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Stem-cell therapy for hepatobiliary pancreatic disease

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Abstract
The transplantation of pancreatic beta cells or hepatocytes represents a potential therapeutic approach for type I diabetes and inherited liver diseases, respectively. Furthermore, acquired liver diseases, particularly acute hepatic failure due to toxic or viral injury, have been treated in limited clinical trials with fetal and adult hepatocytes. However, a major limitation is the insufficient amount of beta cells and hepatocytes available for grafts. Alternative sources of these cells have yet to be determined. During the past few years, progress has been made in the development of new strategies to produce mature beta cells and hepatocytes. In this review, we outline the current state of scientific understanding and controversy regarding the properties of embryonic and adult stem cells in the field of hepatobiliary and pancreatic diseases. Our objective is to provide a framework of understanding for the challenges behind translating fundamental stem cell biology into clinical therapies.

Key words Stem-cell therapy · Hepatobiliary pancreatic disease · Embryonic stem cell · Bone marrow cell · Diabetes mellitus

Introduction

Stem cells are cells that are capable of self-renewal and are multipotent, meaning that they can differentiate into many specific cell types. For a long time, "stem cell" has been a concept in mammalian biology but not a reality that could be seen, manipulated, and expanded in vitro. This has changed with the establishment of murine embryonic stem cell (ESC) culture in 1981.[1,2] Through progress made in stem cell research, not only ESCs but also various stem/progenitor cells derived from adult tissues have been isolated and established. These include hematopoietic stem cells,[3–7] neural stem cells,[8,9] vascular endothelial progenitor cells,[10] and hepatic oval cells.[11–13] Recent stem cell-based cell therapies have shown success in the treatment of various animal models for diseases, such as Parkinson's disease, type I diabetes, and inherited genetic liver diseases.

In this review, we survey the latest development in the study of both ESCs and adult stem cells (specifically stem cells from bone marrow (BM)) for the treatment of hepatobiliary and pancreas diseases, with an emphasis on application to clinical therapies. We summarize and discuss stem cell-based therapies for both pancreas and liver diseases, and focus on the issues related to the mechanism of stem cell adaptation, including "transdifferentiation" and "cell fusion."

Stem cell therapy for pancreas diseases

Type I diabetes, or insulin-dependent diabetes, represents a major pancreatic disease with tremendous appeal as a target for cell replacement therapy. This disorder, which results from the loss of insulin-producing beta islet cells due to autoimmune attack, can be reversed by pancreas or islet cell transplantation together with steroid-sparing immunosuppression.[14,15] Diabetes may be particularly suited to cell transplantation thereby because, unlike in Parkinson's disease where precise connections may be necessary, beta cells can function autonomously, even outside the pancreas (e.g., under the kidney capsule). The chief limitation to the wide application of this potentially curative therapy is the inadequate supply of islets from cadavers.

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Embryonic stem cells for the treatment of diabetes mellitus

During the past few years, various groups have tested the possibility of producing beta cells from ESCs. Soria et al.[16] used a cell-trapping system to select mouse ESCs able to transcribe the insulin gene, and such cells corrected hyperglycemia when implanted in the spleen of diabetic mice. Lumelsky et al.[17] designed a new protocol to generate in vitro insulin containing cells from mouse ESCs. These cells secreted insulin as mature pancreatic beta cells, although they could not reverse hyperglycemia when grafted into diabetic mice. Recently, several groups have further modified in vitro protocols.[16–22] The addition of phosphoinositide 3-kinase inhibitors promotes differentiation of a larger number of ESCs toward functional beta cells.[20] With manipulation of the culture conditions and the use of pax4 or pdx-1, both of which are transcription factors associated with beta-cell lineage, more efficient and defined ways of making ESC-derived beta-like cells have been established.[21,23] Much of this rodent data has been adapted for use on human ESCs. The production of insulin by human ESCs has recently been demonstrated.[19,24] Techniques that do not require murine feeder cells have also been developed, allowing for single species propagation of ESCs, and thus avoiding possible zoonotic infection of cells intended for clinical use.[25]

Several researchers have argued that the insulin-positive cells derived from ESCs may not be true "insulin-producing" beta-like cells.[26,27] Rajagopal et al.[26] showed that contrary to previous reports, no message for insulin was detectable in cultured beta-like cells, which suggested that the cells may be concentrating the hormone from the medium rather than actually producing it. Sipione et al.[27] demonstrated that the main producers of insulin in culture were neurons and neuronal precursors, and further demonstrated that a reporter gene under the control of the insulin I promoter was activated in cells with a neuronal phenotype. In addition, problems in the control of differentiation and teratoma formation from ESC-derived insulin-producing cells remain to be overcome.[18,27] Other investigators claim that since existing ESC lines are not assumed to be identical, and none are ideal for generating islets or beta cells, additional ESC lines must be generated.[28] Ethical concerns about the use of ESCs and policies regarding the funding of ESC research need to be addressed before we can wholly evaluated this powerful new technology. Theoretically, ESCs may represent a cell type from which large quantities of functional insulin-producing cells may be generated. However, this remains to be definitively demonstrated.

Bone-marrow cells for the treatment of diabetes mellitus

The BM harbors cells that can become parenchymal cells after entering the liver, intestine, skin, lung, skeletal muscle, heart muscle, and central nervous system in rodent models[29] and in human recipients of marrow or organ transplantation.[30,31] In rodents, the BM also harbors cells that can differentiate into functional pancreatic endocrine cells.[32–37] One month after BM transplantation, donor-derived cells are found in pancreatic islets of recipient mice.[32] These cells express insulin and genetic markers of beta cells. Although the cells secrete insulin in response to glucose in culture, only about 1% of the islet cells originate form the transplanted marrow.[32] A marrow-derived cell type with pluripotential capacity to differentiate into various phenotypes has been described.[38] This or a similar cell type might be able to differentiate into pancreatic beta cells. Similar experiments have been done in overtly diabetic mice whose beta cells have been destroyed by streptozotocin. After BM transplantation, blood glucose and insulin concentrations were normal, and survival was better.[33]

Recent studies demonstrated that BM cells can differentiate in vitro under controlled conditions into insulin-expressing cells.[38–40] Such cells, when transplanted under the kidney capsule of diabetic rodents, reverse hyperglycemia. The removal of the grafted kidney returned the animals to a diabetic state.[40]

However, the discussion that cell fusion rather than true cellular differentiation might account for the detection of donor cells in regenerating tissues has muted some of the enthusiasm for genuine beta-cell differentiation.[37,41] Although studies involving pancreatic endocrine-cell differentiation from haemopoietic organ derivatives largely rule out cell-fusion events as a mechanism of differentiation,[32–36] much more work remains to be done before this issue is settled.

Stem cell therapy for liver diseases

The practical implications of stem cell therapy for liver diseases are that they might serve as a source of cell transplantation and bioartificial liver devices, as well as targets for gene therapies. Direct transplantation of hepatocytes has already been used to bridge patients to orthotopic liver transplant and as a therapeutic alternative to whole liver transplantation.[42–46] Among the diseases with the highest potential to benefit from stem cell therapy are primary liver diseases with extrahepatic manifestations arising from abnormal gene expression or defective protein production by the liver.[46–48] These diseases include genetic disorders such as alpha-1-

antitrypsin deficiency, hemochromatosis, tyrosinemia type I, and Wilson's disease. Also, metabolic deficiencies such as type I Crigler–Najjar syndrome, familial hypercholesterolemia, oxalosis, and familial amyloidotic polyneuropathy, as well as coagulation defects such as hemophilia, may be particularly suited to stem cell therapies.

To date, hepatocyte transplantation has been accomplished in a relatively small number of such patients.[49,50] Acquired liver disease, particularly acute hepatic failure due to toxic or viral injury, have been treated in limited clinical trials with fetal and adult hepatocytes.[51–54] As seen in islet cell transplantation in patients with type I diabetes, the major limiting factor is our inability to produce large quantity of hepatocytes and to keep them ready for use on demand. While bioartificial liver devices may be in clinical use for bridging patients with acute hepatic failure to survival or transplant, their use in patients with chronic liver disease may also require large numbers of hepatocytes. A stable and expandable stem cell culture is expected to provide the means to create such large numbers of cells.

Embryonic stem cells for the treatment of liver diseases

Several recent reports from different groups mention not only in vitro differentiation mechanism of ESC-derived liver cells but also in vivo differentiation after transplantation to several liver disease models. Using various culture conditions or a beta-galactosidase-activating gene-trapping system, some cells derived from embryoid bodies cultured from mouse ESCs acquire the ability to express message for alpha-feto protein (AFP), albumin, transthyretin, and alpha-1-antitrypsin.[55,56] Yin et al.[57] reported that AFP-positive cells isolated from cultured ESCs differentiate into hepatocytes when transplanted into livers of mice that lack the ability to express either apolipoprotein E or haptoglobin. Other reports from Yamada et al.[58] and Chinzei et al.[59] demonstrate that ESC-derived cells in embryoid bodies that express mRNAs for albumin, AFP, and other mature hepatocyte markers incorporate into hepatic plates, produce albumin protein, and morphologically resemble adjacent hepatocytes when transplanted into female mice. Although some promising data were recently reported, any real clinical impact awaits the clear directed differentiation of appropriate cell populations and further investigation into each targeted disease.

Bone-marrow cells for the treatment of liver diseases

BM contains several types of stem cells, including hematopoietic stem cells (HSC); mesenchymal or stromal stem cells, which include multipotent adult progenitor cells (MAPC), and endothelial stem cells. The transplantation of unfractionated BM into the livers of lethally irradiated rats rescues animals from radiation-induced BM ablation and simultaneously yields small numbers of BM-derived hepatic oval cells (and later hepatocytes).[13,60] Recent results show that HSCs can transform into hepatocytes both in vivo and in vitro,[61–63] and that MAPCs acquire hepatocyte differentiation in cell culture[38,57] and give rise to hepatocytes when the cultured cells are transplanted into the liver.[64]

These findings have led to the speculation that HSCs (rather than endogenous liver epithelial cells) are the major source of stem cells involved in the generation of new liver parenchyma following liver damage.[65] Although recent studies clearly demonstrate that cells originating in the BM contribute to the formation of a few hepatocytes, cholangiocytes, and oval cells in both healthy and diseased livers,[13,60] the frequency of this transformation is low.[66–69] Only the fact that HSCs circulate in the blood[70] and may be found in the adult liver[71] supports the argument that HSCs are a major source of cells for regenerating liver parenchyma. Wang et al.[72] demonstrated that endogenous liver epithelial cells are the most efficient type of cell at mediating liver regeneration upon transplantation to damaged liver. These studies indicate that a variety of stem-like cells from several tissues can transform into hepatocytes when transplanted into the liver in vivo or cultured in vitro.[72] Which of these has the highest potential for therapeutic use remains to be determined.

The mechanism by which BM stem cells transform into hepatocytes has been hypothesized to be direct transdifferentiation, reflecting the phenotypic plasticity of stem cells within different tissue microenvironments. The most impressive generation of hepatocytes from BM cells has occurred after transplantation of BM into mice with lethal hepatic failure resulting from homozygous deletion of the fumaryl acetoacetate hydrolase (*Fah*) gene.[61] Subsequent studies in the $Fah^{-/-}$ model suggest that the apparent transdifferentiation of HSCs into hepatocytes results from the fusion of HSC descendant cells (possibly macrophages) with $Fah^{-/-}$ hepatocytes to yield heterokaryotic cells in which hepatocytic phenotypes are dominant and the $Fah^{-/-}$ genotypes is corrected.[73,74] The metabolically corrected heterokaryotic hepatocytes proliferate rapidly and replace the defective host liver parenchyma.[75] Because such extensive replacement of hepatocytes has not been found in other models of damaged liver, it is possible that the accumulation of high levels of fumaryl acetoacetate and the

strong stimulus for proliferation of cells with corrected metabolic defects may be responsible for this phenomenon, whether by fusion or transdifferentiation. Further research is required to reach a final conclusion.

Discussion

The extraordinary activity and interest that currently characterizes research on stem cells afford an opportunity to reassess concepts of liver stem cells. This reevaluation may alter major paradigms of stem cell biology in fundamental ways.[76,77] Recent research on stem cell therapy for liver and pancreatic diseases has emphasized methods that currently dominate all stem cell research: analysis of stem cell differentiation resulting from the transplantation of freshly isolated or cultured cells into tissues in vivo and/or by subjecting these cells to defined conditions of cell culture in vitro. These studies have led to various hypotheses on the nature of stem cells and their potential clinical application regardless of the initial stem cell source.

Compared with hematopoietic cells and neuronal cells, there may be some delay in the progress of stem cell therapy for liver and pancreatic diseases. There is some difficulty in generating endodermal cells from stem cells, because attempts to apply factors characteristic of late liver and pancreas development to essentially very early-stage cells have not been fruitful. Although challenging, generating endoderm is probably the most rewarding lineage toward which stem cells may be directed. Given the fact that both islet cell and hepatocyte transplantation can restore function in patients with type I diabetes and inherited liver diseases, respectively, stem cell-based therapies for liver and pancreas diseases represent one of the most compelling opportunities in regenerative medicine. Real clinical impact will require the clear directed differentiation of appropriate cell populations. Present claims, many of which may be exaggerated, await further confirmation.

Concerning the BM stem cells such as HSCs, some of the early reports on stem cell "transdifferentiation" show the generation of functional hepatocytes or beta cells after BM transplantation. Several consequent studies showed similar results even when using highly purified HSCs or in vitro cultured cells derived from BM. However, owing to the heterogeneity of BM, the possibility that nonhematopoietic stem cells were contained within the transplanted marrow remains. Even in carefully executed studies, impurities in the enriched stem cells could have accounted for nonhematopoietic regeneration. True evidence, such as clonal analysis studies, will be required before a new understanding of fundamental embryological concepts can be accepted.

The idea that cell fusion may contribute to the endodermal differentiation of stem cells does not preclude stem cell-based therapies. Two recent papers suggest that the fusion of differentiated cells with ESCs in vitro could lead to functional cells with stem cell-like properties.[78,79] This led to the idea that transplanted BM stem cells or their progeny were fusing to matured nonhematopoietic cells, resulting in cells with new phenotype and function. In the liver and pancreas, the fusion of hematopoietic cells and endogenous functional cells does appear to be at least part of the mechanism of the generation of hepatocytes or beta cells from BM cells.[37,41,73,74] Because both cell fusion and differentiation of stem cells have been shown to be possible mechanisms for generating mature hepatocyte and pancreatic beta cell phenotypes, further studies will be required to determine the relative frequency of differentiation and cell fusion in particular settings to define the conditions that regulate each mechanism. In addition, there is little debate that a wide range of frequencies have been reported, and the prevalence of such events is extremely low or undetectable. What the discrepancies really indicate is that we have a great deal of basic research left to do.

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