Pancreatic mesenchymal stromal cells: Characteristics and possible origin

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Abstract
Mesenchymal stromal cells (MSCs) are heterogeneous group of cells with multipotent capability that were primarily isolated from bone marrow. Many reports suggest that MSCs reside in almost all tissues and organs. Pancreatic stem cells are of great clinical interest due to their potential role in treatment of diabetes mellitus (DM). MSCs were isolated from both adult and fetal pancreatic tissues. These cells resemble bone marrow MSCs regarding their surface markers expression and mesodermal differentiation potential. Pancreatic MSCs are also able to differentiate into pancreatic endocrine lineage cells, suggesting a possible source for cell replacement therapy for DM. In this review, we will discuss the characterization of pancreatic MSCs, their possible origin and their use as cell therapy for diabetes.

Introduction
Mesenchymal stromal cells (MSCs) are heterogeneous population of cells that were primarily isolated from the bone marrow [1]. MSC-like cells have been isolated from other tissues like the human placenta [2], peripheral blood [3], umbilical cord [4], adipose tissue [5], endometrium [6] and pancreas [7,8]. According to the definition of the International Society for Cell Therapy, MSCs are characterized by being plastic-adherent in standard culture condition and can be differentiated in vitro into osteoblasts, chondroblasts and adipocytes [9-11]. They express certain surface markers such as CD105, CD90, CD73, while lack the expression of CD45, CD34, CD14, CD11b, CD79 alpha, CD19 and HLA-DR. Animal experiments showed that MSCs could engraft into wounds, and contribute to remodeling of injured tissues [10,11]. MSC transplantation has shown promising results in treatment of diseases such as diabetes [12-14], autoimmune diseases [15-17], brain repair [18,19], and blood malignancies [20,21]

In this review, we will examine the characteristics of pancreatic MSCs, their possible origin and their role in cell replacement therapy for diabetes.

Characteristics and origin of pancreatic MSCs

MSCs isolated from exocrine pancreatic culture

MSCs were isolated from exocrine tissue culture (culture that includes both acinar and duct epithelial cells [7,8,22-24]. They resembled bone marrow MSCs both morphologically and in their capability to differentiate into osteoblasts, adipocytes and chondroblasts [7,8,22,23]. Interestingly, pancreatic MSCs isolated from exocrine pancreatic cultures were also shown to generate pancreatic endocrine progenitors. Upon expansion of MSCs isolated from exocrine pancreatic tissues, they expressed endocrine progenitor markers such as Isl1, Nkx2.2, Nkx6.1, nestin, Ngn3, PDX1, and NeuroD [8]. Likewise, MSCs isolated from pancreatic duct tissue cultures expressed pancreatic endocrine progenitor markers such as PAX4 and Ngn3, when induced to differentiate into cells of endocrine lineage [7].

Origin of MSCs isolated from pancreatic exocrine tissue culture

As bone marrow MSCs- like cells have been isolated from different tissues, it was hypothesized that organ MSCs may represent a population of migrating bone marrow MSCs, that “home” to the pancreas [25,26]. In support of this hypothesis, tracking bone marrow MSCs has shown that they populate the pancreatic tissue, and that pancreatic MSCs found in culture were shown to be derived from marrow MSCs [23,27]. A contradictory proposal was suggested by Lechner et al. [28] who showed that bone marrow do not contribute to pancreatic regeneration nor turnover [28]. This was proven through transplantation of green fluorescent protein positive (GFP), sex-mismatched bone marrow into mice, while blood cells acquired full donor chimerism, however, examination of more than 100,000 β-cells showed no GFP positive β-cells [28].

Epithelial to mesenchymal transition process (EMT) was suggested as a possible mechanism of generation of mesenchymal stem cells from cultured exocrine acinar and duct cells [22,24,29]. EMT is a process in which epithelial cells change their phenotype to become mesenchymal cells [30,31]. For this change to occur, epithelial cells lose many of their specific structures and functions, such as cell-cell contact and their cellular polarity while gain the ability to migrate and invade the surrounding tissues.

For EMT process to happen, certain cellular changes should occur. First, a change in the epithelial cell shape due to loss of cellular polarity; epithelial cells become spindle in shape. This change in the shape is mediated by the decrease in the expression of the adhesion molecules

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Key words: beta cells, endocrine, exocrine, mesenchymal stromal cells, mesenchymal stem cells, pancreas

Received: October 22, 2016; Accepted: December 15, 2016; Published: December 19, 2016
Pancreatic exocrine

ABCG2

Highly process upregulation was also observed in duct epithelial cells, α-anhydrase (reviewed by Thiery et al. [32]). These cellular changes occur under the influence of a network of signals of growth factors, mainly TGFβ, via signaling pathway such as Wnt and Notch pathway and transcription factors such as Snail family (Snail and Slug), Twist, ZEB1 and 2 (reviewed by Thiery et al. [32]).

Lima et al. [24] reported that EMT occurs in cultured exocrine cells giving rise to mesenchymal stromal-like cells. Cultured exocrine cells gradually became spindle shaped, losing exocrine markers (amylose and CK19), and epithelial markers (E-cadherin). Meanwhile, mesenchymal markers such as vimentin and α-SMA were upregulated. Cells expressing both exocrine and mesenchymal markers have been detected as well. Interestingly, the EMT transcription factor Snail2 was upregulated in these epitheli-derived mesenchymal cells [24]. Mesenchymal cells derived from the dedifferentiated exocrine cells were characterized as MSCs, as they expressed markers of MSCs and showed the ability to differentiate into cells of mesodermal lineages (adipose tissue, bone and cartilage). Genetic lineage tracing was used to determine the source of these MSCs in exocrine culture. It showed that pancreatic acinar cells gave rise to MSCs, plausibly via the process of EMT [24] (Table 1).

In another study by DeWaele et al. [29], TGFβ and Activin were suggested to underlie EMT-induced cellular changes in cultured human exocrine cells. Interestingly, suppression of EMT pathway enhanced the reprogramming of these MSCs into insulin-producing cells. These cells have the capability of insulin secretion in vitro and in vivo, when injected into streptozotocin diabetic NOD/SCID mice [24].

A recent study of Corritore et al. [22], in which the human pancreatic duct marker, CA19-9, was used to isolate pancreatic duct epithelial cells, has demonstrated that the isolated CA19-9 cells acquired mesenchymal phenotype via EMT. With the exception of CK19 and SOX9 duct markers, there has been downregulation of other duct epithelial markers such as E-cadherin, CD133, HNF1b, carboxic anhydrase II and in the CA19-9 duct cells [22]. As duct markers expression decreased, mesenchymal markers such as vimentin and α-SMA gradually increased. Switch from E-cadherin into N-cadherin was also observed. These cellular changes were accompanied by upregulation of the EMT marker Snail. Upon inhibition of EMT with the TGFβ signaling inhibitor, A83-01, no such cellular changes were noticed in cultured CA19-9 duct cells, providing an evidence for the generation of mesenchymal cells from CA19-9 duct cells via an EMT process (Table 1).

MSCs isolated from pancreatic endocrine (islet) culture

MSCs were isolated from adult and fetal pancreatic islets [33-35]. Highly proliferative MSCs were also isolated from human fetal islets [33,34]. These cells were positive for the stem cell markers nestin and ABCG2 and showed the expression of pancreatic islets hormones such as insulin, glucagon in serum-free media [33]. Isl-1 and nestin-positive MSCs were also isolated from adult pancreatic islets. These MSCs could be differentiated into pancreatic endocrine lineage cells, expressing pancreatic endocrine markers Ips-1, Isl-1, Ngn-3, Pax4, Pax6, Nkx2.2, and Nkx6.1, in addition to pancreatic endocrine hormones such as insulin, glucagon, and somatostatin. They also capable of differentiation into albumin producing cells upon transplantation into SCID mouse liver [35].

MSCs-like islet precursors generated from dedifferentiated human islet epithelial cells were reported by Gershengorn et al. [36], and termed human islet precursor cells (hIPCs). During expansion in serum-containing media, islet epithelial cells gradually acquired the mesenchymal phenotype, producing hIPCs. Islet epithelial cells showed gradual decrease in the epithelial as well as endocrine lineage markers, and increase in mesenchymal markers. Upon serum deprivation, the resulting mesenchymal cells (hIPCs) gradually acquired the epithelial and endocrine phenotype forming aggregates or clusters. When these clusters were transplanted into mice under the kidney capsule, they matured into insulin-secreting cells [36].

Similarly, dedifferentiation of human fetal insulin-secreting cells cultured in serum-containing medium into MSCs-like cells has been reported [37]. Human fetal insulin-secreting cells gradually acquired fibroblast-like phenotype, and showed increase in the mesenchymal markers (such as vimentin, α-SMA, CD44 and CD90) and decrease in the endocrine, duct and exocrine epithelial markers (such as insulin, glucagon, somatostatin, pancreatic polypeptides, CK19, CK7 and amylase) [37].

Several research teams have characterized hIPCs that exhibit characteristics of conventional MSCs [38-40]. They adhered to plastic, and expressed MSC markers, such as CD105, CD90, CD73, CD44, CD29, and CD13 in addition to nestin and vimentin. Pericyte markers like CD146, NG2, α-SMA and PDGF-Rβ were also expressed. On the other hand, these MSC-like cells were negative for hematopoietic and endothelial markers, such as CD31, CD34 and CD45, and for pancreatic duct markers, CK19 and CA19.9. When induced to differentiate under in vitro culture conditions, hIPCs showed multipotency as they differentiated into cells of osteogenic, chondrogenic and adipogenic lineages. When cultured in serum-free media, hIPCs formed clusters that express insulin, glucagon, and somatostatin [38-40].

Origin of MSCs isolated from pancreatic endocrine tissue culture

The generation of hIPCs through dedifferentiation of islet epithelial cells, primarily reported by Gershengorn et al. [36]; was attributed to an EMT process. During culture in serum-containing media, islet cells acquired a fibroblast-like morphology, gradually losing epithelial marker transcripts (such as E-cadherin, claudin-3, claudin-4 and PECAM-1), as well as endocrine marker transcripts (such as PDX1 and proinsulin). Meanwhile, the mesenchymal marker transcripts (such as vimentin, nestin, α-SMA and matrix metalloproteinase 2) were all increased. By immunostaining, hIPCs showed filamentous expression of vimentin and nestin; a pattern that was similar to that of mesenchymal cells.

### Table 1. Summary of the possible origin of pancreatic MSCs.

| Source of Pancreatic MSCs | Possible Origin of Isolated MSCs | Species | References |
|---------------------------|---------------------------------|---------|------------|
| Pancreatic exocrine tissue culture | EMT of pancreatic acinar epithelial cells | Human | [24,29] |
|                            | EMT of pancreatic duct epithelial cells |         | [22] |
| Pancreatic endocrine tissue culture | EMT of β cells of pancreatic islets |         | [36,43] |
|                            | Not yet specified, but may be resident pancreatic MSCs | Murine | [44-46] |

Ahmed SM (2016) Pancreatic mesenchymal stromal cells: Characteristics and possible origin

Liver Panceeat Sci. 2016 doi: 10.15761/LPS.1000105 Volume 1(1): 2-5
Mesenchymal cells co-expressing epithelial and mesenchymal markers were also observed, suggesting that these cells were in a transitional stage. Most interestingly, the EMT main transcriptional regulators Snail1 and Snail2 were expressed in islet epithelial cells undergoing transition to mesenchymal cells, suggesting that EMT process may be responsible for these cellular changes. Even after dedifferentiation of islet epithelial cells to hIPCs, proinsulin transcripts were still detected and it was concluded that hIPCs originated from β cells through EMT.

The process of EMT was reversed in serum-free media. Mesenchymal-like hIPCs underwent mesenchymal to epithelial transition (MET) in which hIPCs expression of epithelial and endocrine markers started to increase gradually while the expression of mesenchymal markers decreased [36].

Although MSC-like hIPCs, may have originated from β cells by an EMT process, there was also a possibility that these cells represented a population of pancreatic MSCs, contaminating islet culture [38-40]. Hence, it was a necessity to conduct lineage tracing studies to track the fate of β cells in pancreatic islets culture.

Lineage tracing studies conducted on human islets has shown that β cells undergo dedifferentiation and EMT in culture [41, 42]. In a recent study [43], Lineage tracing analysis provided further evidence, as the cultured β cells exhibited fibroblast-like morphology and ability to differentiate into cells of mesodermal lineages. These changes were mediated via EMT of cultured β cells [43].

In contrary to results obtained from human islet culture, a series of lineage tracing studies provided evidence against the proposed model of β cells dedifferentiation into mesenchymal cells or the occurrence of EMT [44-46]. However species difference maybe the cause of discrepancies between results of human and mice islet cultures (Table 1).

Type 1 diabetes resulted from autoimmune destruction of the pancreatic islets. Replacement of the these islets by islet transplantation was considered the best way to restore blood insulin level. However, the need of large number of donors islets is the greatest limitation for islet transplantation [47]. The ability of human β cells to expand in vitro from limited number of islets giving rise to insulin producing cells through dedifferentiation/ redifferentiation process, may be considered a reliable way for increasing cell availability for cell replacement therapy of diabetes [48]. Through the inhibition of Slug, the EMT transcription factor expressed in the dedifferentiated human β cells, researchers were able to increase insulin content of the dedifferentiated cells. Since it has shown that it disturb hormone expression in immortalized endocrine cell line [48,49].

Future perspective

In this review, we discussed the characteristics of MSCs isolated from pancreatic tissues. The differentiation potential and origin of these MSCs is yet to be further investigated.

The process of EMT plays a major role during regeneration of different tissues. Despite the paucity of data on the mechanism of pancreatic regeneration, recent studies showing that pancreatic epithelial cells undergo an EMT-like process in culture provides evidence to the plasticity of the pancreatic epithelial tissue, and suggests a role for this plasticity in pancreatic regeneration. Few studies showed that EMT-derived cells from pancreatic acinar cells express MSC characteristics. Still, the EMT mechanism by which these MSCs are generated, and the conditions that favor their growth and expansion need further investigations.

In endocrine pancreatic tissue cultures, available data supports that differences exist between human and murine islet cells, whereas the evidence for an EMT process as an origin of MSCs in human islet culture is just being explored. In view of new data that MSCs arising in human endocrine culture express EMT markers Snail1 and Snail2 [50], designing experiments in which the role of EMT is directly explored in generation of MSCs in islet culture is fundamental for further understanding of islet biology.

New studies are also needed to address the issue of plasticity of EMT-derived mesenchymal cells in endocrine and exocrine pancreatic cultures. Since the ability of these cells to differentiate into pancreatic and extra-pancreatic lineages needs further investigation.

The role of EMT process has been critical during tissue repair by fibrosis, Fibroblasts, which have mesenchymal markers and origin, are generated during fibrosis by an EMT process. Although they share several properties with MSCs, such as the spindle shape, and expression the alpha smooth muscle actin and vimentin, they lack the multipotency of MSCs, and the mesodermal differentiation potency [51,52]. It is thus essential to properly characterize the pluripotency of EMT derived mesenchymal cells.

Authors' contributions

Authors contributed equally for this work.

Acknowledgement

This work has been supported by grant # 5300 from the Science and Technology Development Fund.

Financial disclosure

This work has been supported by grant # 5300 from the Science and Technology Development Fund.

Competing interest

No competing financial interests exist.

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