PERSPECTIVE

Genetically modified human umbilical cord blood cells as a promising strategy for treatment of spinal cord injury

Spinal cord injury (SCI) continues to be a pressing health and social problem. The injury leads to neuronal and glial cell death accompanied by degeneration of nerve fibers. There are currently no particularly effective treatments. SCI causes profound disability of people affected and has attracted increased attention in the international field of neuroregeneration. For the past two decades, much hope has been placed in cell therapies for the restoration of both structure and function of the injured spinal cord. Embryonic and neural stem cells, olfactory ensheathing cells, microglia-like cells, Schwann cells, mesenchymal stem cells, human umbilical cord blood cells (UCBCs) and many other cell types have been investigated (Xu and Onifer, 2009; Ronaghi et al., 2010; Sabapathy et al., 2015). It has been shown that graft cells support tissue sparing, exert a trophic effect on neurons and glia, promote axonal growth, and participate in their remyelination.

The current literature suggests that after SCI, cell therapy is effective at promoting neuroregeneration. However, the optimal cell type for transplantation after neurotrauma has not been determined due to differences in experimental conditions, such as localization (cervical, thoracic, lumbar), injury type (contusion, compression, hemisection, complete transection, selective shutdown tracts, *etc.*), the severity of the injury, methods of cultivation and preparation of cells for transplantation, time of administration after SCI, site of cell injection (site of injury, intrathecal or intravenous), the presence or absence of immunosuppression, and many other factors. It appears that researchers are guided by the general criteria when choosing a cell intended for transplantation, such as oncogenic and infectious safety, low invasiveness of the fence material, which is important for clinical use. The main limitations of stem and progenitor cell transplants in SCI are their poor survival rates and uncontrolled differentiation. Globally clinical trials suggest that the use of autologous cells, especially cells of mesenchymal origin, have a high likelihood of being effective in treatment of neurotrauma. In addition to fitting the criteria for safety and invasiveness, transplantation studies in model systems and the results of clinical trials collectively suggest that hUCBCs are very suitable for cell therapy and warrant further research into their use for stimulation of regeneration of SCI.

Previously, our group, and others, have demonstrated that hUCBCs possess many of the properties that allow them to overcome many of the major hurdles of SCI regeneration (**Figure 1**). It has been demonstrated that transplantation of hUCBCs in SCI depresses the inflammatory response, provides a neurotrophic influence and stimulates neovascularization (Chen et al., 2008). Injection of hUCBCs into the bloodstream after SCI reduces the expression of pro-apoptotic genes and promotes neuronal survival (Dasari et al., 2009). At 6 weeks after local transplantation of hUCBCs into the area of SCI, Chua et al reported a significant improvement in behavioral recovery (BBB rating scale) with increased levels of cytokines and growth factors with neuroprotective, angiogenic and anti-inflammatory actions (Chua et al., 2010). Using a model of SCI in dogs, Lee at al. (2011) demonstrated the ability of hUCBCs to enhance remyelination by formation of peripheral type myelin sheaths. In this study, axons were remyelinated by P0-positive Schwann cells with functional recovery being maintained for three years after the cell transplantation. Recently, our group demonstrated that transplantation of human umbilical cord blood mononuclear cells (hUCB-MCs) reduced cavity volume and tissue retention at the lesion site (both white and gray matter) (Mukhamedshina et al., 2016).

It should be noted that clinical trials are being conducted with hUCBCs. Kang et al. have shown that transplantation of MSCs from hUCBCs (UCMSCs) into a 37-year-old female with SCI improved both functional recovery and sensory perception (Kang et al., 2005). Transplantation UCMSCs in combination with $CD34^+$ hUCBCs into a 29-year-old male with an L1 SCI resulted in recovery of muscle, bowel, and sexual function along with a decrease in ASIA score from "A" to "D" (Ichim et al., 2010).

For cell therapy to be more effective in stimulation of neuroregeneration and restoration of lost functions of the spinal cord, there is a need for both the continued presence of high levels of transplanted cells in the damaged tissue and the ability of cells to readily home to the injured area. It has been shown that hUCBCs have high survival rates and strong migration potential both by intravenous and intraspinal administration (Garbuzova-Davis et al., 2003; Mukhamedshina et al., 2015).

Despite many important advances in cell transplantation research for the treatment of SCI, it has become clear that standalone cell therapy leads to only a modest improvement in structural parameters and to insufficient restoration of function. Therefore, genetically modified cells with enhanced expression of therapeutic genes (*e.g.* genes for neurotrophic factors genes) have attracted the attention of researchers as potential stimulators of posttraumatic spinal cord regeneration. Simultaneous delivery into the damaged tissues of several neurotrophic factors appears to be the most effective, with co-delivery of neurotrophic and angiogenic factors being the most efficient at stimulating neuroregeneration. As example is the combination of vascular endothelial growth factor (VEGF) and glial-derived neurotrophic factor (GDNF) genes. Until recently the use of such gene combinations had not been investigated.

Gene delivery into cells using viral vectors is currently the leading method of therapy directed to CNS disorders. As a gene delivery system we used adenoviral vectors since they provide high titers of the recombinant virus, efficiently transfer genes into dividing and non-dividing cells, do not integrate into the genome, and have high expression of introduced genes. However, adenoviruses have the potential to induce nonspecific inflammation and cellular immune responses, which reduces the duration of transgene expression to weeks or months. However, other viral vectors also have disadvantages such as transient expression of transgenes (herpes virus), transduction of dividing cells only (retrovirus), small insert size for transgene constructs (adeno-associated virus) and doubts about safety (lentivirus). Therefore, the search for the most efficient and safe gene delivery system is still ongoing.

We investigated the possibility of strengthening posttraumatic spinal cord regeneration by transduction of hUCB-MCs with adenoviral vectors encoding genes for neurotrophic and angiogenic factors such as VEGF and GDNF (hUCB-MCs + Ad5-VEGF + Ad5-GDNF) (Mukhamedshina et al., 2016). Our results indicate that hUCB-MCs administered directly into the lesion site (immediately following SCI) survived 30 days after injection providing the possibility of expressing products of recombinant therapeutic genes for this therapeutic period, which completely overlaps the window during which secondary damage responses rapidly unfold. Examples of such responses include increased levels of proinflammatory cytokines, oxidative stress and excitotoxicity, all of which lead to neural cell death and axonal degeneration. The duration of grafted cell survival in the area of SCI in our experiments is consistent with similar work using transplanted mesenchymal precursors. It should be noted that these positive findings were obtained in the absence of immunosuppression. It is anticipated that further studies will demonstrate longer persistence in the SCI area of grafted hUCB-MCs that are capable of continual expression of therapeutic genes. Further, auto- or paracrine signaling could support the viability of the transplanted cells and/or their proliferation extending the survival of transduced hUCB-MCs in rat spinal cord.

Transplantation of hUCB-MCs transduced with adenoviral

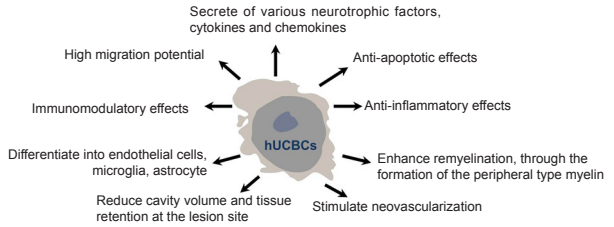

Figure 1 Human umbilical cord blood cells (hUCBCs) possess many properties which allow them to overcome the hurdles of regeneration in spinal cord injury.

vectors expressing VEGF and GDNF at the site of SCI induced behavioral recovery that correlated with tissue sparing. The efficiency of hUCB-MCs + Ad5-VEGF + Ad5-GDNF transplantation into the area of SCI was higher than transplanted hUCB-MCs transduced with adenoviral vectors expressing a non-therapeutic gene (enhanced green fluorescent protein, EGFP) for both pathological cavitation and gray/white matter sparing. These results can be explained by the action of therapeutic gene products in targeting cells in the damaged tissue. An increase in the number of regenerating fibers in the white matter of the spinal cord as a result of gene therapy (unpublished data) could result from the direct effect of neurotrophic factors produced by the grafted cells on oligodendrocytes and remyelination. Amplification of myelinated fiber regeneration may also be a consequence of reducing the volume of cavities and increased white matter sparing.

We have demonstrated that transplantation of hUCB-MCs transduced with adenoviral vectors expressing VEGF and GDNF genes into the site of SCI reduce glial scar formation and induce prominent axonal sparing/regeneration in comparison to the other constructs tested. Thirty days after SCI and transplantation of genetically modified VEGF and GDNF genes in hUCB-MCs, it was observed that growth associated protein 43 positive ($GAP43^{+}$) fibers in the lesion zone had no detectable glial fibrillary acidic protein (GFAP) and grew through small and medium-sized cystic cavities (**Figure 2A, 2A'**) While in the group with transplanted hUCB-MCs transduced with adenoviral vectors expressing a non-therapeutic gene (hUCB-MCs + Ad5-EGFP), GAP43 expression was located in the islet of lesion zone and also distal to the lesion zone (**Figure 2B, 2B"**). Astroglial scars are known to inhibit the regeneration of nerve fibers. We hypothesize that decreased synthesis of chondroitin sulphate proteoglycans due to reductions in glial scar tissue contributed to sprouting nerve fibers after transplantation of the genetically modified hUCB-MCs.

Thus, the transplantation of hUCB-MCs transduced with adenoviral vectors expressing VEGF and GDNF genes after SCI supports functional tissue plasticity and its ability to regenerate. This approach permits the delivery into the damaged area of neurotrophic and angiogenic factors, which reduce the severity of retrograde axonal degeneration, support tissue reconstruction including remyelination, and elongation and collateral branching of axons to form synapses.

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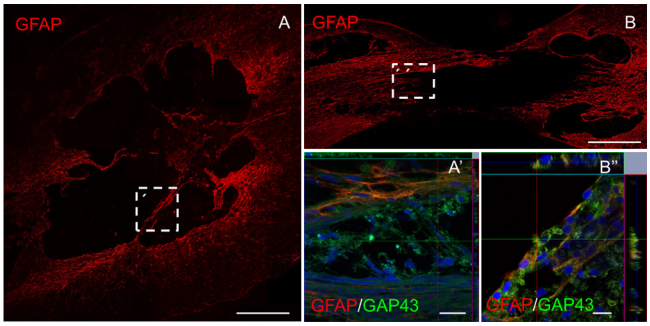

Figure 2 Expression of GFAP and GAP43 in the lesion site of spinal cord in experimental groups.
Panoramic view of glial scar formation at the lesion site using GFAP (red) in experimental groups with transplantation of hUCB-MCs transduced with adenoviral vectors expressing VEGF and GDNF genes after SCI (A, A') and transplantation of hUCB-MCs transduced with adenoviral vector expressing EGFP (B, B"). The dashed boxes indicate enlarged areas of A' and B". (A') $GAP43^{+}$ fibers (green) grew through small and medium-sized cystic cavities. (B") GAP43 expression was located in the islet of lesion zone, surrounded by glial scar (GFAP). Nuclei are stained with DAPI (blue). Scale bars: 750 μm (A), 500 μm (B) and 20 μm (A', B"). Figure 2A, B from Mukhamedshina et al., 2016. hUCB-MCs: Human umbilical cord blood mononuclear cells; GFAP: glial fibrillary acidic protein; GAP43: growth associated protein 43; SCI: spinal cord injury; VEGF: vascular endothelial growth factor; GDNF: glial-derived neurotrophic factor.

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References

Chen CT, Foo NH, Liu WS, Chen SH (2008) Infusion of human umbilical cord blood cells ameliorates hind limb dysfunction in experimental spinal cord injury through anti–inflammatory, vasculogenic and neurotrophic mechanisms. Pediatr Neonatol 49:77-83.

Chua SJ, Bielecki R, Yamanaka N, Fehlings MG, Rogers IM, Casper RF (2010) The effect of umbilical cord blood cells on outcomes after experimental traumatic spinal cord injury. Spine 35:1520-1526.

Dasari VR, Veeravalli KK, Tsung AJ, Gondi CS, Gujrati M, Dinh DH, Rao JS (2009) Neuronal apoptosis is inhibited by cord blood stem cells after spinal cord injury. J Neurotrauma 26:2057-2069.

Garbuzova-Davis S, Willing AE, Zigova T, Saporta S, Justen EB, Lane JC, Hudson JE, Chen N, Davis CD, Sanberg PR (2003) Intravenous administration of human umbilical cord blood cells in a mouse model of amyotrophic lateral sclerosis: distribution, migration, and differentiation. J Hematother Stem Cell Res 12:255-270.

Ichim TE, Solano F, Lara F, Paris E, Ugalde F, Rodriguez JP, Minev B, Bogin V, Ramos F, Woods EJ, Murphy MP, Patel AN, Harman RJ, Riordan NH (2010) Feasibility of combination allogeneic stemcell therapy for spinal cord injury: a case report. Int Arch Med 3:30.

Kang KS, Kim SW, Ohet YH, Yu JW, Kim KY, Park HK, Song CH, Han H (2005) A37-year-old spinal cord injured female patient, transplanted of multipotent stem cells from human UC blood, with improved sensory perception and mobility, both functionally and morphologically: a case study. Cytotherapy 7:368-373.

Lee JH, Chung WH, Kang EH, Chung DJ, Choi CB, Chang HS, Lee JH, Hwang SH, Han H, Choe BY, Kim HY (2011) Schwann cell-like remyelination following transplantation of human umbilical cord blood (hUCB)-derived mesenchymal stem cells in dogs with acute spinal cord injury. J Neurol Sci 300:86-96.

Mukhamedshina YO, Shaymardanova GF, Garanina EE, Salafutdinov II, Rizvanov AA, Islamov RR, Chelyshev YA (2015) Adenoviral vector carrying glial cell-derived neurotrophic factor for direct gene therapy in comparison with human umbilical cord blood cell-mediated therapy of spinal cord injury in rat. Spinal Cord 54:347-359.

Mukhamedshina YO, Garanina EE, Masgutova GA, Galieva LR, Sanatova ER, Chelyshev YA, Rizvanov AA (2016) Assessment of glial scar, tissue sparing, behavioral recovery and axonal regeneration following acute transplantation of genetically modified human umbilical cord blood cells in a rat model of spinal cord contusion. PLoS One 11:e0151745.

Ronaghi M, Erceg S, Moreno-Manzano V, Stojkovic M (2010) Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? Stem cells 28:93-99.

Sabapathy V, Tharion G, Kumar S (2015) Cell therapy augments functional recovery subsequent to spinal cord injury under experimental conditions. Stem Cells Int 2015:132172.

Xu XM, Onifer SM (2009) Transplantation-mediated strategies to promote axonal regeneration following spinal cord injury. Respir Physiol Neurobiol 169:171-182.