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Cell Sources for Treating Diabetes

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1. Introduction

Diabetes is one of the most prevailing metabolic disorders worldwide. The number of individuals suffering from diabetes is increasing significantly. There are two main types of diabetes; Type 1 Diabetes mellitus (T1D) and Type 2 Diabetes mellitus (T2D). T1D results from autoimmune destruction of insulin-producing pancreatic beta-cells. This results in a complete loss of insulin, a hormone that is essential for loading any excess circulating glucose into target organs. Individuals with T1D therefore need daily injections of insulin so as to regulate their circulating blood glucose concentrations and survive with the disease. Although such lifelong insulin therapy supports glucose metabolism, patients often develop complications related to renal function, endothelial damage and retinopathy. T1D usually develops in children or young adults. T2D on the other hand results from insulin resistance, a condition in which insulin is not properly recognized by receptors on cell surface. It is sometimes combined with an absolute insulin deficiency as eventually these patients loose their β cells and are then classified as type 2 diabetic individuals requiring insulin. It usually occurs in people over 40 years of age, but is also being observed at younger age. A cure for diabetes came in with the demonstration to support insulin action and regain the patient's quality of life by transplantation of cadaveric insulin-producing cells (Tzakis et al., 1990). Such a therapy, which in practice includes whole pancreas transplantation or transplantation of the endocrine portion; the islet of Langerhans, has been carried out in several patients suffering from Diabetes. In year 2000, the Edmonton group successfully demonstrated transplantation of cadaveric islets using a steroid-free immunosuppressive regimen, which was a major advance in the field. The Edmonton Protocol involves isolating islets from a cadaveric donor pancreas using enzymatic digestion. Islets from one to as many as three donors were transplanted in to each recipient. Cadaveric islets are infused into the patient's portal vein, and are then kept from being destroyed by the recipient's immune system through the use of two immunosuppressants, sirolimus and tacrolimus (Shapiro et al., 2000). Several hundred people have received islet transplants since 2000. By a year after transplantation, about 50 - 68% of patients were observed off insulin, but by five years after the procedure, less than 10% of total patients were seen to be free of daily insulin injections. One of the major factors in success of these

transplants has been the number of transplants / islet cell grafts received by these individuals. For such a therapy to be available for several millions of people suffering from T1D it is necessary to identify other human cell sources that can be used for replacement therapy in diabetes.

Several possible cell sources have been considered for obtaining insulin-producing cells. Our previous studies demonstrate that lineage committed stem cells such as pancreatic islet-derived are better cell sources for treatment of diabetes. We and others showed that progenitor cells generated from insulin-producing cells retain the active chromatin conformation of pancreatic or islet-specific lineage and hence can be differentiated into insulin-producing cells with better efficiency (Gershengorn et al., 2005; Gershengorn et al., 2004; Joglekar and Hardikar; Joglekar et al., 2009a; Mutskov et al., 2007; Russ et al., 2008; Russ et al., 2009). Apart from beta cells themselves, other pancreatic cell types (endocrine, acinar or ductal) are also demonstrated as potential candidate cell types (Baeyens et al., 2005; Bonner-Weir et al., 2004; Lardon et al., 2004a; Lardon et al., 2004b). Differentiation from human embryonic stem cells is also shown to generate insulin-producing cells (Blyszczuk et al., 2004). D'Amour et al illustrated this phenomenon (D'Amour et al., 2005; D'Amour et al., 2006) by recapitulating the normal sequence of embryonic developmental events that lead to beta-cell differentiation (Van Hoof et al., 2009). It was demonstrated (Aoi et al., 2008; Nakagawa et al., 2008; Okita et al., 2007; Takahashi et al., 2007; Takahashi and Yamanaka, 2006) that forced expression of "reprogramming factors" (also known as the "Yamanaka factors") allowed conversion of fibroblasts or somatic cells to an embryonic stem cell-like state. These cells, referred to as induced pluripotent stem (iPS) cells are popular since they can be derived from adult somatic cells, including those from diabetic patients (Huangfu et al., 2008; Maehr et al., 2009), and could be potentially used for autologous transplantation in diabetes, if efficient differentiation is achieved. Another study (Zhou et al., 2008; Zhou and Melton, 2008) also demonstrated that forced expression of pancreatic transcription factors (Pdx1, Ngn3 and MafA) induced insulin expression in exocrine pancreatic (acinar) cells. We and others have also demonstrated (Bar et al., 2008; Joglekar et al., 2009a; Joglekar et al., 2009b; Russ et al., 2009) the role of several regulators in generation of islet-derived progenitors (via EMT) and their conversion to insulin-producing cells (via reverse-EMT or MET). Mesenchymal stem cells obtained from non-endodermal tissues such as bone marrow, umbilical cord blood placenta, Wharton's jelly, adipose tissue are also observed to differentiate to beta-like cells in vitro or in vivo (Chandra et al., 2009; Kadam et al.; Parekh et al., 2009; Phadnis et al.). Although non-endodermal cells can be differentiated to insulin-producing cells in vitro, they are limited in their ability to efficiently transcribe insulin gene.

Transdifferentiation, is a phenomenon that refers to differentiation of an already differentiated tissue to another differentiated cell type, possibly via de-differentiation and re-differentiation. An example is the conversion of liver cells to pancreas (Ferber et al., 2000). More recently, we demonstrated evidence that human gallbladder epithelial cells contain pancreatic hormone-producing cells (Sahu et al., 2009a). These observations are in line with similar observations made in mice (Coad et al., 2009). Although the mechanisms that contribute to generation of hormone-producing cells in human gallbladder remain to be identified, these observations reveal that gallbladder epithelial cells can be a potentially important source of islet progenitors for cell replacement therapy in diabetes. It is important to note that gallbladder and pancreas are next door neighbours during embryonic development (Figure 1). In fact, the gallbladder develops from the ventral pancreatic bud.

Thus understanding the development of gallbladder-derived hormone-producing immature islet cells will help us in generating an alternate source of pancreatic stem cells for replacement therapy in diabetes. In this chapter, we further emphasis on gallbladder and liver development and cells derived from these organs as potential candidates in cell replacement therapy for diabetes.

2. Liver and gallbladder as cell sources to generate insulin producing cells

Low yield and availability of transplantable islets from cadaveric pancreatic samples is a major concern in the field of cell replacement therapy for diabetes. There is a wider search for cell sources with the potential to produce insulin at physiologically significant levels. Liver and gallbladder are next door neighbors to pancreas during embryonic development and share common expression territories for several transcription factors during embryonic development (Sahu et al., 2009b). In this regard, the role of liver and especially gallbladder has come into picture recently. During embryonic development, the development of pancreas begins with dorsal and ventral out-pouching of the gut tube. Interestingly, the ventral pancreatic bud also gives rise to the gallbladder. In this section we will discuss the developmental plans of liver and gallbladder in context of pancreas development.

2.1 Liver function

The liver is a large glandular organ that is well known due to its' extensive regenerative capabilities as well as ability to trans-differentiate into pancreatic fate. The liver is made up of hepatocytes with their basal surface facing the capillaries invading sinusoidal spaces. The liver secretes bile through its apical surface into the canaliculi that join to form the biliary duct. Biliary duct carries bile to gallbladder via hepatic duct. The function of gallbladder is to concentrate and store bile, which is required for digestion of fats. The adult rat liver consists of 60% hepatocytes while remaining mass consist of cholangiocytes, kuppfer cells, stellate cells and endothelial cells even those existing in sinusoidal spaces. Although majority of liver parenchyma consists of hepatocytes, the interaction of hepatocytes with the non-parenchymal cells is important for normal liver function (Kmiec, 2001). However, rats do not have a gallbladder while mice do. Recently considerable amount of advances have been made in understanding the mechanisms that direct the development of bile duct. Although mechanisms involving gallbladder development have been less explored, studies from liver development have provided us with clues to understand the developmental biology of biliary duct and gallbladder.

2.2 Embryonic development of the liver

Liver development is a complex process involving fate decision, specification, proliferation and differentiation. Development of liver begins with foregut development (involving Wnt/β-catenin and FGF4), hepatic induction (by FGFs and BMPs), hepatic morphogenesis (involving transition from a simple cuboidal to pseudostratified columnar epithelium) and hepatic bud growth and maturation (influenced by signals from developing blood vesels). With the help of molecular genetic tools, tissue explant cultures and molecular biological techniques, liver development has been well understood in mice (Zaret, 1998; Zaret, 2000; Zorn and Wells, 2007). The liver primordium appears as a diverticulum in the ventral region of the anterior intestinal portal at 9.0, 10.5, and 21days of gestation in mouse, rat, and human embryos, respectively (Du Bois, 1963; Shiojiri et al., 1991). This part of the ventral

foregut endoderm lies adjacent to heart and hence receives signals from cardiac mesoderm (Douarin, 1975; Fukuda-Taira, 1981; Fukuda, 1979). As the development proceeds, liver primordium diverges into two portions in mouse and human embryos: the cranial and caudal lobe. The cranial diverticulum develops into the liver parenchyma and hepatic ducts while the caudal portion gives rise to gallbladder and the ventral pancreas (Shiojiri, 1997). At the early somite stage (14-20) or early stage of liver development i.e at embryonic day (e)9.5, the liver primordium is separated from septum transversum by basement membrane (Medlock and Haar, 1983). As the liver bud grows this basement membrane is disrupted gradually and the pre-hepatic cells separate as layers from the foregut and migrate as cords into the surrounding septum transversum (Douarin, 1975; Medlock and Haar, 1983). Fibroblasts and stellate cells of liver are generated form septum transversum mesenchyme (STM). Liver bud undergoes a period of accelerated growth between e10–15 to become the major hematopoietic organ, which is further colonized and vascularized by hematopoietic cells.

The hepatoblasts are bi-potential cells that give rise to different types of cells including the biliary epithelial cells (BECs), intrahepatic bile ducts and hepatocytes depending on their distribution. For example BECs are generated from hepatoblasts residing near portal vein while hepatocytes mainly originate from the hepatoblasts residing in the parenchyma. BECs are also known as cholangiocytes and line the lumen of intrahepatic bile ducts (IHBD) (Lemaigre, 2003; Shiojiri, 1984). The formation of biliary network, which connects to extrahepatic bile duct (EHBD) and maturation of hepatocytes is a gradual process that begins at e13 and continues after birth to achieve the characteristic tissue architecture.

2.3 Endoderm formation

The definitive endoderm emerges as a sheet of cells starting from the anterior end of the primitive streak during gastrulation. The glandular and epithelial cells of lung, thyroid, pancreas, gastrointestinal and respiratory epithelium and of course the hepatocytes and the biliary epithelium originate from multipotent endoderm. Signaling by Nodal, a transforming growth factor beta (TGFβ) family growth factor initiates both endoderm and mesoderm formation in a concentration dependent manner. Low nodal doses are seen to induce mesoderm while high dose induce endoderm (Shen, 2007; Zorn and Wells, 2007). Nodal signaling stimulates core group of endoderm specific transcription factors including Sox17 (HMG domain DNA binding factor) and the fork head domain proteins Foxa1–3 (hepatocyte nuclear factor or HNF 3α/β/γ) (Zorn and Wells, 2007), which in turn regulates the downstream genes committing cells to endodermal lineage.

2.4 Endoderm patterning

The cells that first emerge from the primitive streak give rise to the anterior part while those that arise later give rise to the posterior part (Lawson and Pedersen, 1987). Anterior endoderm development requires higher nodal signaling than the posterior endoderm. It expresses Foxa2, as it is preferentially required for the formation of anterior definitive endoderm (Dufort et al., 1998; Zorn and Wells, 2007). During the early somite stages of development, morphogenetic movement transforms the endoderm into an epithelial gut tube. This epithelial gut tube is surrounded by mesoderm. The mesoderm secretes several factors that regulate pattern formation along the epithelial gut tube along anterior- posterior (A-P) axis to form the foregut, midgut and hindgut regions. These regions of endoderm are distinguishable by the expression of Hhex in foregut, Pancreatic and duodenal homeobox 1

(Pdx1) in midgut and Cdx in posterior endoderm or hindgut (Grapin-Botton, 2005; Moore-Scott et al., 2007). The foregut endoderm contains common precursors for liver, gallbladder and pancreas. Initially the regional identity is "plastic" as recombination of the early foregut endoderm with the posterior endoderm results into intestinal fate instead of liver and pancreas development (Gualdi et al., 1996; Horb and Slack, 2001; Kumar et al., 2003; Wells and Melton, 2000).

2.5 Repression of Wnt/β-catenin and FGF4 is required for foregut development

Overlapping spatiotemporal gradients of Wnt, fibroblast growth factor (FGF), bone morphogenetic proteins (BMP) and retinoic acid secreted from the adjacent mesoderm, patterns the endoderm to express regionally specific genes (Chen et al., 2004; Dessimoz et al., 2006; Kumar et al., 2003; Martin et al., 2005; McLin et al., 2007; Roberts et al., 1995; Stafford et al., 2004; Tiso et al., 2002). For the development of foregut endoderm, inhibition of Wnt and FGF4 signaling by Wnt antagonist like Sfrp5 (secreted by the foregut endoderm) is essential (Dessimoz et al., 2006; McLin et al., 2007; Wells and Melton, 2000). Obviously the posterior endoderm depends on the secretion of Wnt and FGF4 by the posterior mesoderm. Experimentally it has been demonstrated that blocking the canonical Wnt effector β-catenin in the posterior endoderm of Xenopus embryos activates Hex expression and results in ectopic liver bud formation in intestine (McLin et al., 2007).

2.6 Hepatic competence

The foregut endoderm is the only competent candidate in vivo to generate liver and its establishment is the first step towards hepatogenesis (Douarin, 1975; Fukuda-Taira, 1981). This intrinsic property to develop into liver is due to expression of transcription factors including Foxa2, Gata4-6 and Hhex (or Hex). These transcription factors have important role in early organogenesis. Foxa and Gata have been shown to bind to enhancer element of albumin gene and enhances chromatin accessibility thereby increasing the competency of such liver specific genes to be transcribed (Bossard and Zaret, 1998; Bossard and Zaret, 2000; Gualdi et al., 1996). Evidently, deletion of Foxa1 and Foxa 2 prevents induction of liver development in early foregut.

2.7 Hepatic induction by cardiac mesoderm and STM

At an early somite stage of 4-7, FGF signals from the cardiac mesoderm of developing heart and BMPs from the STM induces hepatic fate in the ventral foregut endoderm (Douarin, 1975; Fukuda-Taira, 1981; Gualdi et al., 1996; Jung et al., 1999; Rossi et al., 2001). This has been illustrated in mouse models where the mouse foregut explants isolated at 2-4 somite stage when cultured in the presence of cardiac mesoderm start expressing albumin after 1-2 days. However removal of cardiac mesoderm or blocking FGF signals inhibited liver induction (Calmont et al., 2006; Gualdi et al., 1996; Rossi et al., 2001). It has also been illustrated that exogenous FGF1 and FGF2 can replace cardiac mesoderm and induce albumin expression in foregut endoderm explants. However BMP signaling may act by maintaining Gata4/6 expression and is not sufficient for hepatic induction in the explants (Rossi et al., 2001). Analysis of downstream signal transduction pathway of FGF signaling suggests that P13 kinase pathway promotes hepatic growth while MAP kinase pathway regulates hepatic gene expression (Calmont et al., 2006). Although the target genes of FGF and BMP signal have not been revealed due to early embryonic lethality, it is predicted that

they act cooperatively to regulate liver development. Additionally, wnt2 and wnt2b are also expressed in the mouse lateral plate mesoderm at the time of hepatic induction but their role is yet to be determined. Mouse explant studies have suggested that high, low and medium concentrations of FGFs are critical for segregation of different organs lung, liver and gallbladder respectively from a common progenitor cell. It is not clear whether it is the proximity or the duration that the endoderm is in contact with the cardiac mesoderm that controls FGF doses. *Hhex,* a homeobox protein regulates this process. It is responsible for the proliferation of foregut cells and the movement of epithelium in relation to the FGF-secreting cardiac mesoderm during development (Bort et al., 2004).

2.8 Endothelial cells signals in hepatogenesis

At around e9.5 endothelial cells intersperse and form simple continuous vessels into the future parenchymal cells as it expands into septum transversum mesenchyme. As development proceeds, the morphology of the endothelial cells changes, becoming fenestrated and forming endothelial sheets that line the hepatic sinusoids (Bankston and Pino, 1980; Enzan et al., 1997). The role of endothelial cells in hepatogenesis was illustrated by utilizing Flk-1$^{-/-}$ mouse which lack mature endothelial cells or blood vessels (Shalaby et al., 1997; Shalaby et al., 1995). In control mice, at an early stage, when liver starts developing, the endothelial cells are found between the hepatic bud and the septum transversum however Flk-1$^{-/-}$ mice lack endothelial cells as expected. Comparison of control and Flk-1$^{-/-}$ embryos liver revealed that although hepatic specification occurred hepatogenesis failed and there were no signs of expansion or morphogenetic movement into the septum transversum. Flk expression is restricted to endothelial cells and not in STM and endoderm. Hence it was confirmed that endothelial cell signaling is a crucial aspect for normal hepatogenesis.

2.9 Transcription factors and their roles in controlling hepatogenesis

During embryonic development, hepatogenesis is defined by the precise timing and the location of tissue interactions and the signaling molecules involved therein. However the intracellular response to these signaling events explains the mechanisms through which the cells of endoderm actually adopt a hepatic fate. Large changes in gene expression patters accompany the differentiation of ventral endoderm towards hepatic phenotype. It is thus quite obvious that a lot of transcription factors are involved as gene regulation is tightly regulated by transcription factors (Cereghini, 1996; Duncan, 2000). Molecular biology and molecular genetics have been helpful in identifying a number of transcription factors that are crucial candidates to regulate various aspects of hepatogenesis.

2.9.1 Transcription factors controlling hepatic competency

Competency is described as the innate ability of a cell type to follow a developmental fate given appropriate inductive signals (Gilbert and MA., 2000; Waddington, 1940). The work of Gauldi et al suggests that both ventral and dorsal endoderm have the potential to express albumin gene (Gualdi et al., 1996). However the laboratory of Ken Zaret have suggested a mechanism whereby Hnf3 (FoxA) and Gata4 could modulate competency intracellularly (Bossard and Zaret, 1998; Chaya et al., 2001; Cirillo et al., 2002; Gualdi et al., 1996; Shim et al., 1998). Extracts from e9.5 liver buds when used for in vivo foot printing analysis of *Albumin* enhancer revealed that several binding sites including Hnf3, Gata and Nf-1 sites were occupied. However uncommitted cells from endoderm showed occupancy at Hnf3

and Gata sites only. In addition, in vitro chromatin reconstitution assays elucidated that the binding of either Hnf3 or Gata4 resulted in the opening of compacted chromatin (Cirillo et al., 2002). Thus Hnf3 and Gata were found responsible for remodeling of chromatin structure and hence were able to generate available receptive sites on them for the occupancy of transcriptional activators. Supporting examples of this model is from the finding that Hnf3 can relieve the transcriptional repression of α-fetoprotein gene in transcription assays *in vitro* (Crowe et al., 1999). A similar situation exists for the Gata factor as Gata 4 has been shown to bind the Albumin enhancer. The expressions of these transcriptional activators are in turn controlled by inductive events.

2.9.2 Role of Hex and Prox in early stages of liver development

Hex (or Prh) encodes for divergent homeobox transcription factor known as hematopoietically-expressed homeobox protein**.** At an early head fold stage (pre-somite, e8.0), prior to the initiation of hepatogenesis, expression analysis of the Hex mRNA revealed that it is highly expressed throughout the ventral endoderm. Expression within the ventral endoderm is restricted to two discrete areas that are future sites for liver and thyroid development by e8.5 or 10 somite stage (e8.5) (Keng et al., 1998; Thomas et al., 1998). Hence the early expression of Hex mRNA in ventral endoderm defines Hex as one of the earliest markers of liver development. Role of Hex was discerned using Hex $^{-/-}$ embryos at e9.5 to 10.5. These embryos show thickening of the ventral foregut endoderm but the expansion and morphogenetic outgrowth of liver was undetectable in contrast to the control embryos. In situ hybridization studies on Hex $^{-/-}$ embryos revealed the absence of α-fetoprotein mRNA expression at e9.5 and e10.5. In another study using RT-PCR, absence of albumin was reported in these mutants (Keng et al., 2000; Martinez Barbera et al., 2000). Knockout studies have also revealed the role of Prox 1 in early stages of liver development. Analysis of Prox1-/- embryo found that the liver of these mutant embryos were smaller than the wild types and although these mutants formed distinct liver lobes the hepatic parenchymal cells

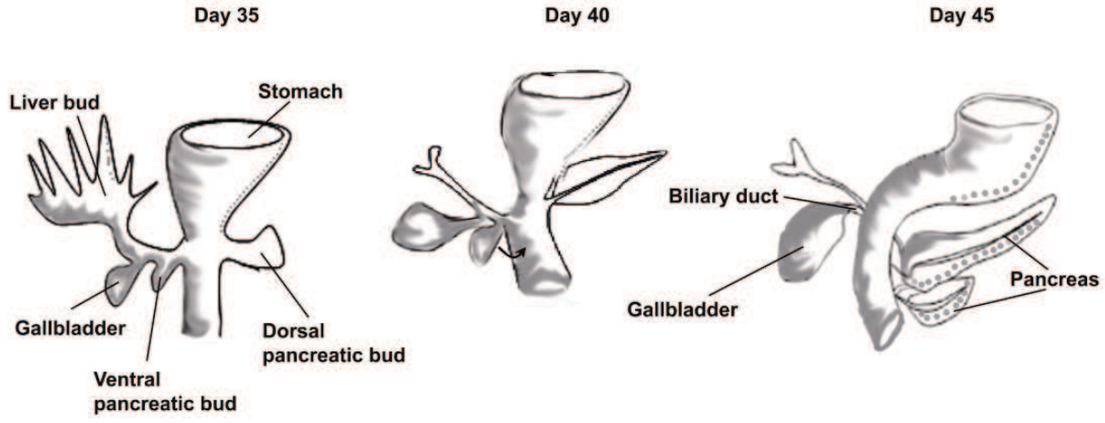

Fig. 1. The human liver, gallbladder and pancreas are next door neighbors during embryonic development. The gallbladder actually develops from the ventral pancreatic bud and is in close proximity to the developing pancreas until the ventral pancreas rotates to fuse with the dorsal pancreatic bud. We therefore believe that this close proximity and overlapping expression pattern of pancreatic transcription factors in the domain of developing gallbladder induces expression of endocrine pancreatic hormones in the gallbladder.

were restricted to central rudiment (Sosa-Pineda et al., 2000). These mutant embryos showed reduced number of hepatoblasts than control embryos. These two characteristic of the mutant phenotype explains why these embryos failed to inhibit the mesenchymal portion of the liver. At e10 Prox1$^{-/-}$ hepatoblasts mice failed to exhibit the delamination of hepatic chords into the septum transversum in contrast to control hepatoblasts which normally migrate as chords into the septum transversum. Although these mutants failed to expand and migrate, they were found positive for albumin and α-fetoprotein implying their specification toward hepatic fate (Sosa-Pineda et al., 2000). In short, Prox 1 is dispensable for hepatic specification but it is required for morphogenic expansion of the liver primordium.

2.9.3 Transcription factors in regulation of hepatocyte differentiation

During 1980s enormous amount of work from several laboratories allowed the identification of the transcription factors that predominantly regulates the liver specific genes (Lai and Darnell, 1991; Sladek and Darnell, 1992). These transcription factors included Hnf1α and β , the c/EBP family, Hnf3α, β, and γ, Hnf4α, and more recently Hnf6 (Lai and Darnell, 1991; Lemaigre et al., 1996; Samadani and Costa, 1996; Sladek and Darnell, 1992). Knockout models for these factors have helped in studying the role of these factors in hepatic differentiation. It was found that loss of a single factor generally has negligible effect. For example disruption of either Hnf3α, Hnf3β or Hnf3γ in the liver does not affect hepatocyte differentiation, although moderate changes in expression of a subsets of hepatocyte genes have been described (Kaestner et al., 1998; Shih et al., 1999; Sund et al., 2000). Functional redundancy could be the reason for this insignificant effect of disruption of single gene but its note worthy that disruption of Hnf3β inhibits β-cell differentiation in pancreas although they express Hnf3α (Kaestner et al., 1998; Sund et al., 2000). Noticeably disruption of some genes has serious effects in liver development. For example the early liver development in c/EBPα$^{-/-}$ embryos is normal although the neonates die from hypoglycemia due to failure of liver to accumulate glycogen. In these mice, expression of glycogen synthase is disrupted and the bile canaliculi form abnormally (Wang et al., 1995). In addition hepatocyte proliferation was increased in the newborns c/EBPα$^{-/-}$ liver. This was supported by the fact that c/EBPα inhibits cell proliferation by regulating S-phase-specific E2F–p107 complexes and by stabilisizing the cyclin dependent kinase inhibitor p21 (Harris et al., 2001; Timchenko et al., 1997; Timchenko et al., 1999). Hnf1α mutants do not show significant effect on liver development, however mice lacking Hnf1α suffer from various different abnormalities depending on the targeted allele. In one case, mice suffer from hepatic dysfunction, Phenylketonuria and renal Faconi's syndrome, while a different gene targeting strategy generated mice that suffered from non-insulin-dependent diabetes mellitus (NIDDM) and Laron dwarfism. Loss of function of phenylalanine hydroxylase (Pah) gene due to the inability of Hnf1α to facilitate modification of chromatin around the Pah promoter results Phenylketonuria (Pontoglio et al., 1996; Pontoglio et al., 1997). Hnf4α mRNA was first detected in the primitive endoderm of the blastocyst at the time of implantation. Hnf4α expression was first detected in the liver bud at around 10 somite stage. Hnf4α expression was restricted to the ventral extraembryonic visceral endoderm which forms the endodermal portion of the yolk sac just before this developmental stage. Hnf4α acts on the important effector in the hepatocyte development. Hnf4α positively regulates Hnf1α expression as evident by the presence of Hnf4α binding sites on the transcriptional

regulatory element of Hnf1α. Hnf4α slightly precedes the expression of Hnf1α in number of tissues in which both factors are expressed. Although hepatoblasts during liver development are relatively immature, they still initiate expression of genes that define mature hepatocyte phenotype. When the mRNA levels of such genes were compared between control and Hnf4α$^{-/-}$ livers it was found that the expression of 14 genes that have important roles in hepatocyte function were either undetectable in some cases or down-regulated in others in the absence of Hnf4α (Li et al., 2000). These results have also been consistent with the adult hepatocytes that lack Hnf4α (Hayhurst et al., 2001). All of these studies strongly support the notion that Hnf4α is a central regulator of hepatocyte differentiation.

2.10 Transcription factors in gallbladder and intrahepatic bile duct development

The gallbladder and liver share several common transcription factors during embryonic development. In developing mouse liver, HNF6 is expressed in hepatocytes and in the epithelial cells of both the intrahepatic and extrahepatic bile ducts (Landry et al., 1997; Rausa et al., 1997). Recent studies illustrate that Hnf6$^{-/-}$ mouse embryos fail to develop a gallbladder and exhibit severe abnormalities in both extrahepatic and intrahepatic bile ducts (IHBD), which is associated with diminished expression of the Hnf1β transcription factor (Clotman et al., 2002; Jacquemin et al., 2000). Thus Hnf6 is essential for regulating the expression of Hnf1β, which in turn plays important role in intrahepatic bile duct and gall bladder development. Foxf1 is another transcription factor involved in gallbladder development. It is expressed in the embryonic gut mesoderm and septum transversum. It participates in the mesenchymal-epithelial cell inductive signaling of the internal organs. Foxf1$^{+/-}$ mice show several developmental abnormalities in gallbladder, esophagus, lung and trachea which supports this hypothesis (Kalinichenko et al., 2001; Kalinichenko et al., 2002; Mahlapuu et al., 2001). Foxf1$^{+/-}$ gallbladder fails to express vascular cell adhesion molecule-1, α5 integrin, and platelet-derived growth factor receptor α and hepatocyte growth factor genes required for mesenchymal epithelial cell induction (Kalinichenko et al., 2002). Foxf1$^{+/-}$ gallbladder shows malformations in external smooth muscle cell layer, reduction in mesenchymal cell number and in some cases, lack of a discernible biliary epithelial cell layer (Kalinichenko et al., 2002). There is little information available regarding the development of gallbladder. Present knowledge is gained based on a few knockout studies that are summarized in the table below. It suggests a need to generate more similar information for better understanding of molecular pathways regulating the development of hormone-producing cells in gallbladder.

No.	Gene name	Phenotype	Ref
1	forkhead box F1a (Foxf1)	Heterozygotes of Foxf1 knockout animals (haplosufficient) have severely abnormal and small gall bladder and they lack epithelial cell layer	Kalinichenko et al, Journal of Biological Chemistry, 2002, 227:12369-12374
2	One cut domain family member 1 (Onecut1/ Hnf6)	Hnf6 null mice lack gall bladder. Extrahepatic as well as intrahepatic bile ducts are abnormally developed	Clotman et al, Development, 2002, 129:1819-1828

No.	Gene name	Phenotype	Ref
3	Hairy and enhancer of split 1 (Hes 1)	Hes1 knockout animals do not develop gall bladder and have severely reduced extrahepatic bile ducts. Such mutant bile ducts express Ngn3 and ectopically differentiate into pancreatic exocrine and endocrine tissue	Sumazaki et al, Nature Genetics, 2004, 36:83-87
4	Hematopoietically expressed homeobox (Hhex)	Conditional deletion of Hhex in hepatic diverticulum is embryonic lethal and results in small, cystic liver. Gall bladder and extrahepatic bile duct are absent. Conditional deletion of Hhex in embryonic liver is not lethal, however it precipitates into irregular development leading to polycystic liver disease in adulthood	Hunter et al, Developmental Biology, 2007, 308:355-367
5	Leucine-rich repeat-containing G protein-coupled receptor 4 (LGR4)	LGR4 hypomorphic embryos develop normal gall bladder bud at e10.25, however it does not elongate in later stages of development. Adult mouse completely lacks gall bladder and cystic duct. Liver and pancreas are unaffected.	Yamashita et al, Developmental Dynamics, 2009, 238:993-1000
6	Hepatocyte nuclear factor beta Hnf1β	HNF1b deletion results into severe jaundice caused by abnormalities in intrahepatic bile ducts and gallbladder. It also leads to reduced expression of Organic Anion Transporting Polypeptide 1 or Oatp1, bile acid transporter and fatty acid dehydrogenase or Very long-chain acyl-CoA dehydrogenase VLCAD.	Coffinier C et al, Development 2002; 129:1829–1838.
7	Hlx -/-	Expression of Hlx is seen in visceral mesenchyme of the developing liver, gallbladder, and gut. Hlx -/- embryos exhibit normal formation of the liver diverticulum and differentiation of hepatocytes , but the cells fail to proliferate and therefore develop into under-sized liver (3% of normal size).	Hentsch B et al, Genes Dev 1996;10:70-79.

Table 1. Phenotypes of the knockout mouse models for the genes involved in gallbladder development.

3. Concluding remarks

Liver, gallbladder and pancreas originate from nearby regions of endoderm and develop in close association with each other (Figure 1). It is quite evident and exciting to note that these organs share several transcription factors (Sahu et al., 2009b). The liver shares expression of genes such as Glut2 and glucokinase and as discussed earlier has the same embryological origin as ventral pancreas. It is hence encouraging that the cells derived from organs outside pancreas can be useful in generating insulin producing cells for cell replacement therapy in diabetes. It has been demonstrated that ectopic expression of Pdx1 in liver ameliorates type 1 diabetes through conversion of hepatocytes in to insulin-producing cells (Ferber et al., 2000). Precisely diabetic CAD-NOD mice treated with Ad-CMV-PDX-1 became normoglycemic and maintained a stable body weight. Ectopic PDX-1 expression induced pancreatic gene expression and insulin production in the mice livers (Ferber et al., 2000). Retrovirally immortalized human fetal liver progenitor cells when transduced using lentiviral vector containing PDX1 underwent successful differentiation into insulin-expressing cells. These cells showed responsiveness to glucose, exhibit a β-cell-like phenotype and are able to restore euglycemia upon transplantation in diabetic mice (Zalzman et al., 2005; Zalzman et al., 2003). Forced expression of Pdx1 is now known to induce conversion of human fetal liver cells to insulin-producing cells. These modified cells express multiple beta cell specific genes. However, they also activated genes expressed in exocrine pancreas and continued expressing hepatic genes as well. Similarly expression of another beta cell transcription factor; NeuroD, in mouse liver cells in vivo resulted in reversal of hyperglycemia (Kojima et al., 2003). This phenomenon was promoted by the presence of betacellulin. Manipulation of the culture conditions (by using activin A in serum free medium for differentiation) of the Pdx1-expressing human fetal liver cells was shown to further promote the differentiation of these cells towards the beta-cell phenotype, as judged by insulin content and gene expression (Zalzman et al., 2005; Zalzman et al., 2003). All of the above studies involve genetic manipulation of stem / propgenitor cells. Such in vitro manipulated cells cannot be used for treatment of diabetes in humans. It is therefore necessary to identify a source of human insulin-producing or islet progenitor cells that can efficiently transcribe, translate and secrete insulin.

At present, human cadaveric pancreas is the ultimate source of such insulin-producing cells for replacement therapy in diabetes. Although this therapy has potential to cure diabetes, there is a significant scarcity in number of cadaveric pancreas available for transplantation (~30 "good" pancreas/year in Australia). In contrast to this, there are several hundreds of gallbladders available every year from just one surgical team in Melbourne, Australia. In other speciality GI centres, this number is over a thousand every year. The level of insulin transcript seen in gallbladder is 1000-fold less (Ct value 22-26) than that observed in pancreas (Ct value 12-16). This level of insulin gene expression is already significantly higher than any of the differentiated stem cells reported until now. Gallbladder cells naturally exhibit insulin expression higher that the differentiated stem cells and hence there is scope for enhancement of expression levels of insulin in gallbladder derived cells upon differentiation/maturation. Further research in understanding the maturation / expansion and differentiation of gallbladder-derived islet progenitor cells will help in providing alternative source for cell replacement therapy in diabetes.

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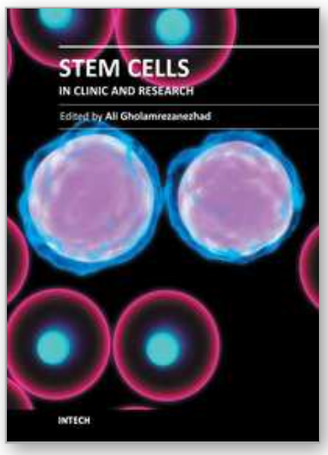

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Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigationally more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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