesothelin (83%), and 2 of the 6 cases labeled for both markers. None of the 3 suspicious cases with benign follow-up labeled for either PSCA or mesothelin. Increasingly, molecular techniques are identifying potential cancer markers that may have

diagnostic utility. In this study, immunohistochemical labeling for 2 of these markers, PSCA and mesothelin, appears highly specific for pancreatic adenocarcinoma in FNA specimens and useful in categorizing cytologically suspicious lesions.

Meier, K., C. M. Lehr, et al. (2009). "Differentiation potential of human pancreatic stem cells for epithelial- and endothelial-like cell types." Ann Anat **191**(1): 70-82.

Pancreatic stem cells (PSC) have proved their high plasticity by differentiating into cell types of all three germ layers after the formation of organoid bodies. Motivated by this high differentiation potential this study focused on the immanent stem cell, endothelial and epithelial characteristics of PSC to elucidate whether PSC are a possible source for a stem cell-based in vitro model for screening of pharmaceutical substances. Furthermore, it was investigated whether marker expression was influenced by application of protocols for inducing endothelial or epithelial differentiation originating from research with mesenchymal stem cells or by cultivation on extracellular matrices (ECM). PSC showed expression of relevant stem cell markers CD 45, nestin, Oct 4 and c-kit. As endothelial characteristics they displayed the markers endoglin, VCAM 1, VEGFR 1 and vWF as well as the formation of vessel-like structures. Those endothelial properties were not further intensified by application of protocols employing vascular endothelial growth factor (VEGF), basic fibroblastic growth factor (bFGF) and endothelial cell growth medium. As typical epithelial characteristics PSC showed expression of keratin 7, 8, 18 and 19, ZO-1 and catenin beta1. In order to induce epithelial differentiation PSC were cocultured with the lung epithelial cell lines A549 and Calu-3 either by the usage of conditioned medium or by cultivation of PSC on fixed epithelial cells. Depending on the applied coculture system and epithelial cell type used expression of the epithelial marker cadherin-1 was altered compared to control. Cultivation on different ECM changed expression of all investigated markers only marginally. These results confirm that PSC possess endothelial and epithelial properties. Furthermore, epithelial characteristics represented by expression of CDH 1 were altered by coculture with epithelial cell lines.

Mimeault, M. and S. K. Batra (2008). "Recent progress on normal and malignant pancreatic stem/progenitor cell research: therapeutic implications for the treatment of type 1 or 2 diabetes mellitus and aggressive pancreatic cancer." Gut **57**(10): 1456-68.

Recent progress on pancreatic stem/progenitor cell research has revealed that the putative multipotent pancreatic stem/progenitor cells and/or more committed beta cell precursors may persist in the pancreatic gland in adult life. The presence of immature pancreatic cells with stem cell-like properties offers the possibility of stimulating their in vivo expansion and differentiation or to use their ex vivo expanded progenies for beta cell replacement-based therapies for type 1 or 2 diabetes mellitus in humans. In addition, the transplantation of either insulin-producing beta cells derived from embryonic, fetal and other tissue-resident adult stem/progenitor cells or genetically modified adult stem/progenitor cells may also constitute alternative promising therapies for treating diabetic patients. The genetic and/or epigenetic alterations in putative pancreatic adult stem/progenitor cells and/or their early progenies may, however, contribute to their acquisition of a dysfunctional behaviour as well as their malignant transformation into pancreatic cancer stem/progenitor cells. More particularly, the activation of distinct tumorigenic signalling cascades, including the hedgehog, epidermal growth factor-epidermal growth factor receptor (EGF-EGFR) system, wingless ligand (Wnt)/beta-catenin and/or stromal cell-derived factor-1 (SDF-1)-CXC chemokine receptor 4 (CXCR4) pathways may play a major role in the sustained growth, survival, metastasis and/or drug resistance of pancreatic cancer stem/progenitor cells and their further differentiated progenies. The combination of drugs that target the oncogenic elements in pancreatic cancer stem/progenitor cells and their microenvironment, with the conventional chemotherapeutic regimens, could represent promising therapeutic strategies. These novel targeted therapies should lead to the development of more effective treatments of locally advanced and metastatic pancreatic cancers, which remain incurable with current therapies.

Mroczko, B., M. Szmitkowski, et al. (2004). "Stem cell factor and macrophage-colony stimulating factor in patients with pancreatic cancer." Clin Chem Lab Med **42**(3): 256-60.

Stem cell factor (SCF) and macrophage-colony stimulating factor (M-CSF) have assumed an increasing importance in cancer biology. In the present study we investigated the serum levels of these cytokines in pancreatic cancer patients in relation to controls and to patients with benign lesions of the pancreas (chronic pancreatitis group). The classical tumor markers, such as carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9) were also tested. We compared the serum levels of cytokines with tumor stage. We also defined the

receiver-operating characteristics (ROC) curve for cytokines and classical tumor markers. The cytokines were measured in 47 patients with pancreatic cancer, in 27 patients with chronic pancreatitis and in 35 healthy subjects. SCF and M-CSF were determined using enzyme-linked immunosorbent assay (ELISA). CEA and CA 19-9 were measured by microparticle enzyme immunoassay. There were significant differences in the levels of circulating SCF, M-CSF, CEA and CA 19-9 in the pancreatic cancer patients compared to the control group, but only the serum levels of M-CSF, CEA and CA 19-9 were significantly higher in pancreatic cancer patients compared to the pancreatitis group. The levels of cytokines and tumor markers were higher in patients with a more advanced tumor stage. The M-CSF serum levels correlated positively with the tested tumor markers. The M-CSF area under the ROC curve was higher than the SCF area. These results suggest that M-CSF is a better candidate for a pancreatic cancer tumor marker than SCF.

Noguchi, H., K. Oishi, et al. (2009). "Establishment of mouse pancreatic stem cell line." Cell Transplant **18**(5): 563-71.

beta-Cell replacement therapy via islet transplantation is a promising possibility for the optimal treatment of type 1 diabetes. However, such an approach is severely limited by the shortage of donor organs. Pancreatic stem/progenitor cells could become a useful target for beta-cell replacement therapy in diabetic patients because the cells are abundantly available in the pancreas of these patients and in donor organs. In this study, we established a mouse pancreatic stem cell line without genetic manipulation. The duct-rich population after islet isolation was inoculated into 96-well plates in limiting dilution. From over 200 clones, 15 clones were able to be cultured for over 3 months. The HN#13 cells, which had the highest expression of insulin mRNA after induction, expressed PDX-1 transcription factor, glucagon-like peptide-1 (GLP-1) receptor, and cytokeratin-19 (duct-like cells). These cells continue to divide actively beyond the population doubling level (PDL) of 300. Exendin-4 treatment and transduction of PDX-1 and NeuroD proteins by protein transduction technology in HN#13 cells induced insulin and pancreas-related gene expression. This cell line could be useful for analyzing pancreatic stem cell differentiation. Moreover, the isolation technique might be useful for identification and isolation of human pancreatic stem/progenitor cells.

Olempska, M., P. A. Eisenach, et al. (2007). "Detection of tumor stem cell markers in pancreatic carcinoma cell lines." Hepatobiliary Pancreat Dis Int **6**(1): 92-7.

BACKGROUND: Cancer of the pancreas is the fourth leading cause of cancer death in industrialized countries. In malignancy, actively proliferating cells may be effectively targeted and killed by anti-cancer therapies, but stem cells may survive and support re-growth of the tumor. Thus, new strategies for the treatment of cancer clearly will also have to target cancer stem cells. The goal of the present study was to determine whether pancreatic carcinoma cell growth may be driven by a subpopulation of cancer stem cells. Because previous data implicated ABCG2 and CD133 as stem cell markers in hematopoietic and neural stem/progenitor cells, we analyzed the expression of these two proteins in pancreatic carcinoma cell lines. METHODS: Five established pancreatic adenocarcinoma cell lines were analyzed. Total RNA was isolated and real-time RT-PCR was performed to determine the expression of ABCG2 and CD133. Surface expression of ABCG2 and CD133 was analyzed by flow cytometric analysis. RESULTS: All pancreatic carcinoma cell lines tested expressed significantly higher levels of ABCG2 than non-malignant fibroblasts or two other malignant non-pancreatic cell lines, i.e., SaOS2 osteosarcoma and SKOV3 ovarian cancer. Elevated CD133 expression was found in two out of five pancreatic carcinoma cell lines tested. Using flow cytometric analysis we confirmed surface expression of ABCG2 in all five lines. Yet, CD133 surface expression was detectable in the two cell lines, A818-6 and PancTu1, which exhibited higher mRNA levels. CONCLUSIONS: Two stem cell markers, ABCG2 and CD133 are expressed in pancreatic carcinoma cell lines. ABCG2 and/or CD133 positive cells may represent subpopulation of putative cancer stem cells also in this malignancy. Because cancer stem cells are thought to be responsible for tumor initiation and its recurrence after an initial response to chemotherapy, they may be a very promising target for new drug developments.

Olerud, J., N. Kanaykina, et al. (2009). "Neural crest stem cells increase beta cell proliferation and improve islet function in co-transplanted murine pancreatic islets." Diabetologia **52**(12): 2594-601.

AIMS/HYPOTHESIS: Long-term graft survival after islet transplantation to patients with type 1 diabetes is insufficient, necessitating the development of new strategies to enhance transplant viability. Here we investigated whether co-transplantation of neural crest stem cells (NCSCs) with islets improves islet survival and function in normoglycaemic and diabetic mice. METHODS: Islets alone or together with NCSCs were transplanted under the kidney capsule to normoglycaemic or

alloxan-induced diabetic mice. Grafts were analysed for size, proliferation, apoptosis and insulin release. In diabetic recipients blood glucose levels were examined before and after graft removal. RESULTS: In mixed transplants NCSCs actively migrated and extensively associated with co-transplanted pancreatic islets. Proliferation of beta cells was markedly increased and transplants displayed improved insulin release in normoglycaemic mice compared with those receiving islet-alone transplants. Mixed grafts survived successfully and partially restored normoglycaemia in alloxan-induced diabetic mice. CONCLUSIONS/INTERPRETATION: Co-grafting of NCSCs with pancreatic islets improved insulin release in mixed transplants and enhanced beta cell proliferation, resulting in increased beta cell mass. This co-transplantation model offers an opportunity to restore neural-islet interactions and improve islet functions after transplantation.

Peshavaria, M. and K. Pang (2000). "Manipulation of pancreatic stem cells for cell replacement therapy." Diabetes Technol Ther **2**(3): 453-60.

The demonstration of the existence of tissue-specific adult stem cells has had a great impact on our understanding of stem cell biology and its application in clinical medicine. Their existence has revolutionized the implications for the treatment of many degenerative diseases characterized by either the loss or malfunction of discrete cell types. However, successful exploitation of this opportunity requires that we have sufficient know-how of stem cell manipulation. Because stem cells are the founders of virtually all tissues during embryonic development, we believe that understanding the cellular and molecular mechanisms of embryogenesis and organogenesis will ultimately serve as a platform to identify factors and conditions that regulate stem cell behavior. Discovery of stem cell regulatory factors will create potential pharmaceutical opportunities for treatment of degenerative diseases, as well as providing critical knowledge of the processes by which stem cells can be expanded in vitro, differentiated, and matured into desired functional cells for implantation into humans. A well-characterized example of this is the hematopoietic system where the discovery of erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF), which regulate hematopoietic progenitor cell behavior, have provided significant clinical success in disease treatment as well as providing important insights into hematopoiesis. In contrast, little is known about the identity of pancreatic stem cells, the focus of this review. Recent reports of the potential existence of pancreatic stem cells and their utility in rescuing the diabetic state now raise the same possibilities of generating insulin-producing beta cells as well as other cell types of the pancreatic islet from a stem cell. In this review, we will focus on the potential of these new developments and how our understanding of pancreas development can help design strategies and approaches by which a cell replacement therapy can be implemented for the treatment of insulin-dependent diabetes which is manifested by the loss of beta cells in the pancreas.

Rovira, M., F. Delaspre, et al. (2008). "Murine embryonic stem cell-derived pancreatic acinar cells recapitulate features of early pancreatic differentiation." Gastroenterology **135**(4): 1301-1310, 1310 e1-5.

BACKGROUND & AIMS: Acinar cells constitute 90% of the pancreas epithelium, are polarized, and secrete digestive enzymes. These cells play a crucial role in pancreatitis and pancreatic cancer. However, there are limited models to study normal acinar cell differentiation in vitro. The aim of this work was to generate and characterize purified populations of pancreatic acinar cells from embryonic stem (ES) cells. METHODS: Reporter ES cells (Ela-pur) were generated that stably expressed both beta-galactosidase and puromycin resistance genes under the control of the elastase I promoter. Directed differentiation was achieved by incubation with conditioned media of cultured fetal pancreatic rudiments and adenoviral-mediated co-expression of p48/Ptf1a and Mist1, 2 basic helix-loop-helix transcription factors crucial for normal pancreatic acinar development and differentiation. RESULTS: Selected cells expressed multiple markers of acinar cells, including digestive enzymes and proteins of the secretory pathway, indicating activation of a coordinated differentiation program. The genes coding for digestive enzymes were not regulated as a single module, thus recapitulating what occurs during in vivo pancreatic development. The generated cells displayed transient agonist-induced Ca(2+) mobilization and showed a typical response to physiologic concentrations of secretagogues, including enzyme synthesis and secretion. Importantly, these effects did not imply the acquisition of a mixed acinar-ductal phenotype. CONCLUSIONS: These studies allow the generation of almost pure acinar-like cells from ES cells, providing a normal cell-based model for the study of the acinar differentiation program in vitro.

Saldeen, J., N. Agren, et al. (2006). "The role of the adapter protein SHB in embryonic stem cell differentiation into the pancreatic beta-cell and endothelial lineages." Methods Mol Biol **330**: 353-72.

Embryonic stem (ES) cells represent an attractive tool not only for the study of the

development of various cell types but also as a potential source of cells for transplantation. Previous studies suggested a role of the signal transduction protein SRC homology 2(SH2) protein of Beta-cells (SHB) for the development of both pancreatic 3-cells and blood vessels. SHB is an SH2 domain-containing adapter protein involved in the generation of signaling complexes in response to activation of a variety of receptors, several of which have been implicated in developmental processes. Moreover, microarray analysis of ES cells expressing mutant SHB has revealed decreased expression of several genes of developmental importance. Here, we present protocols that may be used for transfection of mouse ES cells and to study the differentiation of ES cell-derived embryoid bodies (EBs) into the pancreatic Beta-cell lineage as well as into vascular structures with special reference to the effect of SHB. Moreover, we also provide a protocol that may be used for enrichment by fluorescence-activated cell sorting of specific cell lineages in EBs.

Salnikov, A. V., A. Groth, et al. (2009). "Targeting of cancer stem cell marker EpCAM by bispecific antibody EpCAMxCD3 inhibits pancreatic carcinoma." J Cell Mol Med **13**(9B): 4023-33.

Patients with pancreatic cancer have a poor survival rate, and new therapeutic strategies are needed. Epithelial cell adhesion molecule (EpCAM), suggested as a marker for cancer stem cells, is over-expressed on most pancreatic tumour cells but not on normal cells and may be an ideal therapeutic target. We evaluated the anti-tumour efficiency of bispecific EpCAMxCD3 antibody linking tumour cells and T lymphocytes. In NOD SCID mice, EpCAMxCD3 had a long serum half-life (t(1/2) approximately 7 days). EpCAMxCD3 significantly retarded growth of BxPC-3 pancreatic carcinoma xenografts. For mimicking a pancreatic cancer microenvironment in vitro, we used a three-dimensional tumour reconstruct system, in which lymphocytes were co-cultured with tumour cells and fibroblasts in a collagen matrix. In this in vivo-like system, EpCAMxCD3 potently stimulated production of the effector cytokines IFN-gamma and TNF-alpha by extracorporally pre-activated lymphocytes. Moreover, compared with a bivalent anti-CD3 antibody, EpCAMxCD3 more efficiently activated the production of TNF-alpha and IFN-gamma by non-stimulated peripheral blood mononuclear cells. Most excitingly, we demonstrate for the first time that EpCAMxCD3 induces prolonged contacts between lymphocytes and tumour cells, which may be the main reason for the observed anti-tumour effects. As an important prerequisite for future use in patients, EpCAMxCD3 did not alter lymphocyte migration as measured by time-lapse video microscopy. Our data may open a way to improve the immune response and treatment outcome in patients with pancreatic cancer.

Shim, J. H., S. E. Kim, et al. (2007). "Directed differentiation of human embryonic stem cells towards a pancreatic cell fate." Diabetologia **50**(6): 1228-38.

AIMS/HYPOTHESIS: The relative lack of successful pancreatic differentiation of human embryonic stem cells (hESCs) may suggest that directed differentiation of hESCs into definitive endoderm and subsequent commitment towards a pancreatic fate are not readily achieved. The aim of this study was to investigate whether sequential exposure of hESCs to epigenetic signals that mimic in vivo pancreatic development can efficiently generate pancreatic endodermal cells, and whether these cells can be further matured and reverse hyperglycaemia upon transplantation. MATERIALS AND METHODS: The hESCs were sequentially treated with serum, activin and retinoic acid (RA) during embryoid body formation. The patterns of gene expression and protein production associated with embryonic germ layers and pancreatic endoderm were analysed by RT-PCR and immunostaining. The developmental competence and function of hESC-derived PDX1-positive cells were evaluated after in vivo transplantation. RESULTS: Sequential treatment with serum, activin and RA highly upregulated the expression of the genes encoding forkhead box protein A2 (FOXA2), SRY-box containing gene 17 (SOX17), pancreatic and duodenal homeobox 1 (PDX1) and homeobox HB9 (HLXB9). The population of pancreatic endodermal cells that produced PDX1 was significantly increased at the expense of ectodermal differentiation, and a subset of the PDX1-positive cells also produced FOXA2, caudal-type homeobox transcription factor 2 (CDX2), and nestin (NES). After transplantation, the PDX1-positive cells further differentiated into mature cell types producing insulin and glucagon, resulting in amelioration of hyperglycaemia and weight loss in streptozotocin-treated diabetic mice. CONCLUSIONS/INTERPRETATION: Our strategy allows the progressive differentiation of hESCs into pancreatic endoderm capable of generating mature pancreatic cell types that function in vivo. These findings may establish the basis of further investigations for the purification of transplantable islet progenitors derived from hESCs.

Tai, M. H., L. K. Olson, et al. (2003). "Characterization of gap junctional intercellular communication in immortalized human pancreatic ductal epithelial cells with stem cell characteristics." Pancreas **26**(1): e18-26.

INTRODUCTION: Gap junctional intercellular communication has been implicated in the homeostatic regulation of cell growth, differentiation, and apoptosis. Cancer cells, which have been viewed as "partially blocked stem cells," and which lack the ability for growth control, terminal differentiation, and apoptosis, also lack functional gap junctional communication. AIMS AND METHODOLOGY: A clone of a human pancreatic ductal epithelial cell line, H6c7, derived after immortalization with human papilloma virus, was used to examine gap junctional intercellular communication and the ability to differentiate under different growth conditions. RESULTS: The cells showed characteristic epithelial morphology on standard tissue culture dishes. When placed on Matrigel they showed phenotypical changes with extensive ductal organization and budding structures. In growth medium containing hormones and growth factors, these cells were gap junctional intercellular communication (GJIC)-incompetent. In the presence of c-AMP elevating agents, isobutylmethylxanthine, and forskolin, in basal medium that did not contain the hormones and growth factors, the cells became GJIC-competent and expressed connexin43 gap junction protein within 48 hours after treatment. RT-PCR analyses of the cells under different growth conditions showed that the cells expressed, and genes when cultured in the basal medium with c-AMP elevating agents. They also expressed the gene that did not change with c-AMP treatment. H6c7 cells also have the capacity to turn on an ectopic insulin promoter reporter gene. CONCLUSION: Our data suggest that the immortalized H6c7 cells retain stem-like characteristics and have the potential to differentiate into duct-like structures and perhaps insulin-producing cells.

Takahashi, T., Y. Omuro, et al. (2004). "Nonmyeloablative allogeneic stem cell transplantation for patients with unresectable pancreatic cancer." Pancreas **28**(3): e65-9.

OBJECTIVES: To clarify whether nonmyeloablative allogeneic stem cell transplantation (NST) can produce the graft versus tumor (GVT) effect in patients with pancreatic cancer. METHODS: A pilot trial of NST was conducted in 5 patients with unresectable pancreatic cancer. Preparative conditioning consisted of administration of 60 mg/kg cyclophosphamide on days 6 and 7 before transplantation, followed by 25 mg fludarabine per square meter of body surface on each of the last 5 days prior to transplantation. Cyclosporine was started 4 days before transplantation. Peripheral blood stem cells from the patients' HLA-identical siblings were transfused into the patients. RESULTS: Complete donor T-cell chimerism in peripheral blood was obtained in 4 patients on day 15 after transplantation. NST resulted in tumor reduction in 2 patients as determined by CT, decreasing levels of tumor markers in 2 patients, pain relief in 2 patients, and a decrease in pleural fluid in 1 patient. Two patients developed acute graft versus host disease (GVHD) of grade II or III and 2 had chronic GVHD involving skin and/or liver. Administration of immunosuppressive drugs for the treatment of GVHD resulted in the elevation of tumor marker levels. CONCLUSION: These findings are the first to suggest that NST induces a GVT effect on pancreatic cancer.

Takeshita, F., M. Kodama, et al. (2006). "Streptozotocin-induced partial beta cell depletion in nude mice without hyperglycaemia induces pancreatic morphogenesis in transplanted embryonic stem cells." Diabetologia **49**(12): 2948-58.

AIMS/HYPOTHESIS: It appears that the adult pancreas has limited regenerative ability following beta cell destruction by streptozotocin (STZ). However, it is not clear if this limitation is due to an inability to respond to, rather than an absence of, regenerative stimuli. In this study we aimed to uncouple the regenerative signal from the regenerative response by using an exogenous stem cell source to detect regenerative stimuli produced by the STZ-injured pancreas at physiological blood glucose levels. METHOD: Adult nude mice received 150 mg/kg STZ and 1x10(6) J1 mouse embryonic stem (ES) cells by i.p. injection. Permanent beta cell depletion of 50% was estimated from the ratio of beta:alpha cells in pancreata from STZ-treated mice compared with control animals after 24 days. RESULTS: Transplanted ES cells homed to the STZ-injured pancreas and formed tumours. Immunocytochemical analysis of pancreas-associated ES tumours revealed foci containing insulin/PDX-1 double-positive and glucagon-positive/PDX-1-negative cell clusters associated with PDX-1-positive columnar lumenal epithelium and extensive alpha-amylase-positive pancreatic acini comprising approximately 0.1% of ES tumour volume. CONCLUSIONS/INTERPRETATION: These data indicate that (1) the adult pancreas produces a milieu of regenerative stimuli following beta cell destruction, and (2) this is not dependent on hyperglycaemic conditions; (3) these regenerative stimuli appear to recapitulate the signalling pathways of embryonic development, since both exocrine and endocrine lineages are produced from PDX-1-positive precursor epithelium. This model will be useful for characterising the regenerative mechanisms in the adult pancreas.

Tanaka, M., N. Komatsu, et al. (2007). "Increased levels of IgG antibodies against peptides of the prostate stem cell antigen in the plasma of pancreatic cancer patients." Oncol Rep **18**(1): 161-6.

One of the longstanding challenges in the treatment of pancreatic cancer, the fifth most common cancer worldwide, is to establish a simple and reliable diagnostic marker for the disease. This study examined whether or not the plasma levels of IgG antibodies (IgGs) reactive to peptides derived from the prostate stem cell antigen (PSCA), which is highly expressed in pancreatic cancer cells, were elevated in patients with pancreatic cancer. Fifty-seven kinds of peptides encoded by PSCA were tested for their reactivity to plasma IgGs of pancreatic cancer patients. The results showed that the levels of IgGs specific to each of the 10 different peptides in the plasma of pancreatic cancer patients were significantly higher than those of non-cancer subjects. Eighty percent of subjects with and 18% of subjects without pancreatic cancer were diagnosed as having pancreatic cancer, respectively, when those cases showing significantly elevated levels of IgGs against at least one of the three peptides of PSCA at positions 2-11, 85-95, and 109-118 were judged as positive for pancreatic cancer. These results indicate that the measurement of IgGs reactive to these PSCA-derived peptides can provide novel information on the host-tumor interaction in pancreatic cancer, and could potentially be used as a new diagnostic tool to screen for pancreatic cancer.

Vincent, R., N. Treff, et al. (2006). "Generation and characterization of novel tetracycline-inducible pancreatic transcription factor-expressing murine embryonic stem cell lines." Stem Cells Dev **15**(6): 953-62.

Pancreatic development in mammals is controlled in part by the expression and function of numerous genes encoding transcription factors. Yet, how these regulate each other and their target genes is incompletely understood. Embryonic stem (ES) cells have recently been shown to be capable of differentiating into pancreatic progenitor cells and insulin-producing cells, representing a useful in vitro model system for studying pancreatic and islet development. To generate tools to study the relationships of transcription factors in pancreatic development we have established seven unique mouse ES cell lines with tetracycline-inducible expression of either Hnf4alpha, Hnf6, Nkx2.2, Nkx6.1, Pax4, Pdx1, and Ptf1a cDNAs. Each of the cell lines was characterized for induction of transgene expression after exposure to doxycycline (DOX) by quantitative real-time PCR and immunofluorescence microscopy. Transgene expression in the presence of DOX was at least 97-fold that seen in untreated cells. Immunofluorescent staining of DOX-treated cultures showed efficient (>95% of cells) transgene protein expression while showing <5% positive staining in uninduced cells. Each of the ES cell lines maintained their pluripotency as measured by teratoma formation. Furthermore, transgene expression can be efficiently achieved in vivo through DOX administration to mice. The establishment of ES cell lines with temporally controllable induction of critical pancreatic transcription factor genes provides a new set of tools that could be used to interrogate gene regulatory networks in pancreatic development and potentially generate greater numbers of beta cells from ES cells.

Wente, M. N., A. Jain, et al. (2005). "Prostate stem cell antigen is a putative target for immunotherapy in pancreatic cancer." Pancreas **31**(2): 119-25.

The prostate stem cell antigen (PSCA) is a glycosylphosphatidyl-inositol (GPI)-linked cell surface antigen expressed in normal prostate and overexpressed in the majority of prostate cancers and correlates with tumor grade and disease stage. Because PSCA has been described to be up-regulated in pancreatic cancer, the purpose was to evaluate the expression of PSCA in human pancreatic cancer. Furthermore, the therapeutic efficacy of a monoclonal anti-PSCA antibody in an in vivo pancreatic cancer model was determined. METHODS: The expression of PSCA in human pancreatic cancer tissues was determined and compared with chronic pancreatitis and normal pancreas by quantitative reverse transcriptase-polymerase chain reaction. Therapeutic efficacy of the monoclonal anti-PSCA antibody 1G8 was examined in Capan-1 pancreatic tumors grown as subcutaneous grafts in athymic nude mice. RESULTS: PSCA was strongly up-regulated in human pancreatic cancer compared with chronic pancreatitis and normal pancreas. In addition, the PSCA protein was expressed on the cell surface of pancreatic cancer cells. Treatment with 1G8 significantly reduced tumor growth initiation in an in vivo pancreatic cancer xenograft model. In addition, antibody treatment of established tumors reduced tumor progression. CONCLUSIONS: These results show a potential therapeutic role for anti-PSCA antibodies in the treatment of pancreatic cancer. Furthermore, PSCA might serve as a novel marker in the diagnosis of pancreatic cancer.

Xiao, M., L. An, et al. (2008). "Establishing a human pancreatic stem cell line and transplanting induced pancreatic islets to reverse experimental diabetes in rats." Sci China C Life Sci **51**(9): 779-88.

The major obstacle in using pancreatic islet transplantation to cure type I and some type II

diabetes is the shortage of the donors. One of ways to overcome such obstacle is to isolate and clone pancreatic stem cells as "seed cells" and induce their differentiation into functional islets as an abundant transplantation source. In this study, a monoclonal human pancreatic stem cell (mhPSC) line was obtained from abortive fetal pancreatic tissues. Pancreatic tissues were taken from abortive fetus by sterile procedures, and digested into single cells and cell clusters with 0.1% type IV collagenase. Cultured in modified glucose-low DMEM with 10% fetal bovine serum (FBS), these single cells and cell clusters adhered to culture dishes, and then primary epidermal-like pancreatic stem cells started to clone. After digesting with 0.25% trypsin and 0.04% EDTA, fibroblasts and other cells were gradually eliminated and epithelioid pancreatic stem cells were gradually purified during generations. Using clone-ring selection, the mhPSCs were obtained. After addition of 10 ng/mL epidermal growth factor (EGF) in cell culture medium, the mhPSCs quickly grew and formed a gravelstone-like monolayer. Continuously proliferated, a mhPSC line, which was derived from a male abortive fetus of 4 months old, has been passed through 50 generations. More than 1 x 10(9) mhPSCs were cryo-preserved in liquid nitrogen. Karyotype analysis showed that the chromosome set of the mhPSC line was normal diploid. Immunocytochemistry results demonstrated that the mhPSC line was positive for the pdx1, glucagon, nestin and CK19, and negative for the insulin, CD34, CD44 and CD45 protein expression. RT-PCR revealed further that the mhPSCs expressed transcription factors of the pdx1, glucagon, nestin and CK19. Also, in vitro induced with beta-mercaptoethanol, the mhPSCs differentiated into nerve cells that expressed the NF protein. Induced with nicotinamide, the mhPSCs differentiated into functional islet-like clusters, as identified by dithizone staining, which expressed the transcription factor of the insulin and secreted the insulin and C-peptide. Furthermore, the transplantation of mhPSCs-induced pancreatic islets into the subcapsular region of the kidney in streptozotocin-induced diabetic rats could reduce blood glucose levels and prolong the life time.

Yang, C., J. M. Wang, et al. (2007). "Expression of stem cell markers CK-19 and PDX-1 mRNA in pancreatic islet samples of different purity from rats." Hepatobiliary Pancreat Dis Int **6**(5): 544-8.

BACKGROUND: Islet stem cells are more or less retained in the procedure of islet isolation and purification, and are transplanted together with islet grafts. Keratoprotein (CK-19) and pancreatic duodenal hox gene 1 (PDX-1) are markers of stem cells. This study was undertaken to examine the expression of these markers in pancreatic islet samples of different purity from rats. METHODS: A total of 30 male Sprague-Dawley rats were randomly assigned to 3 groups to undergo perfusion with V-type collagenase via the pancreatic duct, then the pancreas was excised, diced, shaken, digested and centrifuged to obtain islet sediments. The sediment from group A was not purified, while that from group B was purified with 25% Ficoll-400 and that from group C with 25% and 11% Ficoll-400. RNA was extracted from the different islet samples for reverse transcriptase-polymerase chain reaction (RT-PCR). The expression of the pancreatic stem cell markers CK-19 and PDX-1 was assessed. RESULTS: The purity of islets in samples was (43.6+/-6.29)% in group A; (65.3+/-4.40)% in group B; and (77.6+/-6.36)% in group C ($P<0.05$). The expression of CK-19 and PDX-1 mRNA was significantly higher in group A than in groups B and C, but group C showed the lowest level of expression. CONCLUSION: The expression of CK-19 and PDX-1 mRNA in islet samples of different purity suggests the presence of stem cells in all islet samples.

Yasuda, A., H. Sawai, et al. (2006). "The stem cell factor/c-kit receptor pathway enhances proliferation and invasion of pancreatic cancer cells." Mol Cancer **5**: 46.

BACKGROUND: The transmembrane protein c-kit is a receptor tyrosine kinase (KIT) and KIT is expressed in solid tumors and hematological malignancies such as gastrointestinal stromal tumor (GIST), small-cell lung cancer and chronic myelogenous leukemia (CML). KIT plays a critical role in cell proliferation and differentiation and represents a logical therapeutic target in GIST and CML. In pancreatic cancer, c-kit expression has been observed by immunohistochemical techniques. In this study, we examined the influence of c-kit expression on proliferation and invasion using five pancreatic cancer cell lines. In addition, the inhibitory effect of imatinib mesylate on stem cell factor (SCF)-induced proliferation and invasion was evaluated. Finally, we also analyzed KIT and SCF expression in pancreatic cancer tissues using immunohistochemistry and correlated the results with clinical features. RESULTS: RT-PCR revealed that two pancreatic cancer cell lines, PANC-1 and SW1990, expressed c-kit mRNA. By Western blot analysis, c-kit protein was also present in those lines. In KIT-positive pancreatic cancer cell lines, proliferation and invasion were significantly enhanced by addition of SCF. In contrast, SCF did not enhance proliferation and invasion in the three KIT-negative lines (BxPC-3, Capan-2 and MIA PaCa-2). 5 muM imatinib mesylate significantly inhibited SCF-enhanced proliferation to the same extent compared with the control. Similarly, SCF-

enhanced invasive ability was significantly inhibited by 5 muM imatinib mesylate. KIT was expressed in 16 of 42 clinical specimens by immunohistochemistry, and KIT expression was significantly related to venous system invasion. Furthermore, patients expressing both KIT and SCF had a somewhat lower survival. CONCLUSION: Our results demonstrated that the SCF-KIT pathway enhanced the proliferation and invasiveness in KIT-positive pancreatic cancer cell lines and that the enhanced proliferation and invasion were inhibited by imatinib mesylate. We propose that inhibitors of c-kit tyrosine kinase receptor have the potential to slow the progression of KIT-positive pancreatic cancers.

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3/2/2012