Human pancreatic islet progenitor cells demonstrate phenotypic plasticity *in vitro*

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Phenotypic plasticity is a phenomenon that describes the occurrence of 2 or more distinct phenotypes under diverse conditions. This article discusses the work carried out over the past few years in understanding the potential of human pancreatic islet-derived progenitors for cell replacement therapy in diabetes. The phenotypic plasticity exhibited by pancreatic progenitors during reversible epithelial-to-mesenchymal transition (EMT) and possible role of microRNAs in regulation of this process is also presented herein.

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The increasing scarcity in number of human pancreatic islets available for transplantation in type 1 diabetes (Shapiro *et al.* 2005, 2006), has accentuated the need for research in exploring alternative sources of insulin-producing cells for cell based therapy in diabetes. Since *in vitro* culture of islet *β*-cells demonstrates loss in insulin (Beattie *et al.* 1999), several attempts have been made to identify stem / progenitor cells capable of differentiation into insulin-producing cells. Embryonic stem cells, which are known to be pluripotent, have the potential to differentiate into any lineage. However, studies carried out in several laboratories till now (Lumelsky *et al.* 2001; D'Amour *et al.* 2006; Soria *et al.* 2008) have demonstrated that it is difficult to achieve efficient directed differentiation of human ES cells to endocrine pancreatic lineage. Various studies have shown that pancreatic duct epithelial cells (Bonner-Weir and Weir 2005), pancreas derived mesenchymal cells (Gershengorn *et al.* 2004; Zhang *et al.* 2005), human bone marrow-derived precursor cells (Moriscot *et al.* 2005) and human adipose tissue derived cells (Timper *et al.* 2006), may serve as endocrine pancreatic precursors. However, all these cell types differ in their ability to differentiate into endocrine pancreatic fate. Overall, none of the *in vitro* differentiation protocols published till now have succeeded in making 'islet-beta cells' with therapeutic potential to treat diabetes in humans.

Understanding the differentiation potential of human pancreatic precursors to insulin-producing cells not only requires knowledge of developmental biology of the pancreas, but also a clear understanding of the epigenome. All cells in human body contain the insulin gene; however only a few tissues such as the pancreatic islets show robust insulin gene expression. During our initial studies in the lab (Joglekar *et al.* 2009) we observed that cells derived from the human fetal pancreatic islets retain epigenetic marks that indicate an active promoter conformation. We therefore chose to work with cell systems that would be more committed to differentiate into endocrine pancreatic fate. A few years back, we demonstrated that manipulation of culture conditions can induce hormone expression in islet-like cell aggregates (ICAs) obtained from human pancreatic duct cells (Hardikar *et al.* 2003), as well as adult human islet-derived precursor cells (Gershengorn *et al.* 2004). This process involves transition of proliferative cells exhibiting adherent characteristics into spherical ICAs after a change from serum-containing medium (SCM) to serum-free medium (SFM). Recently, it was demonstrated (Gallo *et al.* 2007) that in the presence of fetal bovine serum (FBS), cultures of purified adult human pancreatic islets can generate an intermediate mixed cell population from which mesenchymal cells can be obtained. These cells on

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Supplementary movies 1 and 2 pertaining to this article are available on the *Journal of Biosciences* Website at *http://www.ias.ac.in/jbiosci/Oct2009/pp523–528.suppl.pdf*

subsequent serum removal were shown to differentiate into islet-like clusters (ILCs) that secrete insulin in response to glucose.

In the past few years, there has been tremendous debate concerning the proliferative potential of pancreatic beta cells. It has been proposed that these "terminally differentiated" beta cells within the islets are incapable of proliferation. Recently, there have been several studies, which demonstrate that mouse islet beta cells do not undergo mesenchymal transition *in vitro* (Atouf *et al.* 2007; Chase *et al.* 2007; Kayali *et al.* 2007; Morton *et al.* 2007; Weinberg *et al.* 2007). These investigators used a genetic lineage tracing system in mice to follow the population of cells obtained during *in vitro* expansion of mouse islets. Though insulin-producing cells in adult mice did not show transition to mesenchymal cells *in vitro*, lineage tracing studies in human pancreatic islets have demonstrated that human islet *β*-cells can proliferate *in vitro* (Russ *et al.* 2008*;* Joglekar *et al.* 2009). It appears that the differences in proliferative potential of islet beta cells may be species- and/or specifically age-related.

Several studies on pancreas development and differentiation of embryonic stem cells have demonstrated that differentiation of pancreatic progenitors follows a stepwise commitment of undifferentiated cells to specialized cell types that are organized in functional units; the islets of Langerhans. It has been demonstrated that when mouse ES cells are placed in suspension cultures, they aggregate and differentiate in a manner that resembles development of a mouse embryo. At around 7-8 days, 2-layered embryoid bodies (EBs) appear and eventually begin to differentiate with expression of specific lineage-restricted genes in tightly regulated temporal sequences (Martin 1980; Robertson *et al.* 2000). Recent studies have demonstrated significant advances in directed differentiation of human ES cells (D'Amour *et al.* 2006). These investigators designed protocols that involved addition of mesoderm-derived factors to EBs to generate definitive endoderm and pancreatic progenitor marker expressing cells (*Foxa2*$^+$*, Sox17*$^+$, and *Pdx1*$^+$), some of which also express islet (pro-) hormones. Though these studies have helped us to get significant insight in directed differentiation of human ES cells, the number and amount of insulin producing cells obtained through such differentiation studies limit their use in cell replacement therapy for diabetes (Noguchi 2007; Soria *et al.* 2008). We therefore began to look at the possibility of generating endocrine pancreatic lineage committed cells. We observe that when human pancreatic islets are placed in a growth-promoting medium, cells within them begin to migrate out (figure 1) and transition to a population of mesenchymal-like cells through a process well known as epithelial-to-mesenchymal transition (EMT). Interestingly, the cells derived from such proliferating populations of islet cells do not show any immunopositivity for pancreatic (pro-) hormones, but express the mesenchymal marker Vimentin as well as the intermediate filament protein Nestin (figure 2A,B). Such populations also demonstrate a high degree of proliferation (figure 2C) when exposed to serum or 50 ng/ml EGF. A yet another important observation is that these pancreatic progenitors come together to form islet-like cell aggregates (ICAs) when serum/EGF is withdrawn from the culture (Supplementary movie 1). By around 14 days of exposure to serum-free conditions, we observe better aggregation and appearance of hormone-producing cells in these ICAs (figure 2E). At this time, ICAs show minimal proliferation (figure 2F) and can be maintained as hormone containing islet-like

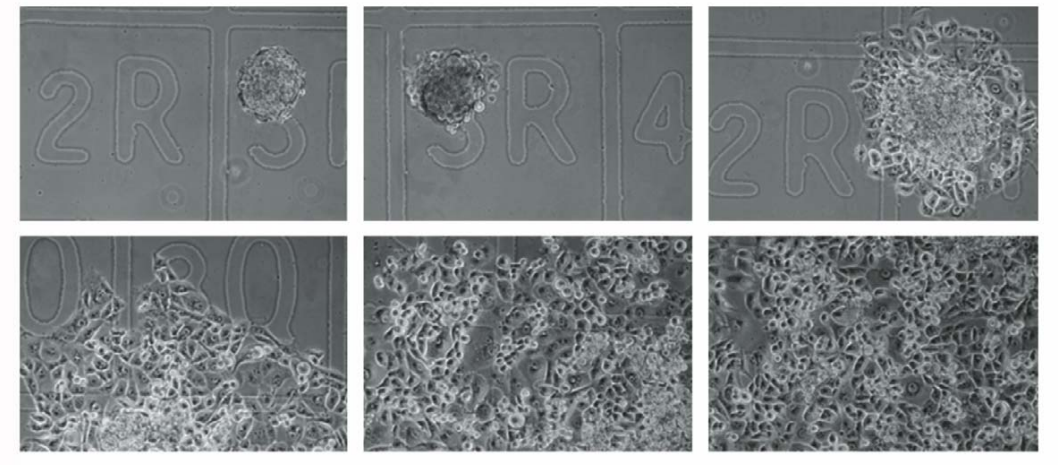

Figure 1. Human pancreatic islet cells migrate to form mesenchymal populations in growth-promoting conditions. Pancreatic islet placed on an alpha-numeric coverslip was observed for every 12 h after exposure to 50 ng/ml EGF in a serum-free medium. By 12-24 h, cells within the islet migrate out and finally result in a monolayer of cells by 60 h of exposure (bottom right). Bar = 50 μm

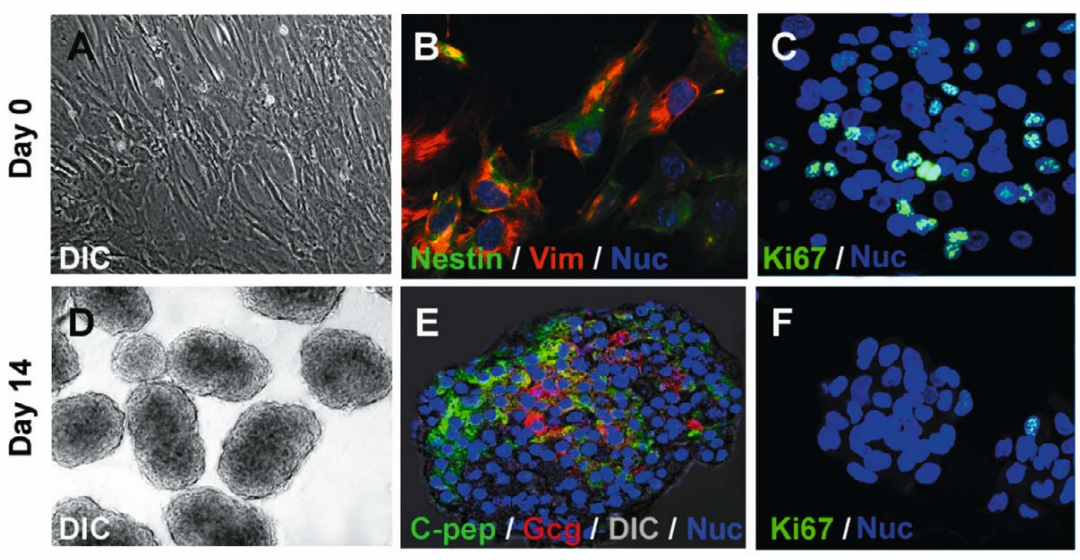

Figure 2. Human islet-derived mesenchymal cells differentiate into hormone-producing cell aggregates. Proliferating populations of mesenchymal-like islet-derived progenitor cells (**A**) produce Nestin (green) and Vimentin (red) (**B**). Under such growth-promoting conditions, these cells show expression of proliferating marker Ki-67 (green, panel **C**). The blue DAPI stain identifies nuclei. On exposure to serum-free (growth inhibiting) conditions, these cells come together to form islet-like cell aggregates (ICAs; **D**), which show immunopositivity to pancreatic islet hormones (**E**). Such ICAs show very few proliferative cells when maintained in serum-free medium. Bar = 50 μm.

aggregates *in vitro*. When these ICAs are subsequently re-exposed to growth-promoting conditions, cells within these ICAs begin to migrate out of the aggregates, generating proliferating populations of mesenchymal-like cells (Supplementary movie 2), driving us to conclude that EMT is reversible in these cell populations.

Members of the Snail superfamily [Snail1 (Cano *et al.* 2000) and Snail2 (Bolos *et al.* 2003)], the basic helix–loop–helix (bHLH) family [E47, also known as TCF3 (Perez-Moreno *et al.* 2001) and TWIST (Yang *et al.* 2004)] and the two zinc-finger E-box-binding homeobox (ZEB) factors [ZEB1 (Eger *et al.* 2005) and ZEB2 (Comijn *et al.* 2001)], are among the various transcription factors that have been found to be involved in EMT. Recently, a new class of non-coding RNAs (microRNAs) has been shown to be involved in mesenchymal transition of human cancer cells (Burk *et al.* 2008; Gregory *et al.* 2008; Kong *et al.* 2008; Korpal *et al.* 2008; Park *et al.* 2008). These investigators demonstrate a role of miR-200 and miR-155 family of microRNAs in regulating epithelial-to-mesenchymal transition during cancer progression *in situ*. Our present studies also demonstrate a role of microRNAs in regulation of mesenchymal transition in primary human pancreatic cells (M V Joglekar and A A Hardikar, unpublished data). Further research in understanding the role of miRNAs in islet cells would provide us with new insights for developing molecular tools to generate lineage committed pancreatic progenitor cells.

The remarkable plasticity demonstrated by these islet progenitor cells in response to serum deprivation is very similar to the behaviour of unicellular *Dictyostelium* amoebae in nutrient-depleted conditions (figure 3). When challenged by adverse conditions such as starvation, these amoebae are induced to form migratory slugs by the aggregation of independent cells, similar to the clustering of islet progenitors in serum-free conditions. Under such adverse conditions, *Dictyostelium* interact with each other via a diffusion-mediated rather than a contact-dependent mechanism, involving the secretion of cAMP by individual cells resulting in cellular aggregation by chemotaxis (Mato *et al.* 1977; Nanjundiah 1998). The cAMP signal perceived by *Dictyostelium* amoebae is relayed via the adenylate cyclase pathway (van Haastert 1985) following binding of cAMP to any of the four adenosine receptor subtypes, ultimately resulting in formation of cellular processes / pseudopodia and cell migration. Analogously, out of the several growth and differentiation factors tested on pancreatic duct progenitor cells (Hardikar *et al.* 2003), fibroblast growth factor 2 (FGF2) secreted by endocrine precursor cells was found to be the most effective chemoattractant in the clustering of these pancreatic precursor cells. FGF2 binds to activate the tyrosine kinase FGF receptors resulting in migration and aggregation. On forming a migratory slug, the *Dictyostelium* aggregate eventually culminates in a fruiting body consisting of a mass of spores and a supporting cellular stalk (Bonner 1971). This transition of *Dictyostelium* amoebae from a proliferation

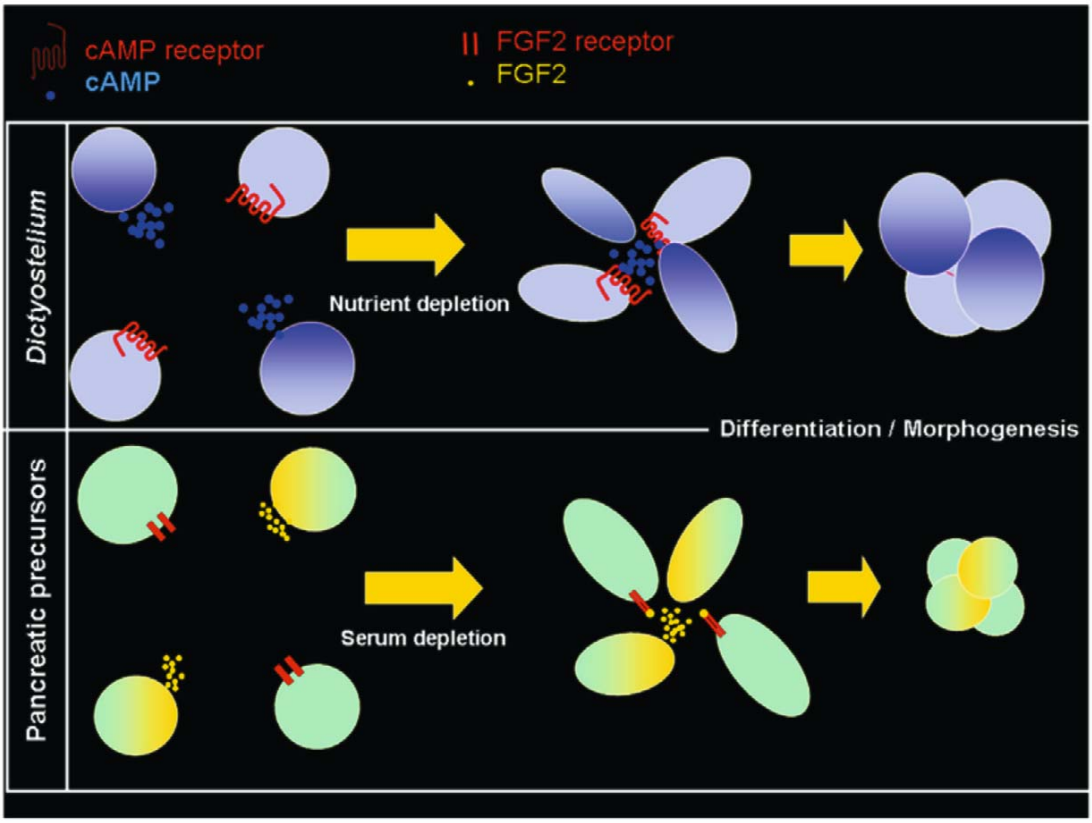

Figure 3. Conservation of phenotypic plasticity. A schematic illustrating the phenotypic plasticity shown by a primitive amoeba; *Dictyostelium*. These organisms multiply as unicellular amoebae in conditions of nutrient abundance but come together to form a specialized structure when placed in nutrient-deprived conditions. The lower panel illustrates the similarity of this primitive system to the phenotypic plasticity demonstrated by pancreatic progenitor cells (see text).

("primitive") state in the presence of nutrients to a differentiation ("specialized") state in conditions of starvation can thus be looked at as a prototype of the mesenchymal-to-epithelial transition seen in our pancreatic progenitors.

The plasticity exhibited by islet cells to undergo reversible epithelial-to-mesenchymal transition under different culture conditions, provides us with the possibility to generate lineage committed cells. We believe that since epigenetic marks of active insulin promoter are retained after million-fold proliferation of islet cells, these mesenchymal cells generated *in vitro* may be better islet progenitors. Further research in understanding the molecular mechanisms that regulate mesenchymal transition of islet epithelial cells will help in generating novel strategies for *in vitro* differentiation of islet derived progenitor cells.

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