Pancreatic Stem Cell Study Literatures

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Abstract: Stem cells are undifferentiated cells that can divide to more stem cells and differentiate into specialized cells, which exit in multicellular organisms. In mammals, there are two types of stem cells, one is embryonic stem cells (from the inner cell mass of blastocysts) and the other one is adult stem cells (in various tissues). In adult organisms, stem cells and progenitor cells act as a repair system for the body. In a developing embryo, stem cells can differentiate into all the specialized cells. The endocrine pancreas produce insulin. One of the major pancreatic diseases, diabetes mellitus is a metabolic disorder caused by having an insufficient number of insulin-producing β cells. The shortage in donor pancreata could be treated by using alternative sources of stem cells. The adult pancreas retains regenerative capacity and it remains unclear whether this organ contains stem cells. Cellular reprogramming or transdifferentiation of exocrine cells or other types of endocrine cells in the pancreas could provide a long-term solution. This introduces recent reports as references in the related studies.
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Stem cells are undifferentiated cells that can divide to more stem cells and differentiate into specialized cells, which exit in multicellular organisms. In mammals, there are two types of stem cells, one is embryonic stem cells (from the inner cell mass of blastocysts) and the other one is adult stem cells (in various tissues). In adult organisms, stem cells and progenitor cells act as a repair system for the body. In a developing embryo, stem cells can differentiate into all the specialized cells. The endocrine pancreas produce insulin. One of the major pancreatic diseases, diabetes mellitus is a metabolic disorder caused by having an insufficient number of insulin-producing β cells. The shortage in donor pancreata could be treated by using alternative sources of stem cells. The adult pancreas retains regenerative capacity and it remains unclear whether this organ contains stem cells. Cellular reprogramming or transdifferentiation of exocrine cells or other types of endocrine cells in the pancreas could provide a long-term solution.

The following introduces recent reports as references in the related studies.

Abate-Daga, D., K. H. Lagisetty, et al. "A novel chimeric antigen receptor against prostate stem cell antigen mediates tumor destruction in a humanized mouse model of pancreatic cancer." Hum Gene Ther **25**(12): 1003-12.

Despite advances in the understanding of its molecular pathophysiology, pancreatic cancer remains largely incurable, highlighting the need for novel therapies. We developed a chimeric antigen receptor (CAR) specific for prostate stem cell antigen (PSCA), a glycoprotein that is overexpressed in pancreatic cancer starting at early stages of malignant transformation. To optimize the CAR design, we used antigen-recognition domains derived from mouse or human antibodies, and intracellular signaling domains containing one or two T cell costimulatory elements, in addition to CD3zeta. Comparing multiple constructs established that the CAR based on human monoclonal antibody Ha1-4.117 had the greatest reactivity in vitro. To further analyze this CAR, we developed a human pancreatic cancer xenograft model and adoptively transferred CAR-engineered T cells into animals with established tumors.

Bhagavati, S. "Stem cell therapy: challenges ahead." Indian J Pediatr **82**(3): 286-91.

Stem cells have generated great interest for their potential therapeutic use because of their capacity to self-renew indefinitely and to generate all cell lineages (pluripotency). Many diseases such as neurodegenerative disorders or diabetes are caused by loss of functionality or deficiency of a particular cell type. Stem cells differentiated into a specific cell type such as pancreatic beta-cells or neurons, for example, thus hold great promise for regenerative medicine. However, many challenges have to be overcome before stem cell therapy can become a viable clinical approach.

Bose, B. and P. S. Shenoy "In Vitro Differentiation of Pluripotent Stem Cells into Functional beta Islets Under 2D and 3D Culture Conditions and In Vivo Preclinical Validation of 3D Islets." Methods Mol Biol.

Since the advent of pluripotent stem cells, (embryonic and induced pluripotent stem cells), applications of such pluripotent stem cells are of prime importance. Indeed, scientists are involved in studying the basic biology of pluripotent stem cells, but equal impetus is there to direct the pluripotent stem cells into multiple lineages for cell therapy applications. Scientists across the globe have been successful, to a certain extent, in obtaining cells of definitive endoderm and also pancreatic beta islets by differentiating human pluripotent stem cells. Pluripotent stem cell differentiation protocols aim at mimicking in vivo embryonic development. As in vivo embryonic development is a complex process and involves interplay of multiple cytokines, the differentiation protocols also involve a stepwise use of multiple cytokines. Indeed the novel markers for pancreas organogenesis serve as the roadmaps to develop new protocols for pancreatic differentiation from pluripotent stem cells. Earliest developed protocols for pancreas differentiation involved "Nestin selection pathway," a pathway common for both neuronal and pancreatic differentiation lead to the generation of cells that were a combination of cells from neuronal lineage. Eventually with the discovery of hierarchy of beta cell transcription factors like Pdx1, Pax4, and Nkx2.2, forced expression of such transcription factors proved successful in converting a pluripotent stem cell into a beta cell. Protocols developed almost half a decade ago to the recent ones rather involve stepwise differentiations involving various cytokines and could generate as high as 25 % functional insulin-positive cells in vitro. Most advanced protocols for beta islet differentiations from human pluripotent stem cells focused on 3D culture conditions, which reportedly produced 60-65 % functional beta islet cells. Here, we describe the protocol for differentiation of human pluripotent stem cells into functional beta cells under both 2D and 3D culture conditions.

Brouwers, B., G. de Faudeur, et al. "Impaired islet function in commonly used transgenic mouse lines due to human growth hormone minigene expression." Cell Metab **20**(6): 979-90.

The human growth hormone (hGH) minigene is frequently used in the derivation of transgenic mouse lines to enhance transgene expression. Although this minigene is present in the transgenes as a secondcistron, and thus not thought to be expressed, we found that three commonly used lines, Pdx1-Cre(Late), RIP-Cre, and MIP-GFP, each expressed significant amounts of hGH in pancreatic islets. Locally secreted hGH binds to prolactin receptors on beta cells, activates STAT5 signaling, and induces pregnancy-like changes in gene expression, thereby augmenting pancreatic beta cell mass and insulin content. In addition, islets of Pdx1-Cre(Late) mice have lower GLUT2 expression and reduced glucose-induced insulin release and are protected against the beta cell toxin streptozotocin. These findings may be important when interpreting results obtained when these and other hGH minigene-containing transgenic mice are used.

Bruin, J. E., N. Saber, et al. "Treating Diet-Induced Diabetes and Obesity with Human Embryonic Stem Cell-Derived Pancreatic Progenitor Cells and Antidiabetic Drugs." Stem Cell Reports.

Human embryonic stem cell (hESC)-derived pancreatic progenitor cells effectively reverse hyperglycemia in rodent models of type 1 diabetes, but their capacity to treat type 2 diabetes has not been reported. An immunodeficient model of type 2 diabetes was generated by high-fat diet (HFD) feeding in SCID-beige mice. Exposure to HFDs did not impact the maturation of macroencapsulated pancreatic progenitor cells into glucose-responsive insulin-secreting cells following transplantation, and the cell therapy improved glucose tolerance in HFD-fed transplant recipients after 24 weeks. However, since diet-induced hyperglycemia and obesity were not fully ameliorated by transplantation alone, a second cohort of HFD-fed mice was treated with pancreatic progenitor cells combined with one of three antidiabetic drugs. All combination therapies rapidly improved body weight and co-treatment with either sitagliptin or metformin improved hyperglycemia after only 12 weeks. Therefore, a stem cell-based therapy may be effective for treating type 2 diabetes, particularly in combination with antidiabetic drugs.

Cogger, K. and M. C. Nostro "Recent advances in cell replacement therapies for the treatment of type 1 diabetes." Endocrinology **156**(1): 8-15.

Exogenous insulin administration is currently the only treatment available to all type 1 diabetes patients, but it is not a cure. By helping to regulate circulating blood glucose levels, it has significantly improved life expectancy, but there are still long-term complications associated with the disease that may be preventable with a treatment strategy that can provide better glycemic control. Isolated islet (or whole pancreas) transplantation, xenotransplantation, and the transplant of human pluripotent stem cell-derived beta-cells provide the potential to restore endogenous insulin production and reestablish normoglycemia. Here, we discuss the latest advances in these fields, including the use of encapsulation technology, as well as some of the hurdles that still need to be overcome for these alternative therapies to become mainstream and/or progress to clinical development.

Czysz, K., S. Minger, et al. "DMSO efficiently down regulates pluripotency genes in human embryonic stem cells during definitive endoderm derivation and increases the proficiency of hepatic differentiation." PLoS One **10**(2): e0117689.

Definitive endoderm (DE) is one of the three germ layers which during in vivo vertebrate development gives rise to a variety of organs including liver, lungs, thyroid and pancreas; consequently efficient in vitro initiation of stem cell differentiation to DE cells is a prerequisite for successful cellular specification to subsequent DE-derived cell types [1, 2]. In this study we present a novel approach to rapidly and efficiently down regulate pluripotency genes during initiation of differentiation to DE cells by addition of dimethyl sulfoxide (DMSO) to Activin A-based culture medium and report its effects on the downstream differentiation to hepatocyte-like cells. MATERIALS AND METHODS: Human embryonic stem cells (hESC) were differentiated to DE using standard methods in medium supplemented with 100ng/ml of Activin A and compared to cultures where DE specification was additionally enhanced with different concentrations of DMSO. DE cells were subsequently primed to generate hepatic-like cells to investigate whether the addition of DMSO during formation of DE improved subsequent expression of hepatic markers.

Dalla Pozza, E., I. Dando, et al. "Pancreatic ductal adenocarcinoma cell lines display a plastic ability to bidirectionally convert into cancer stem cells." Int J Oncol **46**(3): 1099-108.

Pancreatic ductal adenocarcinoma (PDAC) is often diagnosed when metastatic events have occurred. Cancer stem cells (CSCs) play an important role in tumor initiation, metastasis, chemoresistance and relapse. A growing number of studies have suggested that CSCs exist in a dynamic equilibrium with more differentiated cancer cells via a bidirectional regeneration that is dependent on the environmental stimuli. In this investigation, we obtain, by using a selective medium, PDAC CSCs from five out of nine PDAC cell lines, endowed with different tumorsphereforming ability. PDAC CSCs were generally more resistant to the action of five anticancer drugs than parental cell lines and were characterized by an increased expression of EpCAM and CD44v6, typical stem cell surface markers, and a decreased expression of Ecadherin, the main marker of the epithelial state. PDAC CSCs were able to redifferentiate into parental cells once cultured in parental growth condition, as demonstrated by reacquisition of the epithelial morphology, the decreased expression levels of EpCAM and CD44v6 and the increased sensitivity to anticancer drugs. Finally, PDAC CSCs injected into nude mice developed a larger subcutaneous tumor mass and showed a higher metastatic activity compared to parental cells. The present study demonstrates the ability to obtain CSCs from several PDAC cell lines and that these cells are differentially resistant to various anticancer agents. This variability renders them a model of great importance to deeply understand pancreatic adenocarcinoma biology, to discover new biomarkers and to screen new therapeutic compounds.

Diekmann, U., S. Lenzen, et al. "A reliable and efficient protocol for human pluripotent stem cell differentiation into the definitive endoderm based on dispersed single cells." Stem Cells Dev **24**(2): 190-204.

Differentiation of pluripotent cells into endoderm-related cell types initially requires in vitro gastrulation into the definitive endoderm (DE). Most differentiation protocols are initiated from colonies of pluripotent cells complicating their adaption due to insufficiently defined starting conditions. The protocol described here was initiated from a defined cell number of dispersed single cells and tested on three different human embryonic stem cell lines and one human induced pluripotent stem cell line. Combined activation of ActivinA/Nodal signaling and GSK3 inhibition for the first 24 h, followed by ActivinA/Nodal signaling efficiently induced the DE state. Activation of ActivinA/Nodal signaling alone was not effective. Efficient GSK3 inhibition allowed the reduction of the ActivinA concentration during the entire protocol. A feeder-independent cultivation of pluripotent cells was preferred to achieve the high efficiency and robustness since feeder cells hindered the differentiation process. Additionally, inhibition of the phosphatidylinositol 3-kinase (PI3K) signaling pathway was not required, nonetheless yielding high cell numbers efficiently committed toward the DE. Finally, the endoderm generated could be differentiated further into PDX1-positive pan-pancreatic cells and NGN3-positive endocrine progenitors. Thus, this efficient and robust DE differentiation protocol is a step forward toward better reproducibility due to the well-defined conditions based on dispersed single cells from feeder-free-cultivated human pluripotent cells.

Feng, T., L. Li, et al. "Metformin enhances radiation response of ECa109 cells through activation of ATM and AMPK." Biomed Pharmacother **69**: 260-6.

Metformin is a first-line used agent for type II diabetes with few side effects. The antineoplastic

effect of metformin was widely explored recently. Metformin may also be a prospective chemosensitizer or radiosensitizer in cancer treatment. In the present study, we firstly showed that metformin could effectively enhance the anti-proliferation effect of ionizing radiation (IR) on esophageal cancer (EC) cells ECa109. More potent DNA damage was observed by detection of gammaH2AX foci. Metformin synergistically induce apoptosis and cell cycle arrest in ECa109 cells with IR. Furthermore, the mechanisms how metformin sensitized ECa109 cells to IR may be targeting the ATM and AMPK/mTOR/HIF-1alpha pathways. Metformin may be a valuable agent in comprehensive treatment of EC.

Gao, C., S. Li, et al. "SCF, Regulated by HIF-1alpha, Promotes Pancreatic Ductal Adenocarcinoma Cell Progression." PLoS One **10**(3): e0121338.

Stem cell factor (SCF) and hypoxia-inducible factor-1alpha (HIF-1alpha) both have important functions in pancreatic ductal adenocarcinoma (PDAC). This study aims to analyze the expression and clinicopathological significance of SCF and HIF-1alpha in PDAC specimens and explore the molecular mechanism at PDAC cells in vitro and in vivo. We showed that the expression of SCF was significantly correlated with HIF-1alpha expression via Western blot, PCR, chromatin immunoprecipitation (ChIP) assay, and luciferase assay analysis. The SCF level was also correlated with lymph node metastasis and the pathological tumor node metastasis (pTNM) stage in PDAC samples. The SCF higher-expression group had significantly lower survival rates than the SCF lower-expression group ($p<0.05$). Hypoxia up-regulated the expression of SCF through the hypoxia-inducible factor (HIF)-1alpha in PDAC cells at the protein and RNA levels. When HIF-1alpha was knocked down by RNA interference, the SCF level decreased significantly. Additionally, ChIP and luciferase results demonstrated that HIF-1alpha can directly bind to the hypoxia response element (HRE) region of the SCF promoter and activate the SCF transcription under hypoxia. The results of colony formation, cell scratch, and transwell migration assay showed that SCF promoted the proliferation and invasion of PANC-1 cells under hypoxia. Furthermore, the down-regulated ability of cell proliferation and invasion following HIF-1alpha knockdown was rescued by adding exogenous SCF under hypoxia in vitro. Finally, when the HIF-1alpha expression was inhibited by digoxin, the tumor volume and the SCF level decreased, thereby proving the relationship between HIF-1alpha and SCF in vivo. In conclusion, SCF is an important factor for the growth of PDAC. In our experiments, we proved that SCF, a downstream gene of HIF-1alpha, can promote the development of PDAC under hypoxia. Thus, SCF might be a potential therapeutic target for PDAC.

Hannan, N. R., F. Sampaziotis, et al. "Generation of Distal Airway Epithelium from Multipotent Human Foregut Stem Cells." Stem Cells Dev.

Collectively lung diseases are one of the largest causes of premature death worldwide and represent a major focus in the field of regenerative medicine. Despite significant progress, only few stem cell platforms are currently available for cell based therapy, disease modelling and drug screening in the context of pulmonary disorders. Human foregut stem cells (hFSCs) represent an advantageous progenitor cell type that can be used to amplify large quantities of cells for regenerative medicine applications and can be derived from any human pluripotent stem cell line. Here we further demonstrate the application of hFSCs by generating a near homogeneous population of early pulmonary endoderm cells co-expressesing NKX2.1 and FOXP2. These progenitors are then able to form cells representative of distal airway epithelium that express NKX2.1, GATA6, CFTR and secrete SFTPC. This culture system can be applied to hFSCs carrying the CFTR mutation Deltaf508 enabling the development of in vitro model for cystic fibrosis. This platform is compatible with drug screening and functional validations of small molecules which can reverse the phenotype associated with CFTR mutation. This is the first demonstration that multipotent endoderm stem cells can not only differentiate into both liver and pancreatic cells but also into lung endoderm. Furthermore our study establishes a new approach for the generation of functional lung cells that can be used for disease modelling as well as drug screening and the study of lung development.

He, Z. X., Z. W. Zhou, et al. "Overview of clinically approved oral antidiabetic agents for the treatment of type 2 diabetes mellitus." Clin Exp Pharmacol Physiol **42**(2): 125-38.

Type 2 diabetes mellitus (T2DM) is caused by insulin resistance and characterized by progressive pancreatic beta-cell dysfunction. This articles reviews the application and limitations of currently approved oral drugs for the treatment of T2DM. Data were retrieved from the literature and well-recognized drug-related databases. Although lifestyle modifications and metformin are the cornerstones of the initial management of T2DM, there is an increasing array of second- and third-line pharmacological agents, including sulphonylureas, insulin, thiazolidinediones and glitazones, alpha-glucosidase inhibitors, glucagon-like peptide-1 agonists, dipeptidyl peptidase

4 inhibitors and the amylin receptor agonist pramlintide. Current T2DM treatment focuses on reducing blood glucose levels via different mechanisms, including nuclear hormone receptors, nucleic acid binding proteins, transcription factors, voltage-gated K(+) channels, glucosidase, G-protein-coupled receptors and non-receptor serine/threonine protein kinase. Extensive efforts are needed to address the pathogenesis of T2DM, which may facilitate the development of new therapies and the identification of new therapeutic targets to overcome the shortcomings of currently available drugs for T2DM and to achieve therapeutic goals.

Hu, J., M. Jo, et al. "uPAR induces expression of transforming growth factor beta and interleukin-4 in cancer cells to promote tumor-permissive conditioning of macrophages." Am J Pathol **184**(12): 3384-93.

Cancer cells condition macrophages and other inflammatory cells in the tumor microenvironment so that these cells are more permissive for cancer growth and metastasis. Conditioning of inflammatory cells reflects, at least in part, soluble mediators (such as transforming growth factor beta and IL-4) that are released by cancer cells and alter the phenotype of cells of the innate immune system. Signaling pathways in cancer cells that potentiate this activity are incompletely understood. The urokinase receptor (uPAR) is a cell-signaling receptor known to promote cancer cell survival, proliferation, metastasis, and cancer stem cell-like properties. The present findings show that uPAR expression in diverse cancer cells, including breast cancer, pancreatic cancer, and glioblastoma cells, promotes the ability of these cells to condition co-cultured bone marrow-derived macrophages so that the macrophages express significantly increased levels of arginase 1, a biomarker of the alternatively activated M2 macrophage phenotype. Expression of transforming growth factor beta was substantially increased in uPAR-expressing cancer cells via a mechanism that requires uPA-initiated cell signaling. uPAR also controlled expression of IL-4 in cancer cells via a mechanism that involves activation of ERK1/2. The ability of uPAR to induce expression of factors that condition macrophages in the tumor microenvironment may constitute an important mechanism by which uPAR promotes cancer progression.

Jain, N. and E. J. Lee "Islet Endothelial Cells Derived From Mouse Embryonic Stem Cells." Cell Transplant.

The islet endothelium comprises a specialized population of islet endothelial cells (IECs) expressing unique markers such as nephrin and alpha-1 antitrypsin (AAT) that are not found in endothelial cells in surrounding tissues. However, due to difficulties in isolating and maintaining a pure population of these cells, the information on these islet specific cells is currently very limited. Interestingly, we have identified a large subpopulation of endothelial cells exhibiting IEC phenotype, while deriving insulinproducing cells from mouse embryonic stem cells (mESCs). These cells were identified by the uptake of low density lipoprotein (LDL) and were successfully isolated and subsequently expanded in endothelial cell culture medium. Further analysis demonstrated that the mouse embryonic stem cell derived endothelial cells (mESC-ECs) not only express classical endothelial markers, such as platelet endothelial cell adhesion molecule (PECAM1), thromobomodulin, Intercellular adhesion molecule -1 (ICAM-1) and endothelial nitric oxide synthase (eNOS), but also IEC specific markers such as nephrin and AAT. Moreover, mESC-ECs secrete basement membrane proteins such as collagen type IV, laminin and fibronectin in culture and form tubular networks on a layer of Matrigel, demonstrating angiogenic activity. Further, mESC-ECs not only express eNOS, but also its eNOS expression is glucose dependent, which is another characteristic phenotype of IECs. With the ability to obtain highly purified IECs derived from pluripotent stem cells, it is possible to closely examine the function of these cells and their interaction with pancreatic beta cells during development and maturation in vitro. Further characterization of tissue-specific endothelial cell properties may enhance our ability to formulate new therapeutic angiogenic approaches for diabetes.

Jaramillo, M., S. Mathew, et al. "Endothelial cells mediate islet-specific maturation of human embryonic stem cell-derived pancreatic progenitor cells." Tissue Eng Part A **21**(1-2): 14-25.

It is well recognized that in vitro differentiation of embryonic stem cells (ESC) can be best achieved by closely recapitulating the in vivo developmental niche. Thus, implementation of directed differentiation strategies has yielded encouraging results in the area of pancreatic islet differentiation. These strategies have concentrated on direct addition of chemical signals, however, other aspect of the developmental niche are yet to be explored. During development, pancreatic progenitor (PP) cells grow as an epithelial sheet, which aggregates with endothelial cells (ECs) during the final stages of maturation. Several findings suggest that the interactions with EC play a role in pancreatic development. In this study, we recapitulated this phenomenon in an in vitro environment by maturing

the human ESC (hESC)-derived PP cells in close contact with ECs. We find that co-culture with different ECs (but not fibroblast) alone results in pancreatic islet-specific differentiation of hESC-derived PP cells even in the absence of additional chemical induction. The differentiated cells responded to exogenous glucose levels by enhanced C-peptide synthesis. The co-culture system aligned well with endocrine development as determined by comprehensive analysis of involved signaling pathways. By recapitulating cell-cell interaction aspects of the developmental niche we achieved a differentiation model that aligns closely with islet organogenesis.

Jaramillo, M., S. S. Singh, et al. "Inducing endoderm differentiation by modulating mechanical properties of soft substrates." J Tissue Eng Regen Med **9**(1): 1-12.

Early embryonic stem cell (ESC) differentiation is marked by the formation of three germ layers from which all tissues types arise. Conventionally, ESCs are differentiated by altering their chemical microenvironment. Recently however, it was established that a mechanical microenvironment can also contribute towards cellular phenotype commitment. In this study, we report how the cellular mechanical microenvironment of soft substrates affects the differentiation and phenotypic commitment of ESCs. Mouse ESCs were cultured in a fibrin hydrogel matrix in 2D and 3D cultures. The gelation characteristics of the substrates were modulated by systematically altering the fibrinogen concentration and the fibrinogen-thrombin crosslinking ratio. Analysis of the ESCs cultured on different substrate conditions clearly illustrated the strong influence that substrate physical characteristics assert on cellular behaviours. Specifically, it was found that ESCs had a higher proliferation rate in gels of lower stiffness. Early differentiation events were studied by analyzing the gene and protein expression levels of early germ layer markers. Our results revealed that lower substrate stiffness elicited stronger upregulation of endoderm related genes Sox17, Afp and Hnf4 compared to stiffer substrates. While both 2D and 3D cultures showed a similar response, the effects were much stronger in 3D culture. These results suggest that physical cues can be used to modulate ESC differentiation into clinically relevant tissues such as liver and pancreas. Copyright (c) 2012 John Wiley & Sons, Ltd.

Ji, A. T., Y. C. Chang, et al. "Niche-dependent regulations of metabolic balance in high-fat diet-induced diabetic mice by mesenchymal stromal cells." Diabetes **64**(3): 926-36.

Mesenchymal stromal cells (MSCs) have great potential to maintain glucose homeostasis and metabolic balance. Here, we demonstrate that in mice continuously fed with high-fat diet (HFD) that developed non-insulin-dependent diabetes, two episodes of systemic MSC transplantations effectively improve glucose tolerance and blood glucose homeostasis and reduce body weight through targeting pancreas and insulin-sensitive tissues and organs via site-specific mechanisms. MSCs support pancreatic islet growth by direct differentiation into insulin-producing cells and by mitigating the cytotoxicity of interleukin 1 (IL-1) and tumor necrosis factor-alpha (TNF-alpha) in the pancreas. Localization of MSCs in the liver and skeletal muscles in diabetic animals is also enhanced and therefore improves glucose tolerance, although long-term engraftment is not observed. MSCs prevent HFD-induced fatty liver development and restore glycogen storage in hepatocytes. Increased expression of IL-1 receptor antagonist and Glut4 in skeletal muscles after MSC transplantation results in better blood glucose homeostasis. Intriguingly, systemic MSC transplantation does not alter adipocyte number, but it decreases HFD-induced cell infiltration in adipose tissues and reduces serum levels of adipokines, including leptin and TNF-alpha. Taken together, systemic MSC transplantation ameliorates HFD-induced obesity and restores metabolic balance through multisystemic regulations that are niche dependent. Such findings have supported systemic transplantation of MSCs to correct metabolic imbalance.

Jiao, F., H. Hu, et al. "Long Noncoding RNA MALAT-1 Enhances Stem Cell-Like Phenotypes in Pancreatic Cancer Cells." Int J Mol Sci **16**(4): 6677-6693.

Cancer stem cells (CSCs) play a vital role in tumor initiation, progression, metastasis, chemoresistance, and recurrence. The mechanisms that maintain the stemness of these cells remain largely unknown. Our previous study indicated that MALAT-1 may serve as an oncogenic long noncoding RNA in pancreatic cancer by promoting epithelial-mesenchymal transition (EMT) and regulating CSCs markers expression. More significantly, there is emerging evidence that the EMT process may give rise to CSCs, or at least cells with stem cell-like properties. Therefore, we hypothesized that MALAT-1 might enhance stem cell-like phenotypes in pancreatic cancer cells. In this study, our data showed that MALAT-1 could increase the proportion of pancreatic CSCs, maintain self-renewing capacity, decrease the chemosensitivity to anticancer drugs, and accelerate tumor angiogenesis in vitro. In addition,

subcutaneous nude mouse xenografts revealed that MALAT-1 could promote tumorigenicity of pancreatic cancer cells in vivo. The underlying mechanisms may involve in increased expression of self-renewal related factors Sox2. Collectively, we for the first time found the potential effects of MALAT-1 on the stem cell-like phenotypes in pancreatic cancer cells, suggesting a novel role of MALAT-1 in tumor stemness, which remains to be fully elucidated.

Johannesson, B., L. Sui, et al. "Toward beta cell replacement for diabetes." Embo J.

The discovery of insulin more than 90 years ago introduced a life-saving treatment for patients with type 1 diabetes, and since then, significant progress has been made in clinical care for all forms of diabetes. However, no method of insulin delivery matches the ability of the human pancreas to reliably and automatically maintain glucose levels within a tight range. Transplantation of human islets or of an intact pancreas can in principle cure diabetes, but this approach is generally reserved for cases with simultaneous transplantation of a kidney, where immunosuppression is already a requirement. Recent advances in cell reprogramming and beta cell differentiation now allow the generation of personalized stem cells, providing an unlimited source of beta cells for research and for developing autologous cell therapies. In this review, we will discuss the utility of stem cell-derived beta cells to investigate the mechanisms of beta cell failure in diabetes, and the challenges to develop beta cell replacement therapies. These challenges include appropriate quality controls of the cells being used, the ability to generate beta cell grafts of stable cellular composition, and in the case of type 1 diabetes, protecting implanted cells from autoimmune destruction without compromising other aspects of the immune system or the functionality of the graft. Such novel treatments will need to match or exceed the relative safety and efficacy of available care for diabetes.

Ki, C. S., T. Y. Lin, et al. "Thiol-ene hydrogels as desmoplasia-mimetic matrices for modeling pancreatic cancer cell growth, invasion, and drug resistance." Biomaterials **35**(36): 9668-77.

The development of pancreatic ductal adenocarcinoma (PDAC) is heavily influenced by local stromal tissues, or desmoplasia. Biomimetic hydrogels capable of mimicking tumor niches are particularly useful for discovering the role of independent matrix cues on cancer cell development. Here, we report a photo-curable and bio-orthogonal thiol-ene (i.e., cross-linked by mutually reactive norbornene and thiol groups via photoinitiation) hydrogel platform for studying the growth, morphogenesis, drug resistance, and cancer stem cell marker expression in PDAC cells cultured in 3D. The hydrogels were prepared from multi-arm poly(ethylene glycol)-norbornene cross-linked with protease-sensitive peptide to permit cell-mediated matrix remodeling. Collagen 1 fibrils were incorporated into the covalent network while cytokines (e.g., EGF and TGF-beta1) were supplemented in the culture media for controlling cell fate. We found that the presence of collagen 1 enhanced cell proliferation and Yes-associated protein (YAP) translocation to cell nuclei. Cytokines and collagen 1 synergistically up-regulated MT1-MMP expression and induced cell spreading, suggestive of epithelial-mesenchymal transition (EMT) in the encapsulated cells. Furthermore, PDAC cells cultured in 3D developed chemo-resistance even in the absence of collagen 1 and cytokines. This phenotype is likely a consequence of the enrichment of pancreatic cancer stem cells that expressed high levels of CD24, sonic hedgehog (SHH), and vascular endothelial growth factor (VEGF).

Kim, J. and K. S. Zaret "Reprogramming of human cancer cells to pluripotency for models of cancer progression." Embo J **34**(6): 739-747.

The ability to study live cells as they progress through the stages of cancer provides the opportunity to discover dynamic networks underlying pathology, markers of early stages, and ways to assess therapeutics. Genetically engineered animal models of cancer, where it is possible to study the consequences of temporal-specific induction of oncogenes or deletion of tumor suppressors, have yielded major insights into cancer progression. Yet differences exist between animal and human cancers, such as in markers of progression and response to therapeutics. Thus, there is a need for human cell models of cancer progression. Most human cell models of cancer are based on tumor cell lines and xenografts of primary tumor cells that resemble the advanced tumor state, from which the cells were derived, and thus do not recapitulate disease progression. Yet a subset of cancer types have been reprogrammed to pluripotency or near-pluripotency by blastocyst injection, by somatic cell nuclear transfer and by induced pluripotent stem cell (iPS) technology. The reprogrammed cancer cells show that pluripotency can transiently dominate over the cancer phenotype. Diverse studies show that reprogrammed cancer cells can, in some cases, exhibit early-stage phenotypes reflective of only partial expression of the cancer genome. In one case, reprogrammed human pancreatic cancer cells have been shown to recapitulate stages of cancer progression, from early to late stages, thus

providing a model for studying pancreatic cancer development in human cells where previously such could only be discerned from mouse models. We discuss these findings, the challenges in developing such models and their current limitations, and ways that iPS reprogramming may be enhanced to develop human cell models of cancer progression.

Kiuchi, S., S. Ikeshita, et al. "Pancreatic cancer cells express CD44 variant 9 and multidrug resistance protein 1 during mitosis." Exp Mol Pathol **98**(1): 41-6.

Pancreatic cancer is one of the most lethal cancers with high metastatic potential and strong chemoresistance. Its intractable natures are attributed to high robustness in tumor cells for their survival. We demonstrate here that pancreatic cancer cells (PCCs) with an epithelial phenotype upregulate cell surface expression of CD44 variant 9 (CD44v9), an important cancer stem cell marker, during the mitotic phases of the cell cycle. Of five human CD44(+) PCC lines examined, three cell lines, PCI-24, PCI-43 and PCI-55, expressed E-cadherin and CD44 variants, suggesting that they have an epithelial phenotype. By contrast, PANC-1 and MIA PaCa-2 cells expressed vimentin and ZEB1, suggesting that they have a mesenchymal phenotype. PCCs with an epithelial phenotype upregulated cell surface expression of CD44v9 in prophase, metaphase, anaphase and telophase and downregulated CD44v9 expression in late-telophase, cytokinesis and interphase. Sorted CD44v9-negative PCI-55 cells resumed CD44v9 expression when they re-entered the mitotic stage. Interestingly, CD44v9(bright) mitotic cells expressed multidrug resistance protein 1 (MDR1) intracellularly. Upregulated expression of CD44v9 and MDR1 might contribute to the intractable nature of PCCs with high proliferative activity.

Kobayashi, T., M. Kato-Itoh, et al. "Targeted organ generation using Mixl1-inducible mouse pluripotent stem cells in blastocyst complementation." Stem Cells Dev **24**(2): 182-9.

Generation of functional organs from patients' own cells is one of the ultimate goals of regenerative medicine. As a novel approach to creation of organs from pluripotent stem cells (PSCs), we employed blastocyst complementation in organogenesis-disabled animals and successfully generated PSC-derived pancreas and kidneys. Blastocyst complementation, which exploits the capacity of PSCs to participate in forming chimeras, does not, however, exclude contribution of PSCs to the development of tissues-including neural cells or germ cells-other than those specifically targeted by disabling of organogenesis. This fact provokes ethical controversy if human PSCs are to be used. In this study, we demonstrated that forced expression of Mix-like protein 1 (encoded by Mixl1) can be used to guide contribution of mouse embryonic stem cells to endodermal organs after blastocyst injection. We then succeeded in applying this method to generate functional pancreas in pancreatogenesis-disabled Pdx1 knockout mice using a newly developed tetraploid-based organ-complementation method. These findings hold promise for targeted organ generation from patients' own PSCs in livestock animals.

Larijani, B., H. R. Aghayan, et al. "GMP-Grade Human Fetal Liver-Derived Mesenchymal Stem Cells for Clinical Transplantation." Methods Mol Biol **1283**: 123-36.

Stem cell therapy seems a promising avenue in regenerative medicine. Within various stem cells, mesenchymal stem cells have progressively used for cellular therapy. Because of the age-related decreasing in the frequency and differentiating capacity of adult MSCs, fetal tissues such as fetal liver, lung, pancreas, spleen, etc. have been introduced as an alternative source of MSCs for cellular therapy. On the other hand, using stem cells as advanced therapy medicinal products, must be performed in compliance with cGMP as a quality assurance system to ensure the safety, quality, and identity of cell products during translation from the basic stem cell sciences into clinical cell transplantation. In this chapter the authors have demonstrated the manufacturing of GMP-grade human fetal liver-derived mesenchymal stem cells.

Lemper, M., G. Leuckx, et al. "Reprogramming of human pancreatic exocrine cells to beta-like cells." Cell Death Differ.

Rodent acinar cells exhibit a remarkable plasticity as they can transdifferentiate to duct-, hepatocyte- and islet beta-like cells. We evaluated whether exocrine cells from adult human pancreas can similarly respond to proendocrine stimuli. Exocrine cells from adult human pancreas were transduced directly with lentiviruses expressing activated MAPK (mitogen-activated protein kinase) and STAT3 (signal transducer and activator of transcription 3) and cultured as monolayers or as 3D structures. Expression of STAT3 and MAPK in human exocrine cells activated expression of the proendocrine factor neurogenin 3 in 50% to 80% of transduced exocrine cells. However, the number of insulin-positive cells increased only in the exocrine cells grown initially in suspension before 3D culture. Lineage tracing identified human acinar cells as the source of Ngn3- and insulin-expressing cells. Long-term engraftment into immunocompromised mice increased the efficiency of reprogramming to insulin-positive cells. Our data demonstrate that exocrine cells from human

pancreas can be reprogrammed to transplantable insulin-producing cells that acquire functionality. Given the large number of exocrine cells in a donor pancreas, this approach presents a novel strategy to expand cell therapy in type 1 diabetes.Cell Death and Differentiation advance online publication, 5 December 2014; doi:10.1038/cdd.2014.193.

Li, W., L. Huang, et al. "Engraftable neural crest stem cells derived from cynomolgus monkey embryonic stem cells." Biomaterials **39**: 75-84.

Neural crest stem cells (NCSCs), a population of multipotent cells that migrate extensively and give rise to diverse derivatives, including peripheral and enteric neurons and glia, craniofacial cartilage and bone, melanocytes and smooth muscle, have great potential for regenerative medicine. Non-human primates provide optimal models for the development of stem cell therapies. Here, we describe the first derivation of NCSCs from cynomolgus monkey embryonic stem cells (CmESCs) at the neural rosette stage. CmESC-derived neurospheres replated on polyornithine/laminin-coated dishes migrated onto the substrate and showed characteristic expression of NCSC markers, including Sox10, AP2alpha, Slug, Nestin, p75, and HNK1. CmNCSCs were capable of propagating in an undifferentiated state in vitro as adherent or suspension cultures, and could be subsequently induced to differentiate towards peripheral nervous system lineages (peripheral sympathetic neurons, sensory neurons, and Schwann cells) and mesenchymal lineages (osteoblasts, adipocytes, chondrocytes, and smooth muscle cells). CmNCSCs transplanted into developing chick embryos or fetal brains of cynomolgus macaques survived, migrated, and differentiated into progeny consistent with a neural crest identity. Our studies demonstrate that CmNCSCs offer a new tool for investigating neural crest development and neural crest-associated human disease and suggest that this non-human primate model may facilitate tissue engineering and regenerative medicine efforts.

Lin, C. C., C. S. Ki, et al. "Thiol-norbornene photo-click hydrogels for tissue engineering applications." J Appl Polym Sci **132**(8).

Thiol-norbornene (thiol-ene) photo-click hydrogels have emerged as a diverse material system for tissue engineering applications. These hydrogels are cross-linked through light mediated orthogonal reactions between multi-functional norbornene-modified macromers (e.g., poly(ethylene glycol), hyaluronic acid, gelatin) and sulfhydryl-containing linkers (e.g., dithiothreitol, PEG-dithiol, bis-cysteine peptides) using low concentration of photoinitiator. The gelation of thiol-norbornene hydrogels can be initiated by long-wave UV light or visible light without additional co-initiator or co-monomer. The cross-linking and degradation behaviors of thiol-norbornene hydrogels are controlled through material selections, whereas the biophysical and biochemical properties of the gels are easily and independently tuned owing to the orthogonal reactivity between norbornene and thiol moieties. Uniquely, the cross-linking of step-growth thiol-norbornene hydrogels is not oxygen-inhibited, therefore the gelation is much faster and highly cytocompatible compared with chain-growth polymerized hydrogels using similar gelation conditions. These hydrogels have been prepared as tunable substrates for 2D cell culture, as microgels or bulk gels for affinity-based or protease-sensitive drug delivery, and as scaffolds for 3D cell culture. Reports from different laboratories have demonstrated the broad utility of thiol-norbornene hydrogels in tissue engineering and regenerative medicine applications, including valvular and vascular tissue engineering, liver and pancreas-related tissue engineering, neural regeneration, musculoskeletal (bone and cartilage) tissue regeneration, stem cell culture and differentiation, as well as cancer cell biology. This article provides an up-to-date overview on thiol-norbornene hydrogel cross-linking and degradation mechanisms, tunable material properties, as well as the use of thiol-norbornene hydrogels in drug delivery and tissue engineering applications.

Masjkur, J., C. Arps-Forker, et al. "Hes3 is expressed in the adult pancreatic islet and regulates gene expression, cell growth, and insulin release." J Biol Chem **289**(51): 35503-16.

The transcription factor Hes3 is a component of a signaling pathway that supports the growth of neural stem cells with profound consequences in neurodegenerative disease models. Here we explored whether Hes3 also regulates pancreatic islet cells. We showed that Hes3 is expressed in human and rodent pancreatic islets. In mouse islets it co-localizes with alpha and beta cell markers. We employed the mouse insulinoma cell line MIN6 to perform in vitro characterization and functional studies in conditions known to modulate Hes3 based upon our previous work using neural stem cell cultures. In these conditions, cells showed elevated Hes3 expression and nuclear localization, grew efficiently, and showed higher evoked insulin release responses, compared with serum-containing conditions. They also exhibited higher expression of the transcription factor Pdx1 and insulin. Furthermore, they were responsive to pharmacological treatments with the GLP-1 analog Exendin-4, which increased nuclear Hes3 localization. We employed a transfection approach to address

specific functions of Hes3. Hes3 RNA interference opposed cell growth and affected gene expression as revealed by DNA microarrays. Western blotting and PCR approaches specifically showed that Hes3 RNA interference opposes the expression of Pdx1 and insulin. Hes3 overexpression (using a Hes3-GFP fusion construct) confirmed a role of Hes3 in regulating Pdx1 expression. Hes3 RNA interference reduced evoked insulin release. Mice lacking Hes3 exhibited increased islet damage by streptozotocin. These data suggest roles of Hes3 in pancreatic islet function.

McGrath, P. S., C. L. Watson, et al. "The basic helix-loop-helix transcription factor NEUROG3 is required for development of the human endocrine pancreas." Diabetes.

Neurogenin 3 (NEUROG3) is a basic helix-loop-helix transcription factor that is required for development of the endocrine pancreas in mice. In contrast, humans with NEUROG3 mutations are born with endocrine pancreas function, calling into question whether NEUROG3 is required for human endocrine pancreas development. To test this directly, we generated human embryonic stem cell (hESC) lines where both alleles of NEUROG3 were disrupted using CRISPR/Cas9-mediated gene targeting. NEUROG3-/- hESC lines efficiently formed pancreatic progenitors, but lacked detectible NEUROG3 protein and did not form any endocrine cells in vitro. Moreover, NEUROG3-/- hESC lines were unable to form mature pancreatic endocrine cells following engraftment of PDX1+/NKX6.1+ pancreatic progenitors into mice. In contrast, a 75-90% knockdown of NEUROG3 caused a reduction, but not loss, of pancreatic endocrine cell development. We conclude that NEUROG3 is essential for endocrine pancreas development in humans and that as little as 10% NEUROG3 is sufficient for formation of pancreatic endocrine cells.

Mehrabi, M., K. Mansouri, et al. "Differentiation of human skin-derived precursor cells into functional islet-like insulin-producing cell clusters." In Vitro Cell Dev Biol Anim.

Advances in cell-replacement strategies for diabetes have focused on renewable sources of glucose-responsive, insulin-producing cells (IPCs). One of the most proper alternatives is multipotent skin-derived precursors cells (SKPs), which can be differentiated into IPCs. In this study, we reported the isolation and expansion of human skin-derived precursors (hSKPs) followed by their differentiation into IPCs in vitro, through exposure to suitable differentiation factors. The gene expression of endocrine beta cell markers was analyzed by reverse transcriptase-polymerase chain reaction. In addition, insulin production was examined immunocytochemically, and insulin and C-peptide secretion were examined using enzyme-linked immunosorbent assay. Dithizone-stained cellular clusters were observed after approximately 20 d. The clusters were found to be immunoreactive to insulin and expressed multiple genes related to pancreatic beta cell development and function: insulin, Pdx-1, Islet-1, NeuroD, Glut-2, Pax-4, Ngn-3, and Nkx2.2, but not to other pancreas-specific hormones such as glucagon and somatostatin. Cellular clusters were also able to secrete detectable amounts of insulin and C-peptide in a glucose dose-dependent manner. These findings suggest that human SKPs can differentiate into functional IPCs. This may offer a safer cell source for future stem cell-based therapies.

Niederhaus, S. V. "Pancreas transplant alone." Curr Opin Organ Transplant **20**(1): 115-20.

PURPOSE OF REVIEW: The present article aims to review the current state of diabetes, including its treatment options, and highlight current issues in pancreas transplantation. RECENT FINDINGS: Compared with other areas of transplantation, pancreas transplant/transplantation alone in the absence of kidney disease remains a relatively small field. As a consequence, reported new research articles are few in number, and often data regarding pancreas transplant/transplantation alone are mixed in with simultaneous kidney-pancreas and pancreas after kidney transplantation, which are covered separately. The prevalence of diabetes is reaching epidemic levels and continues to increase. New developments in diabetes care include the bionic pancreas and immunotherapy. Persistent efforts at gene and stem cell therapies are ongoing. Pancreas transplantation outcomes are improving, yet numbers are declining, even though pancreas transplantation still offers the most optimal glycemic control. SUMMARY: Pancreas transplantation has improving outcomes but stands in competition with other diabetes management options.

Pandey, P., S. Rachagani, et al. "Amyloid precursor-like protein 2 (APLP2) affects the actin cytoskeleton and increases pancreatic cancer growth and metastasis." Oncotarget **6**(4): 2064-75.

Amyloid precursor-like protein 2 (APLP2) is aberrantly expressed in pancreatic cancer. Here we showed that APLP2 is increased in pancreatic cancer metastases, particularly in metastatic lesions found in the diaphragm and intestine. Examination of matched human primary tumor-liver metastasis pairs showed that 38.1% of the patients had positive APLP2 expression in both the primary tumor and the

corresponding liver metastasis. Stable knock-down of APLP2 expression (with inducible shRNA) in pancreatic cancer cells reduced the ability of these cells to migrate and invade. Loss of APLP2 decreased cortical actin and increased intracellular actin filaments in pancreatic cancer cells. Down-regulation of APLP2 decreased the weight and metastasis of orthotopically transplanted pancreatic tumors in nude mice.

Phillips, F., A. C. Muls, et al. "Are bile acid malabsorption and bile acid diarrhoea an important cause of diarrhoea complicating cancer therapy?" Colorectal Dis.

AIM: Gastrointestinal (GI) symptoms during and after cancer therapy can significantly affect quality of life and interfere with treatment. This study assessed whether bile acid malabsorption [BAM] or bile acid diarrhoea [BAD] are important causes of diarrhoea associated with cancer treatment. METHOD: A retrospective analysis was carried out of consecutive patients assessed for BAM using 75-selenium homocholic acid taurine [SeHCAT] scanning after reporting any episode of loose stool in a gastroenterology clinic in a cancer centre. RESULTS: Between 2009-2013, 506 consecutive patients (54.5% male, age range 20-91 years), were scanned. BAM/BAD was diagnosed in 215 [42.5%]. It was mild in 25.6%, moderate in 29.3% and severe in 45.1%. Pelvic chemoradiation had induced BAM in >50% of patients. BAM was also frequent after treatment for conditions not preciously associated with BAM such nas anal and colorectal cancer and was present in >75% referred after pancreatic surgery. It was also unexpectedly frequent in patients who were treated for malignancy outside the GI tract such as breast cancer and haematological malignancy. CONCLUSION: Bile acid malabsorption/diarrhoea is a very common and an under-appreciated cause of GI symptoms after cancer treatment. Health professionals should have a low threshold in suspecting this condition as diagnosis and treatment can significantly improve quality of life. This article is protected by copyright. All rights reserved.

Pysz, M. A., S. B. Machtaler, et al. "Vascular Endothelial Growth Factor Receptor Type 2-targeted Contrast-enhanced US of Pancreatic Cancer Neovasculature in a Genetically Engineered Mouse Model: Potential for Earlier Detection." Radiology **274**(3): 790-9.

Purpose To test ultrasonographic (US) imaging with vascular endothelial growth factor receptor type 2 (VEGFR2)-targeted microbubble contrast material for the detection of pancreatic ductal adenocarcinoma (PDAC) in a transgenic mouse model of pancreatic cancer development. Materials and Methods Experiments involving animals were approved by the Institutional Administrative Panel on Laboratory Animal Care at Stanford University. Transgenic mice (n = 44; Pdx1-Cre, KRas(G12D), Ink4a(-/-)) that spontaneously develop PDAC starting at 4 weeks of age were imaged by using a dedicated small-animal US system after intravenous injection of 5 x 10(7) clinical-grade VEGFR2-targeted microbubble contrast material. The pancreata in wild-type (WT) mice (n = 64) were scanned as controls. Pancreatic tissue was analyzed ex vivo by means of histologic examination (with hematoxylin-eosin staining) and immunostaining of vascular endothelial cell marker CD31 and VEGFR2. The Wilcoxon rank sum test and linear mixed-effects model were used for statistical analysis. Results VEGFR2-targeted US of PDAC showed significantly higher signal intensities (26.8-fold higher; mean intensity +/- standard deviation, 6.7 linear arbitrary units [lau] +/- 8.5; P < .001) in transgenic mice compared with normal, control pancreata of WT mice (mean intensity, 0.25 lau +/- 0.25). The highest VEGFR2-targeted US signal intensities were observed in smaller tumors, less than 3 mm in diameter (30.8-fold higher than control tissue with mean intensity of 7.7 lau +/- 9.3 [P < .001]; and 1.7-fold higher than lesions larger than 3 mm in diameter with mean intensity of 4.6 lau +/- 5.8 [P < .024]). Ex vivo quantitative VEGFR2 immunofluorescence demonstrated that VEGFR2 expression was significantly higher in pancreatic tumors (P < .001; mean fluorescent intensity, 499.4 arbitrary units [au] +/- 179.1) compared with normal pancreas (mean fluorescent intensity, 232.9 au +/- 83.7). Conclusion US with clinical-grade VEGFR2-targeted microbubbles allows detection of small foci of PDAC in transgenic mice. ((c)) RSNA, 2014 Online supplemental material is available for this article.

Reddi, A. S., N. Kothari, et al. "Human Umbilical Cord Blood Cells and Diabetes Mellitus: Recent Advances." Curr Stem Cell Res Ther.

Stem cell therapy for patients with diabetes is an area of great interest to both scientists and clinicians. Human umbilical cord blood cells (HUCBCs) are being increasingly used as a source of stem cells for cell-based therapy for diabetes because these cells can differentiate into pancreatic islet beta-cells. Administration of HUCBCs has been shown to lower blood glucose levels in diabetic animal models. The use of autologous HUCBC transfusion in type 1 diabetic children has not shown any benefit. However, "Stem Cell Educator" therapy has shown promise in long term lowering of blood glucose levels in both type 1 and type 2 diabetic patients. In this review, we

will briefly discuss recent advances in HUCBC therapy in the treatment of diabetes and some of its complications.

Sakano, D., N. Shiraki, et al. "Pancreatic Differentiation from Murine Embryonic Stem Cells." Methods Mol Biol.

Pluripotent stem cells are considered as a cell source for replacement therapies for pancreatic beta cells and other organs. It was identified tetrabenazine (TBZ), vesicular monoamine transporter 2 (VMAT2) inhibitor as a promoter of late-stage differentiation of Pdx1-positive pancreatic progenitor cells into Ngn3-positive endocrine progenitor cells. A cell-permeable cAMP analog, dBu-cAMP promotes beta cell maturation in late stage of differentiation. The induced beta cells can secrete insulin in a glucose-dependent manner. The protocol consists of a three -step differentiation process. ES cell recapitulate embryonic developmental processes in vitro. Therefore, the ES cell differentiation system is a useful model for the understanding of molecular mechanism of beta-cell differentiation and are useful for application for future regenerative medicine.

Satoh, K., S. Hamada, et al. "Involvement of epithelial to mesenchymal transition in the development of pancreatic ductal adenocarcinoma." J Gastroenterol **50**(2): 140-6.

Pancreatic ductal adenocarcinoma (PDAC) is an intractable disease as a result of its rapid dissemination and resistance to conventional chemotherapy and radiotherapy. Surgical resection is the only curative therapy, but most of the tumors are unresectable at the time of diagnosis. The molecular mechanisms underlying the biological aggressiveness of this tumor type remain to be clarified. Epithelial to mesenchymal transition (EMT) is a developmental process that leads the phenotype shift from an epithelial morphology to a motile, fibroblast-like morphology. Recent studies showed that EMT is involved in the invasion and metastasis of many types of carcinomas including PDAC. In addition, PDAC cells with the EMT phenotype also exhibit chemoresistance and the cancer stem cell property. Various factors such as cytokines, growth factors, or transcriptional factors were found to promote the EMT program in PDAC cells. In this review, we summarize the current knowledge about the EMT in PDAC cells, focusing on the involvement of this process and its regulatory molecules including microRNA during the development of PDAC cells.

Shaer, A., N. Azarpira, et al. "Differentiation of human-induced pluripotent stem cells into insulin-producing clusters." Exp Clin Transplant **13**(1): 68-75.

OBJECTIVES: In diabetes mellitus type 1, beta cells are mostly destroyed; while in diabetes mellitus type 2, beta cells are reduced by 40% to 60%. We hope that soon, stem cells can be used in diabetes therapy via pancreatic beta cell replacement. Induced pluripotent stem cells are a kind of stem cell taken from an adult somatic cell by "stimulating" certain genes. These induced pluripotent stem cells may be a promising source of cell therapy. This study sought to produce isletlike clusters of insulin-producing cells taken from induced pluripotent stem cells. MATERIALS AND METHODS: A human-induced pluripotent stem cell line was induced into isletlike clusters via a 4-step protocol, by adding insulin, transferrin, and selenium (ITS), N2, B27, fibroblast growth factor, and nicotinamide. During differentiation, expression of pancreatic beta-cell genes was evaluated by reverse transcriptase-polymerase chain reaction; the morphologic changes of induced pluripotent stem cells toward isletlike clusters were observed by a light microscope. Dithizone staining was used to stain these isletlike clusters. Insulin produced by these clusters was evaluated by radio immunosorbent assay, and the secretion capacity was analyzed with a glucose challenge test. RESULTS: Differentiation was evaluated by analyzing the morphology, dithizone staining, real-time quantitative polymerase chain reaction, and immunocytochemistry. Gene expression of insulin, glucagon, PDX1, NGN3, PAX4, PAX6, NKX6.1, KIR6.2, and GLUT2 were documented by analyzing real-time quantitative polymerase chain reaction. Dithizone-stained cellular clusters were observed after 23 days. The isletlike clusters significantly produced insulin. The isletlike clusters could increase insulin secretion after a glucose challenge test. CONCLUSIONS: This work provides a model for studying the differentiation of human-induced pluripotent stem cells to insulin-producing cells.

Sharivkin, R., M. D. Walker, et al. "Functional proteomics screen enables enrichment of distinct cell types from human pancreatic islets." PLoS One **10**(2): e0115100.

The current world-wide epidemic of diabetes has prompted attempts to generate new sources of insulin-producing cells for cell replacement therapy. An inherent challenge in many of these strategies is the lack of cell-surface markers permitting isolation and characterization of specific cell types from differentiating stem cell populations. Here we introduce an iterative proteomics procedure allowing tag-free isolation of cell types based on their function.

Our method detects and associates specific cell-surface markers with particular cell functionality by coupling cell capture on antibody arrays with immunofluorescent labeling. Using this approach in an iterative manner, we discovered marker combinations capable of enriching for discrete pancreatic cell subtypes from human islets of Langerhans: insulin-producing beta cells (CD9high/CD56+), glucagon-producing alpha cells (CD9- /CD56+) and trypsin-producing acinar cells (CD9- /CD56-). This strategy may assist future beta cell research and the development of diagnostic tools for diabetes. It can also be applied more generally for function-based purification of desired cell types from other limited and heterogeneous biological samples.

Shi, G., C. Sun, et al. "Free fatty acid receptor 2, a candidate target for type 1 diabetes, induces cell apoptosis through ERK signaling." J Mol Endocrinol **53**(3): 367-80.

Recent reports have highlighted the roles of free fatty acid receptor 2 (FFAR2) in the regulation of metabolic and inflammatory processes. However, the potential function of FFAR2 in type 1 diabetes (T1D) remains unexplored. Our results indicated that the mRNA level of FFAR2 was upregulated in peripheral blood mononuclear cells of T1D patients. The human FFAR2 promoter regions were cloned, and luciferase reporter assays revealed that NFkappaB activation induced FFAR2 expression. Furthermore, we showed that FFAR2 activation by overexpression induced cell apoptosis through ERK signaling. Finally, treatment with the FFAR2 agonists acetate or phenylacetamide 1 attenuated the inflammatory response in multiple-low-dose streptozocin-induced diabetic mice, and improved the impaired glucose tolerance. These results indicate that FFAR2 may play a protective role by inducing apoptosis of infiltrated macrophage in the pancreas through its feedback upregulation and activation, thus, in turn, improving glucose homeostasis in diabetic mice. These findings highlight FFAR2 as a potential therapeutic target of T1D, representing a link between immune response and glucose homeostasis.

Wang, F., H. Li, et al. "Alisertib induces cell cycle arrest and autophagy and suppresses epithelial-to-mesenchymal transition involving PI3K/Akt/mTOR and sirtuin 1-mediated signaling pathways in human pancreatic cancer cells." Drug Des Devel Ther **9**: 575-601.

Pancreatic cancer is the most aggressive cancer worldwide with poor response to current therapeutics. Alisertib (ALS), a potent and selective Aurora kinase A inhibitor, exhibits potent anticancer effects in preclinical and clinical studies; however, the effect and underlying mechanism of ALS in the pancreatic cancer treatment remain elusive. This study aimed to examine the effects of ALS on cell growth, autophagy, and epithelial-to-mesenchymal transition (EMT) and to delineate the possible molecular mechanisms in human pancreatic cancer PANC-1 and BxPC-3 cells. The results showed that ALS exerted potent cell growth inhibitory, pro-autophagic, and EMT-suppressing effects in PANC-1 and BxPC-3 cells. ALS remarkably arrested PANC-1 and BxPC-3 cells in G2/M phase via regulating the expression of cyclin-dependent kinases 1 and 2, cyclin B1, cyclin D1, p21 Waf1/Cip1, p27 Kip1, and p53. ALS concentration-dependently induced autophagy in PANC-1 and BxPC-3 cells, which may be attributed to the inhibition of phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR), p38 mitogen-activated protein kinase (p38 MAPK), and extracellular signal-regulated kinases 1 and 2 (Erk1/2) but activation of 5'-AMP-dependent kinase signaling pathways. ALS significantly inhibited EMT in PANC-1 and BxPC-3 cells with an increase in the expression of E-cadherin and a decrease in N-cadherin. In addition, ALS suppressed the expression of sirtuin 1 (Sirt1) and pre-B cell colony-enhancing factor/visfatin in both cell lines with a rise in the level of acetylated p53. These findings show that ALS induces cell cycle arrest and promotes autophagic cell death but inhibits EMT in pancreatic cancer cells with the involvement of PI3K/Akt/mTOR, p38 MAPK, Erk1/2, and Sirt1-mediated signaling pathways. Taken together, ALS may represent a promising anticancer drug for pancreatic cancer treatment. More studies are warranted to investigate other molecular targets and mechanisms and verify the efficacy and safety of ALS in the treatment of pancreatic cancer.

Xia, S., Z. Feng, et al. "Clinical implication of Sox9 and activated Akt expression in pancreatic ductal adenocarcinoma." Med Oncol **32**(1): 358.

Pancreatic ductal adenocarcinoma (PDAC) is one of the most leading causes of cancer-related death. Cancer stem cell is responsible for tumor initiation, metastasis and relapse. Sox9 is a pancreatic stem cell marker. PI3K/PTEN/Akt/mTORC is an important signal for maintaining stem cells. The purpose of this study is to determine the expression pattern of Sox9 and p-Akt in human PDAC and its correlation with prognosis. Immunohistochemical analysis was used to explore the expression of Sox9 and p-Akt in 88 human PDAC patients. The Pearson's test was used to compare the clinicopathological parameters between negative and positive expressors. The Pearson's correlation analysis was used to explore the relationship between Sox9 and p-Akt expression.

Kaplan-Meier's method and Cox regression analysis were used to analyze patients' survival. The results showed that Sox9 and p-Akt overactivated in PDAC ($p = 0.011$, $p = 0.008$). Sox9-positive expression is significantly associated with distant metastasis ($p = 0.046$). p-Akt-positive expression is significantly associated with distant metastasis ($p = 0.000$), TNM stage (0.001) and PCNA expression ($p = 0.000$). Sox9 expression is positively correlated with p-Akt expression ($r = 0.314$, $p = 0.003$). In 54 patients with survival information, both Sox9- and p-Akt-positive expressions are associated with unfavorable prognosis ($p = 0.002$, $p = 0.000$). Sox9 and p-Akt double-positive expressor showed much poorer prognosis ($p = 0.000$). Cox regression analysis showed that Sox9- or p-Akt-positive expression and LN metastasis were independent prognostic factors. This study provides the first evidence that Sox9 and p-Akt are both relevant to distant metastasis and proliferation. Our data suggest the potential of Sox9 and p-Akt as prognostic biomarkers for PDAC.

Zhan, H. X., J. W. Xu, et al. "Pancreatic cancer stem cells: new insight into a stubborn disease." Cancer Lett **357**(2): 429-37.

Resistance to conventional therapy and early distant metastasis contribute to the unsatisfactory prognosis of patients with pancreatic cancer. The concept of cancer stem cells (CSCs) brings new insights into cancer biology and therapy. Many studies have confirmed the important role of these stem cells in carcinogenesis and the development of hematopoietic and solid cancers. Recent studies have shown that CSCs regulate aggressive behavior, recurrence, and drug resistance in pancreatic cancer. Here, we review recent advances in pancreatic cancer stem cells (PCSCs) research. Particular attention is paid to the regulation mechanisms of pancreatic cancer stem cell functions, such as stemness-related signaling pathways, microRNAs, the epithelial-mesenchymal transition (EMT), and the tumor microenvironment, and the development of novel PCSCs targeted therapy. We seek to further understand PCSCs and explore potential therapeutic targets for pancreatic cancer.

Zhao, X., W. M. Puszyk, et al. "Small molecule inhibitor YM155-mediated activation of death receptor 5 is crucial for chemotherapy-induced apoptosis in pancreatic carcinoma." Mol Cancer Ther **14**(1): 80-9.

Despite much effort, pancreatic cancer survival rates are still dismally low. Novel therapeutics may hold the key to improving survival. YM155 is a small molecule inhibitor that has shown antitumor activity in a number of cancers by reducing the expression of survivin. The aim of our study is to understand the mechanisms by which YM155 functions in pancreatic cancer cells. We established the antitumor effect of YM155 with in vitro studies in cultured cells, and in vivo studies using a mouse xenograft model. Our data demonstrated that YM155 reduced the expression of survivin; however, downregulation of survivin itself is insufficient to induce apoptosis in pancreatic cancer cells. We showed for the first time that treatment with YM155 increased death receptor 5 (DR5) expression in pancreatic cancer cells. We found that YM155 induced apoptosis by broad-spectrum inhibition of IAP family member proteins (e.g., CIAP1/2 and FLIP) and induced proapoptotic Bak protein upregulation and activation; the antitumor effect of YM155 treatment with either the DR5 agonist lexatumumab or gemcitabine on pancreatic cancer cells was synergistic. Our data also revealed that YM155 inhibits tumor growth in vivo, without apparent toxicity to the noncancerous human pancreatic ductal epithelial cell line. Together, these findings suggest that YM155 could be a novel therapeutic agent for pancreatic cancer.

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