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Umbilical cord blood stem cells: what to expect

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Umbilical cord blood (UCB) is a valuable alternative source of hematopoietic stem cells (HSCs). It has unique advantages of easy procurement, absence of risk to donors, low risk of transmitting infections, immediate availability, greater tolerance of human leukocyte antigen (HLA) disparity, and lower incidence of inducing severe graft-versus-host disease (GVHD). In the last several years, these features of UCB permit the field of UCB transplantation (UCBT) to move at a faster pace for both children and adults with malignancies and nonmalignancies. However, new strategies and novel developments are expected to improve engraftment and reconstitution, and to enable *in utero* transplantation for early therapy, as well as to allow the therapy for a wide spectrum of human diseases.

Keywords: hematopoietic stem cells; umbilical cord blood; human leukocyte antigen disparity

Introduction

Stem cells can renew themselves and differentiate into a range of specialized cell types, making them fascinating to research and medical care. There are generally two types of stem cells, namely embryonic stem cells (ES) and non-ES cells. The pluripotent nature of ES presents a significant potential in clinical applications, however ES therapies are currently limited by ethical, political, biological, and regulatory hurdles.[1] Non-ES cells can be found in several tissues, such as bone marrow (BM), skin, ovary, sperm, adipose tissue, and pregnant products of umbilical cord blood (UCB), amniotic fluid, and placenta. The use of UCB-derived stem cells is expanding in the medical field owing to the facts that UCB is easy to procure from waste products without risk to the donor, and the cells are "younger" than those obtained from adult BM and more tolerant to human leukocyte antigen (HLA) mismatches for lowering the risk of graft-versus-host disease (GVHD).[2] This review focuses on stem cells derived from UCB, their applications in malignant/nonmalignant diseases and what to expect in future, for example, regarding the subject of developing strategies to improve the engraftment and reconstitution, *in utero* transplantation for early therapy before extensive damage to various tissues and organs ensues, as well as new uses of special cell types and technologies to perform therapy for a wide spectrum of human diseases.

Umbilical cord blood stem cells

Transfusion with placental blood was firstly reported in 1939.[3] Decades later, in 1972, Ende and Ende used multiple aliquots of fresh UCB to treat a child with leukemia after conventional chemotherapy. A temporary engraftment of stem and progenitor cells were identified through the changed blood group of the patient after the therapy.[4] Subsequently, *in vitro* studies showed that hematopoietic stem cells (HSCs), such as multipotential (CFU-GEMM), erythroid (BFU-E), and granulocyte-macrophage (CFU-GM) progenitor cells exist in human cord blood.[5] *In vivo* studies on animal experiments also provided evidence that neonatal/placental blood would be sufficient for engraftment.[6] Importantly, the UCB stem cells and progenitors could be easily cryopreserved and stored without significant loss of the features.[7] In 1988, Gluckman *et al.* at the Hospital St. Louis in Paris performed the world's first UCB transplantation (UCBT) with cryopreserved UCB from a related donor, an HLA-identical sibling, to a child with Fanconi anaemia with a successful engraftment.[8] In 2008, the recipient

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participated the celebration of the twentieth anniversary of UCBT.[9] After the first case, several groups also reported UCBT from related donors with matched and unmatched HLA to patients with hematopoietic conditions. The importance of banking the UCB was realized on the basis of the successful UCBT. The first public UCB banking program was established in 1991 at the New York Blood Center supported by US National Institute of Health (NIH).[10] Rubinstein *et al.*, the grant receiver, initiated and performed the world's first UCBT from unrelated donors to two children with leukaemia.[11] Their positive experience on the further allogeneic UCBT among 562 cases confirmed the feasibility and the usefulness of the treatment for clinical applications.[12] Recently, donation and storage of UCB is available by cord blood bank, both private and public. Vita 34 international AG is the first European private cord blood bank located in Germany having 40,000 UCB units in cooperation with Holzgreve, Steinhoff, Wobus, and Emmrich *et al.* In 1998, the first cord blood bank in Switzerland was established at the University of Basel under the initiation of Tichelli, Holzgreve, and Gratwohl *et al.*[13] Today, worldwide, around 600,000 of UCB have been banked and close to 20,000 of allogeneic/autologous UCBTs from unrelated/related donors have been performed for the treatments of patients with malignancy and nonmalignancy.[9]

UCBT for malignant diseases

UCBT has been used for the treatment of hematological malignancies, such as leukaemia and lymphomas.[14] Early reports of UCBT mostly reflected the outcomes of pediatric patients, largely because of the limitation in cell dose available from a single UCB unit. The Eurocord group has reported prognostic factors and outcomes of UCBT from unrelated donors for children with acute myeloid leukemia (AML).[15] This study has shown that unrelated donor UCBT in children was able to reconstitute hematopoiesis and achieve sustained engraftment in most cases, was associated with a low incidence of GVHD, and did not result in a higher relapse risk. Another study from same group has reported retrospective analyses comparing outcomes after UCB transplantation (UCBT) and unrelated BM transplantation (UBMT) in children with acute leukemia.[16] Recipients of UCBT had delayed neutrophil and platelet recovery in a shorter time; decreased acute and chronic GVHD; and decreased relapse rate compared with those of UBMT. However, long-term leukemia-free survival (LFS) and overall survival (OS) were not significantly different between the two groups. These data strongly suggest that the use of UCB, as a source of HSCs, is a reasonable option for children with acute leukaemia lacking an acceptably matched unrelated marrow donor. In recently completed analysis, Eapen *et al.*[17] conducted a comprehensive comparison study through collaborative efforts between New York Blood Center (NYBC) and the Center for International Blood and Marrow Transplantation Research (CIBMTR), comparing the outcomes of children with acute leukemia who received HLA-matched and mismatched UCB ($n = 503$) or 8/8 allele HLA-matched unrelated donor BM ($n = 116$). Five-year LFS was similar for recipients of allele matched BM and UCB mismatched at one or two loci, when matched UCB showed even superior results. This new intriguing finding may indicate that HLA-matched or high dose mismatched UCBT can potentially be a front line therapy for pediatric acute leukemia patients, even if HLA-matched bone marrow donors are available.

Recently, using UCBT for adults with malignant hematological diseases has been increasing rapidly. Several studies compared the outcomes of adults with hematological malignancies undergoing UCBT or UBMT after myeloablative conditioning.[18,19] The conclusion is that mismatched UCBT resulted in delayed engraftment, decreased or the comparable incidence of acute and chronic GVHD, comparable relapse rate and transplant related mortality (TRM) when compared to matched UBMT. Overall, in terms of the crucial end point-event free survival, no significant difference was found between the two groups, suggesting that the utilization of UCB as an alternative source of HSCs for adult patients with no HLA-matched BM donor is available.

Allogeneic HSCT, as a means to develop immune-mediated graft-versus-tumor (GVT), has been proposed as an adoptive immunotherapeutic treatment for different nonhematological malignancies, such as gynecological cancer, breast, colorectal, pancreatic cancer and renal cell carcinoma (RCC).[20] Recently UCB has been considered as a feasible alternative source of hematopoietic progenitors (CD34+) for allogeneic stem cell transplantation, but few clinical experience concerning UCB-based treatments

for solid tumors was reported. Takami *et al.*[21] reported a single case of reduced-intensity of allogeneic hematopoietic unrelated UCBT for treatment of cytokine-resistant metastatic RCC. The patient achieved durable donor engraftment with minimal GVHD after two times UCBT and showed regression of metastatic disease, providing the first evidence of a GVT effect on a solid tumor resulting from cord blood graft.

UCBT for nonmalignant diseases

Nonmalignant diseases can be also treated by UCBT, such as inherited metabolic disorders (IMDs), primary immunodeficiency diseases (PIDs), hemoglobinopathies, and BM failure syndromes.

The majority of IMDs is due to defects of single genes resulting dysfunction of enzymes that facilitate conversion of various substrates into products. Several reports provided support to the argument that UCBT is an appropriate and viable option for infants and children with IMD.[22,23] Martin *et al.* reported the availability of UCBT to treat patients with lysosomal and peroxisomal storage diseases, such as mucopolysaccharidoses, mucolipidoses (ML) II, adrenoleukodystrophy, metachromatic leukodystrophy, Krabbe disease, and Tay-Sachs disease on 69 cases. UCBT revealed high levels of near-total chimerism, enzyme recovery in the blood with low risks of graft failure as well as GVHD despite significant donor–recipient HLA mismatching. A 72% of 1-year survival was achieved.

PIDs are caused by inherited defects in our immune system leading to immune-dysfunction and increased susceptibility to infections. HSC transplantation (HSCT) is curative in most children with different types of PIDs, if a suitable donor is available in an appropriate time. However, a majority of patients will not have a suitable matched sibling BM donor. Owing to the mentioned advantages of UCBT especially ready availability and less stringent HLA matching requirements, it offers an attractive option. A number of reports support the use of UCB as a graft source in patients with PID.[12,24] UCBT pointed promise for treatment of chronic granulomatous disease,[25] severe combined immunodeficiency 11 (SCID 11), X-linked lymphoproliferative syndrome 2, Omenn's syndrome 1 and Wiskott-Aldrich syndrome 1.[24] The UCBT for treating PIDs performed by Diaz *et al.* archived a 5-year survival of 73%. All surviving patients presented complete immunologic reconstitution, supporting a valid option of using UCB for children with immunodeficiency who lack an HLA-identical sibling donor.[24]

Hemoglobinopathies are inherited single gene disorders based on the genes, which code hemoglobin. Defects in these genes can produce abnormal hemoglobins and anemia, such as thalassemia and Fanconi anemia. Reports of matched related donor UCBT since the first description of it showed a low risk of GVHD and a high probability of engraftment in most patients with hemoglobinopathies, as well as BM failure syndromes.[26] Unrelated UCBT has been also applied to children with severe aplastic anemia, Fanconi anemia, thalassemia, and sickle cell disease.[26,27] While clinical data have shown excellent outcomes after HLA-identical sibling UCBT for genetic hematologic disorder and BM failure syndromes, results are also promising after unrelated donor UCBT. With proper selection of the UCB unit, the results after UBMT and UCBT from unrelated donors are comparable. Cell dose, HLA compatibility and infections are still important factors for outcomes.[26] For severe aplastic anemia, mainly due to primary graft failure, recent studies suggested that UCBT using the optimal conditioning regimen can be a salvage treatment for patients without a suitable bone marrow donor.[12,28]

What to expect in future

Improving engraftment, reconstitution, and procurement

Delayed or failed engraftment due to low cell dose represents the main restriction of cord blood transplants. To overcome this obstacle, several strategies have been developed, including transplantations with double cord blood units,[29,30] using nonmyeloablative conditioning,[31] intrabone marrow injection (IBMI) of UCB graft,[32] and *ex vivo* expansion of UCB-derived stem cells.[33] To enhance immune reconstitution, *ex vivo* expansion of common lymphocyte progenitors, reduction of pharmacological immunosuppression and adoptive transfer of pathogen specific cytotoxic T lymphocytes have been suggested.[34] To reduce potential risks of infectious or genetic disease transmission, cord blood collection requires screening of

maternal blood for infectious diseases and review of a detailed maternal and family medical history. Donors at high risk for infectious disease transmission or with first-degree relatives with cancer/a blood disorder/immunodeficiency should be excluded. In addition, some obstetrical factors including duration of labor, fetal distress, fetal gender, delivery methods, and birth weight of new born and gestational age were suggested to influence the content of UCB and should be taken into consideration during UCB stem cell application.[35]

Prenatal diagnosis and earlier therapy

Prenatal diagnosis has allowed the early detection of fetal diseases before child birth. Therefore, *in utero* stem cell transplantation is considerable in treating fetal diseases at early stage before extensive damage to various tissues and organs ensues prenatally. Furthermore, fetus is immunologically naïve during early gestation and more acceptable to foreign antigen as compared to post-natal transplantation. Also, during second trimester of gestation, fetal bone marrow is still relatively empty. Hence, it is suitable for the homing of stem cell population without the need of marrow ablation prior to transplantation. Our studies on animal experiments showed that stem cells derived from UCB could be potential candidates for applications in *in utero* transplantation.[36] The fetal sheep model could be used to study *in utero* stem cell transplantation because of its similarity to human fetus in its scale and immunocompetence development relative to gestational age, as well as its relatively longer gestation period.[37] A minimally invasive technique using percutaneous ultrasound for *in utero* transplantation of UCB progenitor cells suggested by our group has significantly reduced the fetal loss rate normally caused by conventional surgical transplantation techniques.[38] However, *in utero* transplantation is still unable to provide significant levels of engraftment and clinical applications. To improve the engraftment level of transplantation, the mechanisms and factors involved in stem cell homing to its destination is an important field to further explore in future. Recently, noninvasive prenatal diagnosis (NIPD) based on the fetal genetic materials in maternal circulation derived from placenta and fetus offers a risk-free tool applicable for *in utero* stem cell transplantation. The NIPD technology is also useful for early identification of suitable UCB before delivery allowing a reduction of costs for the collection, storage and characterization of specimen.

New uses of UCB cells and novel technologies for wider spectrum of human diseases

Lymphoid effectors cells and non-HSCs, such as mesenchymal stem cells and stromal progenitors are being explored to expand the transplantation indications. UCB-derived mesenchymal stem cells could be isolated by *in vitro* study and applied to *in vivo* therapy on animals.[37]

Novel technologies, such as tissue engineering and gene therapy can be explored to improve the UCBT. Some studies have showed the availability of using fetal cells, which can be obtained from extra-embryonic structures and maternal blood, to explore applications from tissue engineering.[39]

Patients with specific disorders, such as PIDs, who lack a suitable HLA-matched donor, gene therapy is likely to be an alterative option. HSCs are attractive targets for gene therapy owing to their ability to produce progeny cells with lifelong therapeutic gene. Currently, gene therapy involves *ex vivo* retroviral transduction of HSCs followed by an autologous transplantation and *in vivo* therapy by transferring the gene-containing-vector directly to recipients. HSCs derived from UCB may be an alterative source for developing gene therapy in future because of the features of the specimen.[40]

Conclusion

Stem cells from UCB are promising in the treatment of various diseases. However, many areas remain to be explored especially regarding the immunological aspects after transplantation, optimal period and conditions for successful engraftment, transplantation in immunologically privileged fetus, optimal *ex vivo* expansion and application of the current research in *in vivo* animal models, with the ultimate aim of future application in clinical studies.

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Conflicts of interest

The authors declare no conflicts of interest.

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