Chapter 6

Discussion and Concluding Remarks

Spinal cord injuries result in permanent anatomical and functional damage which are so far untreatable. The present thesis aimed to enhance our understanding of BMSC therapy for spinal cord injury and to investigate approaches to increase the therapeutic efficacy of BMSC transplants for spinal cord repair. Using an adult rat model of spinal cord contusion, which is clinically the most relevant type of spinal cord injury, the efficacy of BMSCs to repair the contused spinal cord was studied. The results confirmed and further expanded previous data demonstrating that a BMSC transplant results in neuroprotection (i.e., tissue sparing) and improved motor, sensorimotor, ands sensory function recovery. Most functional improvements were strongly correlated with the neuroprotective effects, which included sparing of descending raphespinal axons from the brainstem. Moreover, increased blood vessel density at the injury epicenter was identified as a potential mediator of BMSC-mediated tissue sparing. Although BDNF was not found to be a necessary factor in BMSC-mediated tissue sparing, genetically modifying BMSCs to hypersecrete BDNF were found to further increase the neuroprotective effects. Importantly, increasing BMSC transplant survival using ESHU, a reverse thermal gel with anti-oxidant abilities, was found to augment the effects of a BMSC transplant on anatomical and functional repair of the contused spinal cord.

PARACRINE FUNCTIONS OF BMSCs

The efficacy of BMSCs is thought to be due to the paracrine actions of secreted factors. BSMCs secrete a variety of growth factors and cytokines that can be grouped into three repair-promoting categories. The first group is comprised of factors that affects blood vessels. Among these factors are vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF) and angiopoietin-1 (ANG-1).[1,2] We found increased blood vessel density at a contusion site after BMSC transplantation (Chapter 2) suggesting that BMSC-mediated angiogenesis is involved in its neuroprotective actions. Further studies need to elucidate which of the abovementioned BMSC-derived factors play a role in the formation, repair, or sparing of blood vessels near an injury site. One possible mechanism by which BMSC transplants may elicit vascular repair is by secretion of Ang-1 which is known to stabilize blood vessels thereby decreasing their permeability.

The second group consists of factors that affect cell survival. This group includes BDNF, glial-derived neurotrophic factor (GDNF), nerve growth factor (NGF) and ß-fibroblast growth factor (ß-FGF).[3,4] We found that BDNF is not a necessary factor for BMSC-mediated neuroprotection but the therapeutic efficacy of BMSC transplants can be enhanced using BDNF-hypersecreting BMSCs (Chapter 3). A specific repair-related event may be affected by many different trophic factors as was confirmed in Chapter 3. Conversely, a particular trophic factor may affect multiple events. It is important to acquire thorough understanding of the role(s) of a particular repair-supporting factor, including the benefits and detriments, before approaches can be developed to enhance BMSC-based spinal cord repair.

The third group of repair-supporting factors secreted by BMSC affect the immune response. This group includes interleukin-10 (IL-10) and transforming growth factor ß-1 (TGF-ß1). The inflammatory response plays a dual role in spinal cord injury. After the initial impact, a massive influx and proliferation of macrophages is evident. These macrophages are needed to clear cellular debris and reorganize tissue at the injury site. In doing so, they secrete molecules that increase oxidative stress and exacerbate secondary tissue degeneration. Currently, the differential role of M1 macrophages, with mainly deteriorating effects, and M2 macrophages, with predominantly beneficial effects, are being investigated. Within the setting of this complex inflammatory response, the role of immune modulatory factors secreted by BMSCs remains to be elucidated.

FACTORS DETERMING OUTCOME

Interestingly, investigations of BMSC transplants in spinal cord injury have led to different and at times conflicting conclusions. Many groups, but not all, have reported anatomical or functional improvements after BMSC transplantation in the injured spinal cord. Some groups report BMSC-mediated effects on axonal regeneration in the injured spinal cord. Many different aspects can influence the effects of BMSCs on repair in models of spinal cord injury. Firstly, the age of BMSCs affects their genetic expression profile, including expression of genes involved in neural repair. Previously, we characterized the gene

expression profiles of BMSCs that were passaged three (P3) or fourteen (P14) times and revealed a decrease in plasticity and repair aptitude of long-term cultured BMSCs .[5] In addition, the age of the rat from which the BMSCs are harvested affect BMSC plasticity and their proliferative life span. BMSCs from younger rats have higher telomerase activity and higher expression of Sox-2 and Nanog, increasing their proliferative life span and cell plasticity, respectively.[6.7] Also, human bone marrow stromal cells exhibit donor variations in secretion patterns of growth factors and cytokines, affecting axons growth and functional recovery in rat spinal cord injury.[8] Clearly, determination and standardization of the optimal BMSC age and donor lot is necessary to validly compare studies and move forward with BMSC therapy research.

Another major factor determining outcome after BMSC transplantation is the model system used. The strain and gender of the rats used affect the immune response to the transplanted BMSCs. In the present thesis, female rats were used because their short urethra makes manual bladder expression more practical, and their more gentile temperament makes handling easier. Sprague Dawley rats were used, which is an outbred strain, resulting in greater surgery survival rates and less complications. Allogeneic transplantations in Sprague Dawley rats could possibly result in different immune responses than using more inbred strains, thus allowing for more syngeneic transplantations. Different injury devices, injury types and injury levels used result in different baseline functional deficits. Future research needs to determine which types of injury model best predicts functional recovery in humans, and the site, dose and timing of BMSC injection influence cell survival and cell dynamics. Differences in any of these factors can impact the observed outcome. A golden standard model system for testing cellular transplants for spinal cord injury does not exist and further research is needed to determine the true therapeutic efficacy of BMSCs. On the other hand, there is a high degree of variation between humans and having differences between models of spinal cord injury may in fact support our understanding of the potential of BMSC-based spinal cord repair.

Although the rat contusion model of spinal cord injury shows considerable anatomical similarities to human spinal cord injury, and is generally considered to be a suitable model system, there are limitations that affect the interpretation of the repair effects of BMSCs, especially in light of their potential for human spinal cord repair. Firstly, rats show some degree of functional recovery even in the complete absence of supraspinal input, likely due to the presence of a locomotor central pattern generator (CPG) in the lumbar spinal cord segments. Within days after injury, rats will start to show hindlimb joint movements, followed by stepping movements, and, depending on the severity of the injury, weight supported stepping. Reorganization of the CPG is believed to underlie this functional recovery. Humans do not seem to reorganize their lumbar spinal neurons in a way that leads to functional recovery, even though a lumbar CPG is present. Rats are quadrupeds and following awakening from anesthesia after a spinal cord injury, they begin to move around using their forelimbs while dragging their hind limbs. This constant sensory input to the hind limbs is believed to positively affect functional recovery. Indeed, a recent study shows that hind limb immobilization and hind limb stretching therapy in rats hinders the functional recovery of spinal cord injured rats.[9] Treatments tested in rats that improve functional repair, might do so by positively affecting spinal cord reorganization below the level of injury. Humans might be more dependent on supraspinal input for effective functional recovery. The widely used BBB locomotor recovery scale used in rats, also used in this thesis, may not adequately reflect these differences. In the 21-point BBB scale small changes in tissue can be correlated to changes on the scale. In humans, no scale exists in which the extent of tissue damage/sparing can be correlated with a functional rating scale. Although it seems plausible that neuroprotective interventions that are so closely correlated to functional recovery in rats would also be beneficial for humans, no such evidence exists to date.

Understanding the factors underlying the observed differences in recovery between rats and humans as well as gaining insight in the mechanisms of action of proposed treatments will help us predict which (combination of) therapies may restore function in humans. Conversily, data from the few spinal cord injured patients injected with cellular transplants

so far, both from the discontinued Geron trial, as well as from the ongoing StemCell trial may provide us with insights regarding the questions we need to focus on in the laboratory. However, caution is warranted when efforts to translate therapies into the clinic are taken too prematurely, since lack of efficacy in unfully understood treatments, might unduely discourage patients, the scientific community as well as funding agencies, and decrease the progress of basic research.

COMBINATION STRATEGIES

Clearly, BMSCs or any stem cell by itself will not provide the 'silver bullet' for restoring functional repair after spinal cord injury. The neuroprotective properties of these cells will have to be combined with regenerative and rehabilitative strategies to regain function after paralysis. One recent study showing the additive strength of combination strategies was by Van Den Brand et al[10]. Monoamine agonist, epidural electrical stimulation and neurorehabilitation within a robotic harness were successfully combined to considerably improve walking after spinal cord injury in a rat model.

CONCLUSION

BMSC transplantation is a promising cell-based strategy to promote repair of the injured spinal cord. The knowledge we gain from studying BMSC transplants within spinal cord injury models, provide valuable insights into cell-based treatments for central nervous system disorders that can one day be translated into the clinic providing treatments to improve the quality of life of spinal cord injured patients.

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