

## The Application of Umbilical Cord Blood Cells in the Treatment of Diabetes Mellitus

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### ■ Abstract

In recent years, human umbilical cord blood (HUCB) has emerged as an attractive tool for cell-based therapy. Although at present the clinical application of HUCB is limited to the fields of hematology and oncology, a rising number of studies show potential for further application in the treatment of non-hematopoietic diseases. HUCB, with its real abundance, simple collection procedure and no serious ethical dilemmas, represents a valuable alternative to the use of

other stem cell sources. The aim of this article is to review the literature on HUCB and to assess its eventual usability in the treatment of diabetes mellitus. This review presents some recent reports concerning pancreatic endocrine stem cells and their identification, HUCB stem cells and their advantages and, finally, the potential for converting HUCB-derived stem cells into insulin-producing  $\beta$ -cells.

**Keywords:** diabetes · umbilical cord blood · differentiation ·  $\beta$ -cells

### Introduction

In spite of recent advances in diabetes care, chronic hyperglycemia persists despite all the available means of insulin therapy, causing serious long-term complications in most patients. Pancreas or islet transplantation are the only methods available to date which are able to establish long-term normoglycemia or near-normoglycemia. Due to the potential side effects of immunosuppressive drugs, their use remains restricted to subjects in whom a simultaneous transplantation of the kidney is needed or to those with impaired glycaemic control who are at a high risk of hypoglycemic episodes on insulin therapy. Should the risks of the surgical procedure and chronic immunosuppression improve, there is still an enormous discrepancy between the availability of suitable pancreatic grafts for organ or islet transplantation and the number of dia-

betic subjects who could profit from transplant therapy.

The hope of obtaining an unlimited supply of insulin-producing tissue revived after the first reports on successful isolation of human embryonic and specific adult stem cells emerged [1, 2]. These cells were able to proliferate and differentiate into various cell types including those bearing a phenotype of insulin-secreting  $\beta$ -cells [3-7]. Although these pioneering works have initiated intensive research, the progress made to date has been insufficient for the clinical application of a cell-based therapy for diabetes. The fundamental question in this field remains as to which of the potential cell types will be the most suitable for therapy. Apart from uncertainty concerning their real plasticity and safety, most of the cell types still have drawbacks due to ethical and immunological problems arising from their biological source. In this respect, stem cell-rich

umbilical cord blood represents a promising and so far inadequately studied therapeutic tool.

### Human umbilical cord blood

The umbilical cord serves as a route for the transport of nutrients and oxygen between mother and fetus. Although at first sight it could appear that umbilical cord blood differs from the rest of the infant's blood, the term umbilical cord blood only describes blood remaining in the cord after birth. The blood may be harvested from the cord and cryopreserved for further processing.

In the past, HUCB samples have usually been discarded following birth. However, this situation may change in the near future, as a growing number of reports demonstrated a greater potential of cells present in HUCB. Umbilical cord blood is known as a rich source of hematopoietic stem cells (HSC) [8, 9], which makes it a valuable alternative to bone marrow (BM) transplantation in hematology and oncology. Although cord blood is mainly used for the treatment of malignant and genetic blood diseases, the therapeutic potential of HUCB cells can go beyond blood system therapy. In addition to the HSC, a variety of different stem cell types have been identified within HUCB. Mesenchymal stem cells (MSC) [10], endothelial stem cells (ESC) [11] and a not yet fully characterized population of multipotent cells can be found in the cord blood. Although these multipotent stem cells are very rare and may differentiate into a limited number of cell types, they may represent a promising tool for cell therapy.

### Advantages of HUCB as a source for stem cell therapy

HUCB as a source of stem cells has a number of significant advantages over other stem cell sources. First of all, non-invasive collection without any risk for the donor and a real abundance with more than 100 million births annually worldwide make the HUCB a readily available source of stem cells. Furthermore, a rising number of public and private banks, which can store huge quantities of cord blood units, allow an optimal HLA match for the allogeneic or potentially autologous transplantation. Last but not least, HUCB stem cells, unlike the more controversial embryonic stem cells, do not involve ethical issues. Thus, HUCB has now emerged as an attractive source of stem cells for research and clinical application in treating a variety of diseases.

The biological advantages of HUCB stem cells are even more apparent. Cord blood is collected immediately after the baby is born, which means that HUCB stem cells are among the youngest types of cell that can be isolated from a human being. This is important, as cells from adult donors may have an impaired DNA quality caused by environmental and endogenous factors during their lifetime. Moreover, the HUCB cells carry a lower risk of viral contamination due to the minimal exposure of donor babies to viruses during prenatal life [12, 13].

One of the main advantages of HUCB cells is hidden in their core. The potential of normal cells to replicate is determined by the length of telomeres, the sequences of the DNA at the chromosome ends. The telomeres abbreviate with each cycle of cell division and critically short telomeres finally lead to cell arrest or cell death. HUCB stem cells have very long telomeres as they undergo a limited number of cell divisions prior to birth. The length of HUCB cell telomeres is 11-13 kbp, compared with 8-9 kbp in BM cells of 16-59 year old donors [14]. Moreover, HUCB stem cells contain telomerase, the enzyme which elongates telomeres. Thus, telomere-shortening caused by cell replication is further decreased in cells from HUCB [15].

A critical disadvantage that HUCB has in common with other allogeneic cell-based therapies is the potential to induce graft-versus-host disease (GVHD), which may develop as a result of immune cell transplantation to the recipient. This complication is usually seen in BM transplantations (BMT) even if a high degree of HLA match is achieved. However, compared to BMT, the incidence of GVHD is lower in HUCB transplantation, which could be due to the lower number of T-cells, immaturity of B-cells and decreased function of dendritic cells. As a concomitant strategy, the depletion of lymphocytes and other immune cells could further limit the incidence of GVHD [16]. However, the elimination of some immune cells may impair the establishment of cell chimerism, which is speculated to be a crucial mechanism for the achievement of a long lasting tolerance of the host immune system to transplanted cells.

### Cord blood-derived stem cells

The potential of HUCB stem cells for the treatment of non-hematopoietic diseases is certainly limited by the presence of multipotent stem cells within the cord blood. Several studies have already shown that HUCB indeed contains stem cells that can differentiate into multiple cell types. The unrestricted somatic stem

cell (USSC) has recently emerged as the most promising candidate for such multipotent stem cells [17]. In spite of a very low frequency in cord blood, USSC can easily be expanded without spontaneous differentiation to at least  $10^{15}$  cells, while retaining a normal karyotype. Under appropriate culture conditions, USSC are able to turn into many types of cells with characteristics of osteoblasts, chondroblasts, adipocytes, neural precursors and hematopoietic cells in a homogeneous fashion. Upon transplantation, USSC differentiated into the same cell types with the absence of cell fusion. The proliferative potential of USSC was confirmed by a high number of culture passages and a decreased shortening of telomeres.

Another candidate for multipotent HUCB stem cells is a lineage-restricted cell subset that bears the markers of embryonic stem cells [18]. These so-called "cord-blood-derived embryonic-like stem cells" (CBEs) can expand hematopoietic progenitors and differentiate into hepatocytes and neuroglial progenitors [19]. CBEs as well as USSC comprise a small subpopulation of HUCB cells with no more than 0.25% of total HUCB cells (Table 1).

Due to the facts that (a) different cell markers have been determined and (b) different isolation and cultivation conditions have been applied in experiments, it is still questionable whether USSC and CBEs are two distinct stem cell populations or whether they are only subsets of each other. At least one difference between these two populations is that USSC have a higher self-

renewal capacity than CBEs. On the other hand, the multilineage differentiation capacity of USSC and CBEs is very similar and appears to be determined by the presence of cells with almost the same differentiation potential.

### Sources of pancreatic stem cells in the regenerating organism

During postnatal life, the loss of  $\beta$ -cell mass due to apoptosis is compensated by the replication of existing  $\beta$ -cells and the formation of new  $\beta$ -cells from stem/progenitor cells. Although the concept of  $\beta$ -cell regeneration from postnatal stem cells has recently been challenged [20], it remains very likely that stem cells differentiate into insulin-producing cells even in adult life. Because the pancreatic stem cell has not yet been fully characterized, the precise origin of these cells is not known. The main sources of such stem cells are thought to be specific pancreatic duct cells and islet cells themselves [21]. Nevertheless, cells with a potential to differentiate toward insulin-producing cells have also been identified in organs other than the pancreas, such as the spleen [22], liver [7] and BM [23]. Moreover, insulin-producing cells were found within the same organs and adipose tissue in diabetic animals [24].

The exact mechanism by which BM-derived cells become insulin-producing cells is not clear. Authors of an *in vivo* study ruled out that cells adopting a  $\beta$ -cell-like

**Table 1.** Comparison of HUCB-derived cells and pancreatic progenitor cells identified so far

Property	Cord blood-derived cells			Pancreas-derived cells	
	HUCB	USSC	CBE	MPP	PMP
Self-renewal capacity	+++	+++	++	+	+
Differentiation into multiple cell types		Hematopoietic, hepatocytes, osteoblasts, chondroblasts, adipocytes and neural cells	Hematopoietic, hepatocytes and neuroglial progenitors	Endocrine acinar and ductal pancreatic cells	Endocrine acinar, stellate pancreatic, neurons, glial cells
Expression of stem cell markers	Oct-4, SSEA-3, SSEA-4, Tra 1-60, Tra 1-81, Rex-1, Sox-1, Sox-2, FGF-4	Not done	Oct-4, SSEA-3, SSEA-4, Tra 1-60, Tra 1-81	Not done	Not done
Markers of pancreatic precursor cells	Ngn-3, nestin, Pax-4, Isl-1	Negative	Not done	Ngn-3, nestin, PDX-1	Ngn-3, nestin, PDX-1
Markers of oval stem cells	CK-18, CK-19, $\alpha$ -fetoprotein, albumin	CK-18	CK-18, $\alpha$ -fetoprotein, albumin	CK-18, $\alpha$ -fetoprotein, albumin	Not done

**Legend:** Self-renewal capacity is defined as follows: +: less than  $10^6$ -fold increase. ++:  $10^6$ - $10^{15}$ -fold increase. +++: more than  $10^{15}$ -fold increase in cell number. MPP: multipotent pancreatic progenitors. PMP: pancreas derived multipotent precursors.

phenotype could emerge through cell fusion. Their assumption is supported by *in vitro* studies [25, 26], which also preclude fusion of BM cells with differentiated  $\beta$ -cells.

The transdifferentiation or dedifferentiation of committed hematopoietic stem cells (HSCs) is one possible explanation. A second explanation is based on the presence of cells with the differentiation capabilities of embryonic stem cells. Recently identified multipotent adult progenitor cells (MAPCs) isolated from BM with their multilineage differentiation and self-renewal potential may represent such a type of cells [2]. Another hypothesis postulates that multiple tissue-specific stem cells reside within the BM and the circulation. Upon the release of specific cytokines and

growth factors, these cells may migrate to related tissue and differentiate into the required cell type.

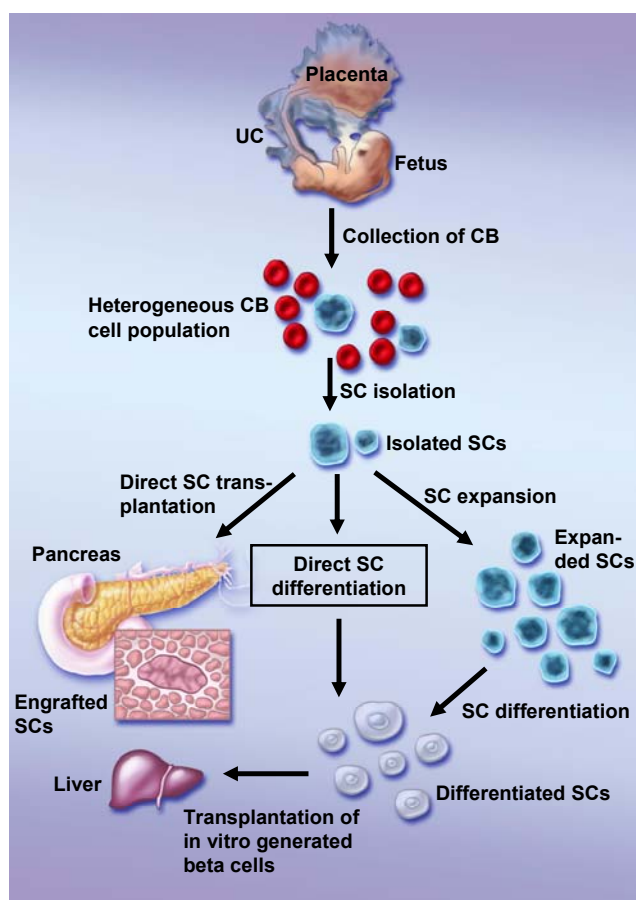
## HUCB for diabetes treatment

In conjunction with the application of HUCB for diabetes treatment, the observation that BM-derived cells may become insulin-producing cells is of enormous importance. Since the HUCB and BM contain similar cell populations and HUCB-derived USSC share most of the cell markers and properties with MAPCs, it should be considered that HUCB may contain cells with a potential to develop into insulin-producing cells (Table 1).

The identification of adult endocrine pancreatic progenitors is a very hard task. Markers involved in endocrine pancreas development and  $\beta$ -cell neogenesis are widely used for this purpose. Hitherto, one study carried out by Pessina and colleagues has investigated the expression of endocrine pancreatic progenitor markers in HUCB cells [27]. They found that undifferentiated HUCB cells were positive for nestin, cytokeratin 19 and transcription factors, such as neurogenin-3 (Ngn-3), Pax-4 and Isl-1, which are considered to be important for  $\beta$ -cell differentiation (Table 1). Since the controversial marker nestin and the detected transcription factors are also expressed by neural cells and their progenitors, which have already been found in HUCB [28], the detection of these markers is not surprising. Moreover, the evidence that HUCB does not contain pancreatic precursors was confirmed, since the expression of the PDX-1 gene, one of the key transcription factors of pancreatic precursors, was not detected.

Although it is not likely that HUCB contains pancreatic progenitors, it is possible that HUCB stem cells are able to differentiate toward pancreatic cells. The candidates for the endocrine pancreatic precursor have a similar phenotype with hepatic [29] and neural [30] stem cells, which could already be reproduced from HUCB cells [28, 31]. Therefore, further *in vitro* or *in vivo* manipulation could result in the differentiation of HUCB cells into pancreatic cells (Figure 1). However, this ability must be proven in further experiments.

Partial clarification of this question has arisen from a few *in vivo* studies which investigated the effect of HUCB transplantation in diabetic animals. One of them indicated that transplantation of HUCB cells resulted in the improvement of blood glucose levels and the survival of diabetic mice. Furthermore, a regression of glomerular hypertrophy and tubular dilatation, common complications attributed to diabetes, was ob-



**Figure 1. Possible methods of generating HUCB-derived  $\beta$ -cells.** After isolation of a specific stem cell population three alternative approaches are possible to obtain mature  $\beta$ -cells: a) direct transplantation of not manipulated stem cells and their homing to the pancreas with their subsequent differentiation, b) *in vitro* differentiation of isolated stem cells and their transplantation to the liver, c) expansion of stem cells prior to their differentiation and transplantation. UC: umbilical cord. CB: cord blood. SC: stem cell.

served in HUCB-treated mice [32]. In another study, transplantation of HUCB cells into type 1 diabetic mice delayed the onset of autoimmunity and insulinitis and improved blood glucose levels. The authors reported that the transplanted mice did not show any clinical or histological evidence of GVHD, despite the omission of immunosuppressive therapy [33]. Because the persistence of human cells was confirmed only in the spleens of a few mice and due to the fact that human cells could not be detected in the pancreas or other organs, it remains unclear which mechanism engenders the improvements that have been achieved.

Finally, a recent report has focused on the capability of HUCB to generate insulin-producing cells *in vivo* [34]. Following the intravenous transplantation of HUCB into NOD/SCID mice, insulin-producing cells of human origin were found in mouse islets. Although the human origin and the insulin-producing potential of the identified cells are almost indisputable, the mechanism underlying their transformation remains questionable. Insulin-producing cells containing both human and mouse chromosomes, as well as only human chromosomes, were identified using species-specific probes. Based on these results, the authors suggested that the generation of HUCB-derived insulin-producing cells can be mediated through both fusion-dependent and -independent mechanisms.

The number of HUCB cells that transdifferentiated and the rate of such an event are critical aspects. The proportion of HUCB-derived insulin-producing cells per total number of islet cells was 0.65%, which was less than in the case of BM-derived insulin-producing cells [23]. However, it is worth noting that the animals were not diabetic after treatment in both experiments. Under diabetic conditions, the demand for the neogenesis of insulin-producing cells might be increased and the higher rate of HUCB cell differentiation could be a compensatory effect in the regeneration of  $\beta$ -cell mass.

Another line of evidence suggesting that HUCB may differentiate toward insulin-producing cells comes from reports on HUCB differentiation into hepatocyte-like cells (Table 1). The liver and the pancreas are derived from an endodermal lineage and share a lot of functional and developmental similarities. During development, the liver and ventral pancreas are formed from the same part of the endoderm. Therefore, it is not surprising that endoderm contains bipotential precursor cell populations for the development of these organs. Interestingly, the default fate of this common endoderm region is to activate the pancreas development program. However, the signals from cardiac

mesoderm induce development of the liver instead of the pancreas in the major portion of this region [35]. Thus, the generation of endodermal progenitors or eventually liver/pancreas bipotential precursor cells could be one of the first steps in the differentiation of HUCB stem cells into insulin-producing cells from any multipotent stem cell.

Not only was the potential of HUCB cells to differentiate towards cells of endodermal origin proven by the detection of functional human hepatocytes in the liver of the USSC-transplanted animals [17], it could also be observed in other experiments. *In vitro* cultivation of unmanipulated HUCB cells in the presence of specific growth factors resulted in the generation of cells with a hepatocyte-like phenotype [31]. The same result could be achieved by the differentiation of CBEs after previous expansion [18] (Figure 1). It is worth noting in this context that hepatocyte-like cells derived in all of these studies expressed albumin, cytokeratin 18 and alpha-fetoprotein, which are regarded as markers of hepatic oval stem cells. Although hepatic oval stem cells reside within the liver and mainly contribute to liver regeneration, they may transdifferentiate into bile duct cells, myocytes and even into the cells of endocrine pancreas [7]. The transdifferentiation of hepatic oval stem cells into pancreatic endocrine cells, including insulin-producing cells, is another potential bridge on the route from HUCB stem cells to the pancreatic  $\beta$ -cell island (Figure 1).

The effect of HUCB cells in the treatment of various diseases, such as myocardial infarction, CNS and spinal cord injuries [36-38], has already been proven. We can conclude from most of these reports that the beneficial effect is supposed to be mediated by the indirect participation of HUCB cells in the regenerating process. HUCB cells are able to secrete mediators critical for the migration, proliferation and homing of endogenous progenitors and participate in a neovascularization of injured or ischemic tissue. While the majority of mediators are produced by mesenchymal cells derived from MSC, the neovascularization is achieved by the proliferation of endothelial cells derived from ESC.

Diabetic neuropathy is one of the most frequent complications in diabetes. Potential factors that may lead to this complication are insufficient nerve blood flow and a decreased amount of vessels around the peripheral nerves. Based on a recent study that employed HUCB-derived EPCs in order to reverse diabetic neuropathy, HUCB can be considered as a possible option for the treatment of this complication [39]. The injection of HUCB-derived EPCs into the legs of diabetic

mice improved nerve function and blood flow at the injected side compared with the untreated legs.

As mentioned above, the reversal of other diabetic complications, such as glomerular hypertrophy and tubular dilatation, could be observed after transplantation of HUCB cells. However, it is not evident whether this effect was due to a decrease in blood glucose levels or due to the trophic influence of HUCB.

### Clinical application and possible approaches

Different strategies should be considered for the clinical application of HUCB in the treatment of diabetes, since many factors may affect the whole procedure. However, the most crucial steps are well documented and will be determinative for the successful application of this therapeutic approach. They include the isolation of suitable cell populations and subsequent differentiation towards a  $\beta$ -cell phenotype. With regard to studies published so far, the problem of cell isolation appears to be almost resolved. Identification and selection of specific cell types based on surface markers and the array of *in vitro* manipulation procedures may lead to the generation of a homogenous population of multipotent cells. Cells with a potential to differentiate into endoderm-derived hepatocytes may represent starting material for further manipulation.

With respect to the high number of islet  $\beta$ -cells required for a single patient and the low frequency of multipotent stem cells in HUCB, *in vitro* expansion prior to transplantation appears to be necessary. Moreover, it is important to consider that this therapy will mainly be used in young patients. The ability of HUCB cells to multiply *in vivo* and *in vitro* appears to be satisfactory for the generation of a sufficient number of cells and their maintenance during the patient's life. Therefore, the most challenging task will be to direct the cell fate toward a  $\beta$ -cell phenotype. There are two

strategies that are worth testing. The more simple approach is the direct transplantation of cultivated stem cells or partially differentiated precursor cells. HUCB cells have the capacity to migrate to various tissues and could be applied for this purpose. However, the exact mechanisms that drive the homing of manipulated cells in the pancreas and determine the phenotype of the potentially engrafted cells are not yet known.

An alternative approach may be the *in vitro* generation of pancreatic precursors or mature  $\beta$ -cells. The employment of exogenous factors that have been shown to be effective in  $\beta$ -cell differentiation could provide desirable outcomes. The main drawback of this approach is that the obtained cells could not be transplanted into the pancreas. Thus, the restoration of pancreatic  $\beta$ -cell mass as the most optimal scenario would not be achieved.

### Conclusion

In the future, umbilical cord blood may be used as a therapeutic tool in the treatment of many diseases. The usability of HUCB in diabetes therapy depends on the ability of its cells to develop into insulin-producing  $\beta$ -cells, as well as on the respective research effort. As HUCB-derived cells have shown the potential to differentiate into insulin-producing cells, further goals include the identification of the suitable cell population and the development of methods for the differentiation of HUCB stem cells into insulin-producing  $\beta$ -cells. Successful achievements of this goal might result in the wide-scale production of  $\beta$ -cells and thus the treatment of diabetes. Although, at the present time, research on UCB stem cells for a potential treatment of diabetes is still in its early stages, we believe that the evidence we have presented here gives some reason for optimism.

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