Clinical Characteristic and Lingering Challenges of Umbilical Cord Blood (UBC) Banking: Future Perspective to Improve Quality of Hematopoietic Stem Cells (HSC)

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ABSTRACT

Both in malignant and non-malignant disorders stem cell based therapies are increasingly being utilized with promising results. hematopoietic reconstitution comprises mainly of 3 types of cells bone marrow (BM), peripheral blood (PB), and umbilical cord blood (UCB). The readily available and abundant resource of stem cell is umbilical cord blood. However, the typical single UCB unit could fetch relatively low numbers of hematopoietic stem- and progenitor cells (HSPCs) and the associated delay in procuring them restrict its routine applicability. In the past decade, the clinical applications of UCB-based cell therapies have broadened with a growing number of diseases treated with hematopoietic stem cell (HSC) transplantation. The assessment of UCB unit hematopoietic stem cells (HSCs), such as CD34+ cells or CFUs (Colony forming units), may provide a more direct estimate of the hematopoietic potential of the unit than the TNC count. As interlaboratory standardization of these assays has been achieved, the selection of UCB units for banking based on quantitation of hematopoietic progenitors is currently not feasible. However, CFU assays provide the only reliable assessment of the viability of the CB unit, although great efforts have been made to develop flow cytometry methods for viability staining. Despite of extreme efforts to find strategies that would enable the *ex vivo* amplification of stem cells for transplantation globally, it has been proven difficult to culture the HSCs in the labs. Therefore, it is imperative to have clear understanding regarding integration of HSC self-renewal, proliferation, and differentiation, molecules participating in their regulation and clinical benefit after their modification. Attempts have been made to improve the quality of UCB units through e.g. standardization of bank procedures, of stem cell enumeration, and of the assessment of HPCs viability. Over 4000000 UCB units are currently available in international registries. However, a significant proportion of patients is still left without a suitable donor, necessitating further development of UCB banking process.

Key words: Umbilical Cord Blood; hematopoietic stem cells

الملخص

لوحظ في الاونة الاخيره تزايد استخدام العلاج بالخلايا الجذعية للقضاء عى اضطرابات الاورام الحميدة و الخبية منها, وظهور نتائج طبية واعدة من خلال إعادة مكونات الدم عن طريق ثلاثة انواع وهي : خلايا نخاع العظم، و الدم المحيطي ، ودم الحبل السري. ومن اكثر الموارد المتاحة بسهولة ووفرة هي الخلايا الجذعية من دم الحبل السري. ومع ذلك، فان وحدة واحدة نموذجية من دم الحبل السري تعطي اعداداً قليلة نسياً من الخلايا الاصلية والخلايا الجذعية المكونة للدم, حيث تاخر تطبيق الحصول على هذه الاعداد القليلة مع وجود الاجراءات الروتينية الكثيرة خلال العقد الماضي. ومع تزايد اعداد الامراض سعت التطبيقات الاكلينيكية استناداً للوحدة النوذجية للعلاج من خلال زراعة الخلايا الجذعية المكونة للدم. ان عملية تقييم الوحدة النموذجية للخلايا الجذعية لمكونات الدم مثل ترميز جينات البروتين (CD34) بالاضافة الى وحدات تشكيل المستعمرات من الفطريات والبكتيريا (CFUs) قد توفر تقديرات مباشرة اكثر من احصاء مجموع انوية الخلية (TNC). وتم العمل على توحيد هذه القياسات بين المختبرات. اما عملية اختيار الوحدات النموذجية للخلايا الجذعية وتخزينها وحفظها على أساس الكميات المكونة للدم هي حالياً غير مجدية. ومع ذلك، فعملية فحوصات وحدات تشكيل المستعمرات من الفطريات والبكتيريا (CFUs) توفر تقييم موثوق وأجدى من وحدة دم الحبل السري، على الرغم من أنه قد بذلت جهود كبيرة لتطوير طرق قياس التدفق الخلوي لبقاء التلوث, وعلى الرغم من الجهود المضنية لإيجاد استراتيجيات من شأنها أن تمكن من استخدام زراعة الخلايا الجذعية بشكل اوسع على الصعيد العالمي، فقد ثبت عملياً أنه من الصعب نشر ثقافة الخلايا الجذعية لمكونات الدم في المختبرات. ولذلك، فمن الضروري أن يكون هناك فهم واضح بشأن دمج الخلايا الجذعية لمكونات الدم مع التجديد الذاتي، والانتشار، والتمايز، والجزيئات المشاركة في تنظيمها والاستفادة الاكلينيكية بعد تعديلها. وقد بذلت محاولات لتحسين نوعية وحدات دم الحبل السري من خلال- على سبيل المثال- توحيد معايير وأعداد واجراءات حفظ وتخزين الخلايا الجذعية، وتقييم قدرتها على البقاء. فهناك اكثر من (4000000) وحدة من الخلايا الجذعية المكونة للدم متاحة حالياً في السجلات الدولية ومع ذلك، ما زال هنالك نسبة كبيرة من المرضى تترك لعدم وجود متبرع مناسب، مما يستدعي مواصلة تنمية وتطوير عملية حفظ وتخزين الخلايا الجذعية للحبل السري.

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INTRODUCTION

Stem cells exist in a wide array of plasticity and self-renewing abilities, from embryonic stem cells that can divide indefinitely and recapitulate the entire organism to adult stem cells, which are limited to a few cell divisions and differentiation into a small number of cell types; each stem cell has its niche both in the life of the animal and in the world of therapeutic medicine. Umbilical cord blood is a widely available, often discarded source of stem cells. This population of stem cells has the potential to be an intermediate population between the naïve embryonic stem cell and the less plastic adult stem cell. Owing to its immature source, umbilical cord blood derived stem cells may be a more malleable population of stem cells with less of the inherent risk of tumor formation found in embryonic stem cells.[1]

Human umbilical cord blood is the blood found in the blood vessels of umbilical cord and placenta. The cord blood is regarded as the "life line" that supplies the developing fetus with the important nutrition elements and oxygen required for proper fetal development. Beside its role in development, umbilical cord blood has been also involved in therapeutic applications, which reported for the first time in 1972, by the pioneer doctors in the United States, Ende and Ende, to treat a patient of acute lymphoblastic leukemia.[1] It was later used regularly for transplantation in hematology setting for bone marrow replacement, following either hematological malignancy or bone marrow failure after any chemotherapy. Umbilical cord blood was, for many years, considered to be restricted to blood disease therapy.[2,3] However, advances in the production of tissue groups, from the three germ layers, has highlighted the additional potential of umbilical cord blood in treatment of other pathological disorders and medical applications including regenerative medicine and tissue engineering.[4-7]

Umbilical cord blood offers an alternative source of stem cells with both research and clinical advantages over other sources of stem cells. Moving toward effective clinical applications requires a readily abundant supply of stem cells to provide the needed amounts of stem cells. With the global birth rate reaching 200 millions/year, umbilical cord blood can be considered as one of the most abundant sources of stem cells.[4,5] In addition, unlike embryonic stem cells, umbilical cord blood stem cells collection does not raise any ethical or religious concerns, which makes it more appealing to both the research and clinical fields.[5,6,8]

Stem cell-based therapy

Given their role in tissue maintenance and repair, stem cells are prime targets for regenerative medicine. The hematopoietic system's liquid nature and underlying easy delivery of HSC to their host tissue has made HSCs the prototype of organ regenerating stem cells and the paradigm for other stem cell-based therapies. Transplantation of HSCs is a conceptual and elegant example of stem cell therapy, and provides means to treat hematopoietic malignancies such as leukemias, lymphomas and immunodeficiencies. The beginning of hematopoietic cell transplantation (HCT) dates back to the late 1950's but clinical success and subsequent breakthrough came with the discovery of the human leukocyte antigen (HLA) system and the selection of immunologically compatible donors in the beginning of the 70's.[9] The basis for this prototype of cellular therapy is the treatment with radio- and/or chemotherapy to breakdown the patient's own hematopoietic system, followed by infusion of new stem- and progenitor cells, which gradually reestablish hematopoiesis.[9]

The time to donor engraftment, also called time to neutrophil recovery (TNR) or immune reconstitution, is a critical period as it leaves the patient susceptible to infection with increased risk of transplant-related mortality. hematopoietic cell transplantation (HCT) can either be autologous, re-infusing the patient's own HSCs; or allogeneic, involving a donor, which in turn can be related or unrelated. The success of allogeneic transplantations greatly depends on the degree of immunological compatibility between donor and recipient tissues as mediated by the HLA genes, and the related occurrence of graft-versus-host disease (GVHD) and graft-versus-leukemia (GVL) effect.[10,11] Traditionally, all stem cell transplants performed used BM as sole source of HSCs.

Today, HSCs obtained from mobilized peripheral blood (mPB) are more commonly used[12]. HSC mobilization can be achieved with e.g. chemotherapy, the cytokine granulocyte colony-stimulating factor (G-CSF) and/or AMD3100, a drug that inhibits binding of the homing molecule CXCL12 to its receptor CXCR4.[13,14,15]

Umbilical cord blood as a third stem cell source was introduced in 1988. Whenever possible, the choice of HSC source is tailored to meet the needs of each individual patient, as they differ with respect to engraftment potential and kinetics, immunogenic characteristics, as well as development and severity of GVHD.[16,17]

Classification of stem cells according to their origin

The use of different definitions and terminology, in scientific and other literature, is often confusing. It is to be expected that as stem cell research progresses, more accurate nomenclature will develop. Stem cells may be derived from fluids and tissues from the moment of conception until after death. Generally, stem cells are classified as: adult, fetal or embryonic stem cells. More recently, umbilical cord blood stem cells, sometimes called 'neonatal' stem cells, have been added as a separate category.

Each stem cell has a unique ability to differentiate into numerous cell types dependent on its potency. Only zygotes are considered totipotent cells, since they are the only cells, which give rise to both every cell type of the embryo and the trophoblast of the placenta. This ability distinguishes them from pluripotent stem cells, which are able to differentiate into all cell types of the embryo except the trophoblast. These cells are usually referred to as embryonic stem cells. In contrast, the differentiation potential of multipotent stem cells, which are usually adult stem cells, is limited to a subset of cell types. Another accepted nomenclature of different stem cell types is related to their tissue specificity. For example, adult stem cells of the nervous system are known as neural stem cells (NSC) whereas blood-forming hematopoietic stem cells (HSC) circulate in the blood.

The potential of stem cells to generate various cell types has emerged great interest for pre-clinical and clinical investigations to cure and repair injured tissues. In the last decade, extensive progress has been made on characterization, isolation, in vitro differentiation and transplantation of multiple stem cell types into various pre-clinical animal disease models. Replacement of lost endogenous cells either by transplantation or by recruitment of resident stem cells is the main goal of stem cell research. Investigations focus on cell replacement strategies for degenerative disorders such as Alzheimer´s or Parkinson´s disease, to cure diabetes with pancreatic cells differentiated from stem cells, to replace damaged cells after myocardial infarction or to replenish immune deficiencies. It is further suggested that stem cells could offer several other approaches for tissue repair as they might facilitate regeneration by providing trophic factors or a permissive substrate for regeneration of endogenous cells.

1. Adult stem cells

In some organs, such as the bone marrow, hair follicle, epidermis, gut and brain, stem cells reside throughout lifetime. In other organs, stem cells divide only after stimulation, usually in response to stress. It has usually been assumed that adult stem cells are multipotent, that is, only capable of developing into cell types of the associated organ or area of the body. Over the past six years, studies have suggested that some adult stem cells have greater plasticity than previously believed, especially stem cells found in the bone marrow (haematopoietic stem cells, mesenchymal stem cells and endothelial progenitor cells) (Verfaillie, Pera and Lansdorp). Haematopoietic stem cells (HSC) are the best characterized adult stem cells.[18, 19]

HSC reside in the adult bone marrow, which further contains different haematopoietic and non-haematopoietic cells. Osteoblasts (mesenchymal cells producing bone matrix), which are localized close to the bone, are known to form and maintain the function of the HSC niche.[20,21,22] HSC can be directly isolated from bone marrow but also after peripheral blood mobilization and from human umbilical cord blood which are successfully used for reconstitution of the haematopoietic system after bone marrow ablation.

In addition to HSC, another stem cell population resides in the bone marrow, named mesenchymal stem cells (MSC). These cells give rise to bone, cartilage, adipose and fibrous tissue.[23] It has been suggested that MSC are in close association with HSC to retain their quiescence[24] and maintenance.[25] However, purification of a homogenous MSC population from bone marrow is lacking due to an incomplete cell marker profile. In contrast, crypt base columnar cells were recently characterized as gut epithelial stem cells by an exclusive expression of Lgr5 (leucine-rich-repeat-containing G-protein-

coupled receptor 5, also known as Gpr49). These cells self-renew and give rise to differentiated cells that constantly repopulate the villi.[26]

Another stem cell source is the bulge of a hair follicle where multipotent stem cells differentiate to all epithelial cells within the hair follicle[27] (Morris et al., 2004) and contribute to wound repair in the epidermis.[28] Interestingly, transplanted hair follicle stem cells are able to generate hair follicles, hair and skin epidermis[29]. These findings provide potential for the treatment of hair loss and other disorders of hair and skin.

The existence of neural stem cells in the adult mammalian brain is well established for two different regions: the subventricular zone (SVZ) and the subgranular zone (SGZ) of the hippocampus. In the SVZ, slowly dividing, radial glia-like cells have been identified as primary neural stem cells. Once activated, these cells generate rapidly dividing transient amplifying precursor cells, which subsequently give rise to neuroblasts that migrate to the olfactory bulb (OB).[30] In the OB, neuroblasts primarily differentiate into GABAergic and dopaminergic interneurons. NSC are also found in the SGZ generating actively self-renewing progenitors and neuroblasts, which mainly differentiate into local neurons. Although NSC have been isolated from other brain regions, active neurogenesis seems to be restricted to SVZ and SGZ in the adult mammalian brain. NSC are the most promising stem cells for clinical applications in neurodegenerative diseases as they can easily give rise to mature neurons and oligodendrocytes.[30]

Adult stem cells have one enormous advantage over stem cells from most other sources: they can be harvested from the patient, ruling out the possibility of immune rejection after transplantation. Immune rejection occurs when the recipient's body fails to accept a transplanted tissue or organ because it is recognised as foreign, and consequently attempts to destroy it. It is the most serious problem faced in surgery involving organ or tissue transplants. Because adult stem cells can be used for autologous transplantation, which refers to a graft in which the donor and recipient area are in the same individual, immunorejection can be avoided. Another advantage of adult stem cells is their limited ability to proliferate, which would reduce the risk of malignancy in therapeutic use.[31] Instead of isolating/culturing/replacing adult stem cells, it has been suggested that adult stem cells present in the body could be triggered to migrate to and regenerate the damaged body-part. This would give us the capacity to re-grow our own tissues and organs, just like zebra fish can re-grow entire limbs and organs (Lanza and Rosenthal). Much research, mainly on the roles played by chemical signals that lead stem cells to damaged body parts, is still required to reach this goal.[31]

2. Neonatal stem cells from the umbilical cord blood

Of all adult stem cells that have been identified, hematopoietic stem cells are the most versatile, and most easy to obtain. Hematological malignancies often require the transplantation of hematopoietic stem cells, which can be isolated from bone marrow, from blood after peripheral blood mobilization or from human umbilical cord blood (hUCB). Umbilical cord blood (UCB) is a rich source of hematopoietic stem cells. UCB stem cells are sometimes categorized as adult stem cells. Others refer to them as fetal cells. More recently, they have been categorized as 'neonatal stem cells'. The first successful transplantation of UCB stem cells was reported by Gluckman and Boxmeyer in 1989, for the treatment of a patient with Fanconi anemia, an inherited form of anemia which leads to bone marrow failure[32]. Up to now, cord blood has emerged as an accepted alternative source of HSC for transplantation in patients.[32]

As suitable HLA (human leukocyte antigen) matched related donors are unavailable for many patients, cord blood from unrelated donors have been increasingly used as an alternative stem cell source for adult patients.[33,34] A lower risk for acute and chronic graft-versus-host-disease (GVHD) despite major HLA disparity compared to bone marrow transplants, the immediate availability and the risk-free donation are further advantages of umbilical cord blood. Unfortunately, it is difficult to receive a single cord blood unit of satisfactory nucleated cell dose for adult patients. In order to overcome that limited number of cells, double cord blood transplantation from different donors has been recognized as one of the most attractive strategies.[35] Another approach to get a sufficient cell number for transplantation is the ex vivo expansion of cord blood cells.[36] In addition to HSC, mesenchymal stem

cells (MSC) can be isolated from umbilical cord blood and expanded as adherent, fibroblastic-like cells in vitro, which is similar in characteristics to MSC populations from bone marrow. MSC are defined as multipotent cells, which can be differentiated into diverse cell types, e.g. osteocytes, chondrocytes and adipocytes.[37, 38] On the other hand, ectodermal and endodermal differentiation potential of MSC from both bone marrow and umbilical cord blood is controversially discussed since different in vitro differentiation protocols have been described to induce marker expression but in vivo confirmation is still lacking. Instead of cell replacement, MSC are thought to provide trophic support, which might influence the recruitment of endogenous stem cells to the injured tissue. Moreover, MSC are known to have immunomodulatory properties as they can inhibit T-cell proliferation in vitro and in vivo.[39] In animal models of clinical relevant diseases, MSC transplantation was shown to have beneficial effects. Interestingly, it has been demonstrated that MSC can decrease oligodendrocyte apoptosis, demyelination and clinical signs after transplantation into an animal model of multiple sclerosis.[40] In a model of amyotrophic lateral sclerosis (ALS), MSC transplantation has been shown to reduce astrogliosis and microglial activation while increasing motor neuron survival and motor performance. Rather little is known about the involved mechanisms but the promising results make MSC attractive for further investigations.[41]

3. Fetal stem cells

In 1998, John Gearhart and his team at the Johns Hopkins University School of Medicine was the first to establish pluripotent stem cells from human fetuses or post-implantation embryos.[42] His team isolated and cultured primordial germ cells (precursors of eggs and sperm) from the gonadal ridges and mesenteries of 5 to 9 week fetuses obtained by therapeutic abortion. The embryonic germ (EG) cells thus obtained have been shown to give rise to cell types of the three germ layers, and can thus be called pluripotent. EG cells represent important in vitro models for cells and developmental biology.[43] However, because pregnancy terminations happen at various times EG cells can be difficult to harvest. There is only a limited time span during which EG cells can be obtained - within the first 8 to 9 weeks after conception. Moreover, EG cells have limited proliferation capacity. Despite these disadvantages, research results suggest EG cells could be therapeutically useful. Human EG cell derivatives have lead to regenerative repair when implanted into rat brains and spinal cords, which offers hope for treatments for neurodegenerative diseases, such as Parkinson's disease.[44] Apart from primordial germ cells, two other sources of fetal stem cells have been investigated: trophoblast stem cells and fetal tissue stem cells.[45] Stem cells derived from human fetal tissue have shown long-term promise in treating strokes in rats.[46]

4. Human Embryonic stem cells (hES)

hES are pluripotent cells derived from the inner cell mass from mammalian blastocyts or from germ cells which can be differentiated into clinically relevant cell types of ectoderm, mesoderm and endoderm in vitro. Further, ESC can be cultured under defined conditions on a large-scale while they maintain their broad differentiation capacity. These features appear to be ideal for clinical cell replacement approaches and regenerative medicine.

Injection of undifferentiated ESC into immunodeficient mice results in growth of teratomas (non-malignant tumours), which contain cell types of all three germ layers, demonstrating their pluripotent nature (Verfaillie, Pera and Lansdorp). Unfortunately, teratocarcinoma formation, which was observed after transplantation of ESC in different mouse models, limits the clinical translation. Tumor formation is supposed to arise by direct transformation of the transplanted ESC followed by uncontrolled proliferation.[47] Currently, it is believed that a pre-differentiation into defined phenotypes and a purification of progenitor cells would probably obviate teratoma formation.

Many studies also have demonstrated the differentiation potential of hES cells in vitro. For instance, hES cells have been shown to give rise to neural progenitors,[48] to insulin-producing cells[49] and cardiomyocites,[50] and endothelial cells.[51] Recent findings suggest that it is also possible to generate in vitro germ cells from hES cells in a Petri dish.[52] If it can be demonstrated that these gamete-forming cells can become mature and are capable of functioning in fertilization and subsequent embryonic development, this would have enormous potential

for infertility treatment, as well as for the shortage of eggs in therapeutic cloning.[53]

A public debate has emerged focusing on the ethical problems associated with the destruction of the embryo during harvesting of hES. Therefore, alternative methods of establishing pluripotent stem cells that do not interfere with the developmental potential of embryos are studied extensively. Interestingly in this regard, hES can be established without any reduction of the developmental capacity of the embryo with a single cell embryo biopsy, which could possibly circumvent the ethical concerns.[54]

Why choose Cord Blood Stem Cells? (Transplantation for hematological diseases)

The increasing interest in umbilical cord blood after its involvements in hematological clinical applications in the past couple of decades focused efforts on analyzing and characterizing the constituents of umbilical cord blood. Beside the blood cells including erythrocytes, leukocytes and thrombocytes, the umbilical cord blood was found to contain different populations of stem cells, a unique feature not shared with peripheral blood. Scientists and researchers have characterized the following stem cell populations from umbilical cord blood; hematopoietic stem cells (HSCs), multipotent non-hematopoietic stem cells and mesenchymal stem cells (MSCs).

Cord blood transplantation in adults was initially hampered by the low-cell dose and high rates of infection.[55] Two advances have improved these results. First, the use of double cord blood transplants has increased the cell dose administered to patients, allowing blood counts to return more rapidly[56]. Second, non-myeloablative or reduced intensity transplants utilize chemotherapy drugs that are better tolerated and safer, particularly for older patients. These changes have improved survival to 40–60% for adult patients with high-risk leukemia.[57]

Umbilical cord blood has been shown to contain a population of hematopoietic stem cells (HSCs) at different stages of hematopoietic commitment characterized by their differential expression of hematopoietic antigens CD133, CD34 and CD45 according to a previously described model.[58] It has been shown that cord blood hematopoietic stem cells can be selectively induced into specific hematopoietic lineages in-vitro including erythroid, megakaryocytic and monocytic lineages.[59]

There has been great debate on the stem cell source of choice for research and clinical applications. Embryonic stem cells, the least committed stem cells, have been shown to have high proliferation and extensive differentiation capacities, which make them a powerful research platform for studying differentiation pathways and lineage commitment of stem cells.[60,61] Nevertheless, when considering potential clinical applications, embryonic stem cells have shown some major limitations. Embryonic stem cells have high tumorigenic characteristics, which might limit and delay any potential clinical use[62]. Further to this, embryonic stem cells often lack the proper imprinting patterns and regulation of certain genes, which might lead to spontaneous uncontrolled differentiation and developmental abnormalities.[63] It has also been found that embryonic stem cells increaseb their immunogenicity by gaining human leukocyte antigens (HLA) during and after differentiation, which might increase the risk of rejection.[64-66] Nevertheless, the ability to generate pluripotent stem cells from the patient's own cells (iPS cells) should offer an alternative to bypass this limitation. Moreover, the isolation of embryonic stem cells from the inner cell mass of the blastocyst involves the destruction of an embryo which itself creates ethical, religious and political problems.[64-66]

Advantages of Umbilical Cord Blood Transplantation

Umbilical cord blood stem cells also show a number of advantages over adult stem cells sources like bone marrow. The collection of umbilical cord blood stem cells is a safe and non-invasive procedure, unlike the collection of adult stem cells from bone marrow[67]. Moreover, umbilical cord blood stem cells occupy an intermediate age stage between embryonic stem cells and adult stem cells, which leads to higher proliferating potential and longer telomeres than other adult stem cells.[68-70]

Another advantage of umbilical cord blood is the ability to store and cryopreserve cord blood units in cord blood banks for future use. This feature provides clinicians and patients with an immediate and abundant supply of cord blood units for transplantation. It also increases the chance of finding the right HLA-matched units for patients requiring

allogeneic transplantation. Many cord blood banks have been established in the U.K, France and many other countries worldwide for such purposes.[71-73]

Adult stem cell plasticity, however, has been called into question, in part because, (i) most of the studies that have demonstrated adult stem cell plasticity have not been confirmed by independent research teams; (ii) because of the low frequency at with which apparent cell transdifferentiation occurs, and (iii) because most studies cannot prove that the plasticity is the result of a single stem cell that differentiates into more than one functionally characterized lineages.

In an effort to prove the plasticity of umbilical cord blood derived stem cells in relation to other stem cell populations, in vitro and in vivo differentiation protocols have been performed. UCB have been successfully differentiated into cells from all three germ layers. Mesodermal cell types, such as osteoblasts, chondrocytes, and adipocytes, are most commonly used to identify a stem cell's ability to differentiate. However, differentiation into cells of the ectodermal or endodermal layer tends to be more difficult. Umbilical cord blood has added advantages over other sources of stem cells, highlighting its potential as a promising therapeutic tool for many diseases and disorders for which the current form of treatment is inadequate.[71-73]

Cord blood banks and networks

Worldwide, efforts are being undertaken to collect UCB cells and store them in freezers for later use in transplantation. The first cord blood bank was established in New York (New York Blood Center, NYBC) by Dr Pablo Rubinstein[74] (National Cord Blood Program). Since then, large-scale UCB-banking has been established worldwide. Cord blood banking programs have since been initiated at several places in United States,[75,76] Europe,[77] Australia[78] and Asia.[79,80] Forty cord blood banks or registries in 24 countries participate in WMDA[81]. To date, more than 100,000 UCB units are registered and available for transplantation in more than 50 banks worldwide and more than 3000 patients, most of which are children, have received UCB stem cells from these banks.[82]

There is a growing consensus among scientists on the great value of UCB stem cells for transplantation. They are easy to obtain and have been shown to be more versatile than other adult stem cells. Kogler and colleagues, for example, identified human adult stem cells from the umbilical cord blood with intrinsic pluripotent differentiation potential.[83]. UCB has several advantages over bone marrow transplantation: the large donor pool, the low incidence of viral infection at birth, the low incidence of graft versus host disease due to the immune immaturity of the newborn (UCB stem cells are less mature than hematopoietic stem cells found in the bone marrow), the increased speed of availability in stem cell banks and the less costly use.[84] One disadvantage is that the number of UCB stem cells in one umbilical cord is too small to treat an adult. However, research is being pursued to overcome this problem and adults have been successfully treated with UCB stem cells.[85]

To enable an HLA type-based search of donors from bone marrow and peripheral blood stem cell donor registries across the world, Bone Marrow Donors Worldwide (BMDW) was established by EBMT.[81] BMDW released its first listing of 156 000 donors in February 1989. Currently, more than 9 million potential donors are listed. Large national registries in the USA, e.g. the National Marrow Donor Program (www.nmdp.org) and the Caitlin Raymond International Registry (www.crir.org), also participate in BMDW. Although BMDW was first established to allow for international searches of tissue typed adult donors, cord blood units are also currently listed. At present, 37 cord blood registries in 21 countries (with a total of 172 550-cord blood units) participate in BMDW (www.bmdw.org/Database/Donors.htm, as of July 2004).

Netcord, founded in May 1998, is an international non-profit joint effort of leading cord blood banks which has issued statutes and guide-lines with the primary aim of improving the quality of cord blood transplants for clinical cell therapy on the international level,[86] NETCORD and FAHCT, 2001) (www.unmc.edu/Community/fahct). To enable efficient cord blood unit data exchange and allocation, the Netcord inventory is available for searches in a virtual office via the internet (office. de.netcord.org). In addition, cord blood banks in Asia are now associated under the name of Asiacord.[79] To enable coordinated management of complicated

international hematopoietic stem cell donor search processes, search activities in each country have been centralized to national hubs.

Private, public or hybrid banking?

UCB banks can be private or public. Private UCB banks store UCB for the family's own use. Parents have to pay for storage of the UCB of their child. The idea is that should the child or another family member at some point in his/her life need a haematopoietic stem cell transplant, these cells will be immediately available, and will (very likely) be an HLA-match. Public UCB banks collect UCB after consent of the mother. Storage is free, but the UCB is intended for any patient in need of a UCB transplant. It is not reserved for the family's private use. Doctors all over the world search the National Marrow Donor Program Registry of donors and cord blood units to find a match for their patients who need a transplant. Since hematopoietic stem cells from umbilical cord blood have greater potential as a collective asset than as an individual asset, eligible pregnant women should be encouraged to consider public cord blood donation by their prenatal care providers.

Average volume of cord blood for banking

Cord blood volume obtained from the umbilical vein during collection may vary between 0 and 255 ml depending on the collection technique and mode of delivery.[87] Banks routinely use a cut-off limit of collected cord blood, e.g. 40ml, to increase the cell yield available for banking.[87] Depending on the selected cut-off limit, an average collected cord blood volume reported has been 68-107ml.[87,88]

Collected total cell amount naturally also varies according to the collected volume. Total nucleated cell counts, such as 102-169x10^7/unit, have been reported[87,88], which may reflect the actual cut-off limits set for collected volume and nucleated cell concentrations analysed by the individual banks.

Lingering Challenges to stem cell transplantation

Challenges for hES cell research

Science of pluripotent hES cells is still in its infancy. Many technical issues remain to be solved.[89] More research is needed into the self-renewing capacity of hES cells, and into means to ensure stability of genotype, epigenetic status, and phenotypic properties of hES cells. hES cells need to be generated in pure form and in sufficient numbers to be therapeutically useful. Another challenge is to direct the differentiation of hES cells down a particular pathway to generate the desired cells that are restricted to specific developmental fates. In addition, a better understanding is required of what stem cell type to supply for treating a particular pathology, and how to deliver it. For instance, by simply injecting hES cells into the damaged body part, or by first coaxing them into progenitor cells or fully differentiated cells. Transplanted hES cell progeny may not function normally in organs and might retain tumorigenic potential, a characteristic of hES cells. As said before, the avoidance of immunological rejection remains one of the biggest challenges. There are numerous unanswered questions as to the control of ES cell growth and differentiation. ES cells have the potential to be tumorigenic, growing into teratomas and teratocarcinomas when injected into mice. Research is being done on this worldwide and progress is being made.[90] Recent research shows there may be infectious and other risks, such as occurred with BSE, of transplanting such tissue back to people, when it is grown on foreign culture material.[91]

Challenges for UCB cell research

Despite the undisputed role of UCB as an abundant and readily available alternative source of stem cell, the relatively low numbers of stem- and progenitor cells in a single unit limit the use of UCB in HCT. Given the low cell numbers, prime targets for cord blood transplantation (CBT) are pediatric group of patients. According to the Center for International Blood and Marrow Transplant Research (CIBMTR), UCB accounts for more than 40% of allogeneic unrelated HCT in children, while the corresponding number in adults is only approximately 10%.[92] There of apparent link between infusion of low cell numbers and delayed immune reconstitution, which in turn increases the risk of early transplant-related mortality. Subsequently, the main challenge is to overcome the cell-dose limitation.[93]

Simplified, the more cells transplanted, the faster recovery, the better outcome. Substantial clinical benefit may be achieved through generation of sufficiently high numbers of progenitors that ensure faster hematopoietic recovery and thus counteract the transplantation-induced neutropenia, while at the

same time providing stem cells for stable long-term engraftment. To make UCB as a donor choice more feasible for adult patients, extensive research has been devoted to find strategies that would improve the numbers, potency, and engraftment capabilities of transplantable HSPCs prior to transplantation.[93]

There is also the potential for transfer of genetically abnormal cells. In addition, clinical results show that the frequency and rate of myeloid and platelet engraftment are slower than that observed with comparably matched bone marrow, leading to the possibility of increased rates of engraftment failure and transplant-related mortality.[94,95] In contrast to bone marrow or peripheral blood progenitor cell transplantation, where it is possible to seek subsequent donations if needed, the unrelated cord blood donor cannot offer a second donation in the event of marrow failure or relapse of the disease.[94,95]

As the transplant recipient is at a significant risk for morbidity before engraftment, speedy recovery of neutrophils and platelets is essential. Engraftment can be evaluated by assessing the degree of chimerism i.e., the proportion of donor-derived myeloid and lymphoid cells versus those of patient origin.[96] In primary graft failure, engraftment fails to occur and the graft never produces an adequate number of blood cells. In secondary graft failure, blood cells are initially produced but the function of the graft ceases later. RBC recovery may also be delayed after hematopoietic stem cell transplantation.[97]

HLA matching is essential for the success of hematopoietic stem cell transplantation, including minimizing the risk of GVHD.[98] In addition, the direction of the HLA mismatch has recently been suggested to be better tolerated in UCB than in BM transplantation.[100] Importantly, however, a better HLA match has been reported to decrease the TNC dose required to achieve the same level of transplant related mortality in UCB transplantation.[99]

Engraftment has been reported to be slower after UCB than after BM transplantation; in contrast, the risk of acute GVHD is reportedly lower.[101] The risk of chronic GVHD may be similar or slightly reduced in UCB transplantation compared to BM transplantation. As the UCB unit is available, immediately, without the delays of contacting and testing a potential donor, UCB may be a better alternative in emergency situations. On the other hand supporting cells or a new hematopoietic stem cell graft can often be collected from a BM donor if needed, an alternative not available for UCB recipients.[101]

Altogether, survival after UCB transplantation has been reported to be comparable to that after BM transplantation.[100] However, most comparative studies of UCB and BM transplantation have been retrospective, and the patients have not been matched for diagnosis, disease stage, pre-transplant conditioning, the grade of HLA matching, or other factors affecting the outcome of the hematopoietic stem cell transplantation. UCB transplantation results have been analyzed in heterogeneous groups of patients, and the recipients have often had a poor transplant prognosis due to e.g. advanced disease. Incomplete HLA matches have had to be accepted, and the TNC dose/kg patient weight has varied widely even within the studies. Thus comparative studies of CB and BM transplantation are scarce.[100]

In recent studies of one-unit transplantation, the median TNC count of the transplanted UCB units has been approximately 90-160 x 10^7. However, the increasing proportion of adult UCB recipients has led to rising requirements for CB unit HPCs and thus, TNCs. The minimum HPC dose required for successful transplantation also depends on the degree of HLA mismatching.[104] Target TNC doses of 3.0x10^7/kg for HLA matched units and 4.0x10^7/kg for HLA-mismatched units have been applied[102]. To overcome HLA mismatches at 1-2 loci, a cryopreserved TNC dose of at least 2.5-5.0x10^7/kg patient weight has been reported to be required to minimize UCB transplant-related mortality[99]. Thus, both the available TNC does and the degree of HLA matching have to be considered when selecting the best UCB unit for each patient.

The transplantation of two UCB units to increase the TNC dose has yielded promising results in adults.[99,102] However, the probability of finding two UCB units both HLA-mismatches have had to be accepted. The risk of acute GVHD may be increased after the transplantation of two UCB units.[103] The CD34+ cell dose[104] and the CFU dose[105] have been suggested to be better determinants of neutrophil and platelet engraftment than the TNC dose. In some studies,

neither the TNC dose nor the CD34+ cell or the CFU-GM doses have correlated with engraftment.[106] Due to problems with the interlaboratory standardization of CD34+ cell and CFU enumeration, the TNC count is still applied in international registries for the selection of UCB units for transplantation. Finally, cord blood collection, storage, and transplantation raises numerous financial, ethical, and regulatory issues for health care providers and society.

Safety Aspects of Cord Blood Banking

Once collected, cord blood units are labelled and shipped to the bank, where they undergo safety testing, human leukocyte antigen typing, and cryopreservation. Public banks generally follow procedures in accordance with established standards.[106] Procedures for transfer of cord blood units from birth hospital to private cord blood banks are less well controlled and defined. There is currently no requirement for registration or regulation of cord blood collection centres, banks, or transplant centres in Canada. Testing cord blood and maternal blood for infectious agents (HIV, cytomegalovirus, human lymphotropic virus, hepatitis viruses, and syphilis) is required by public cord blood banks. Cord blood units are initially placed in quarantine until infectious testing is completed. If new units test negative for infectious diseases, they are placed in long-term storage banks. Public banks also obtain detailed maternal and family history of genetic diseases, travel to countries with high rates of transmissible infections, and other high-risk behavior regarding intravenous drug use and sexual behavior. Cord blood unit screening is the same as that used by the Canadian Red Cross for blood donors.[106]

CONCLUSION

The scientific community has access to a widely varied population of stem cells with which to investigate the biology and potential application of the stem cell. Undoubtedly, certain cell types are more suited for use in different situations. Human umbilical cord blood is probably the largest, but under-utilized source of stem cells with the yearly global birth rate of 200 million per year.[66] Cord blood non-hematopoietic multipotent stem cells, which demonstrated high potential for neural differentiation, are easily accessible, immunologically naive and are free from ethical controversies associated with other sources of stem cells. These added advantages made umbilical cord blood stem cells a potential candidate for disorders and clinical applications of those whose current mode of therapy is inadequate. One disadvantage is that the number of UCB stem cells in one umbilical cord is too small to treat an adult. However, in order to assure potentially effective treatments and the banking of autologous cord blood in private cord blood banks is based on the hope that it may, at some point in the future, be of therapeutic benefit in treating or curing any form of chronic or degenerative disease in its donor or in others. At present, this is entirely speculative. Applications of umbilical cord blood stem cells, more research should be directed toward understanding the molecular properties of umbilical cord blood. This requires the development of effective purification and enrichment strategies of such cells to allow accurate analysis of the molecular mechanisms.

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