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Bone marrow stromal cell : mediated neuroprotection for spinal cord repair

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Chapter 7

Summary

INTRODUCTION

A spinal cord injury leads to loss of neural cells and interruption of axonal tracts, resulting in partial or complete motor, sensory, and autonomous dysfunction below the level of injury. The most prevalent causes of traumatic spinal cord injuries include traffic accidents, sport injuries, and violence. Non-traumatic causes include tumor compression and infections. The initial impact results in immediate death of neurons and glial cells, and damage to axons and blood vessels. The impact further sets off a series of pathophysiological events causing progressive secondary damage which continues for days to months. Contributing events to secondary damage are inflammatory cells and release of cytotoxic molecules. The presence of growth-inhibitory molecules in scar tissue at the injury site and the gradual formation of a fluid-filled cyst contribute to a hostile environment for repair-supporting events including axonal regeneration. Currently, there is no therapy available that effectively restores the damaged axonal tracts, so that most people with spinal cord injury suffer permanent paralysis.

STEM CELL THERAPY

Chapter 1 provides a general introduction on stem cells for repair of the central nervous system. Different types of stem cells can be used for repair of the central nervous system; each type with its specific advantages and disadvantages. Embryonic and fetal stem cells have the advantage of the potential to differentiate into almost any cell type and can potentially replace damaged and lost cells in the nervous system. Disadvantages of these cells include ethical considerations regarding cell harvest and the risk of tumor formation as a result of uninhibited proliferation. Adult stem cells are less tumorigenic but also have less differentiation capacity. Stem cells can exert their beneficial effect by secreting growth factors with protective and/or proliferative effects on neural tissue. One easy to harvest adult stem cell is the bone marrow-derived mesenchymal stem cell, also called bone marrow stromal cell (BMSC). Part two of the introduction specifically focuses on these BMSCs as a therapy for spinal cord repair. BMSCs are relatively easy to harvest from adult bone marrow and can be cultured quickly in defined growth medium. They secrete

various growth factors which include neurotrophic factors and have a protective effect on neural tissue after transplantation into the injured adult rat spinal cord. This thesis has two main goals: (1) to expand our knowledge of BMSC therapy for spinal cord repair, and (2) to investigate approaches to enhance the therapeutic efficacy of intraspinal BMSC transplants. The experiments in this thesis are conducted using an adult rat model of spinal cord injury involving a T9 laminectomy and subsequent contusion of the exposed spinal cord using an automated impactor which results in hindlimb and tail paralysis. Anatomically, the impact results in loss of neurons and glia cells, damage to axons and blood vessel, and an influx of inflammatory cells which contribute to progressive secondary tissue loss. This model mimics the consequences of spinal cord injury in humans.

BMSC-MEDIATED NEUROPROTECTION AND REPAIR

Chapter 2 examined whether BMSC-mediated neuroprotection enhances motor and sensory function recovery after transplantation into an adult rat model of spinal cord contusion. The relationship between BMSC-mediated tissue sparing and different aspects of functional recovery were investigated. The results showed that BMSC transplantation positively affects different parameters for (sensori)motor function (BBB subscore, foot print analysis, horizontal ladder walking) and sensation (thermal hyperalgesia and mechanical allodynia) and that these effects are correlated with the volume of spared nervous tissue in the contused segment. In addition, we found that the BMSC-transplanted segment contained more blood vessels than control (not-transplanted) rats, which may contribute to the increased volumes of spared tissue. We also found that rats with BMSCs contained more axons originating from the raphe nuclei in the brain stem, which may contribute to the observed improvements in motor recovery.

THE ROLE OF BDNF IN BMSC-MEDIATED NEUROPROTECTION

In **chapter 3** we studied the role of BDNF, one of the growth factors secreted by BMSCs, in BMSC-mediated neuroprotection by increasing and decreasing the expression of BDNF in

BMSCs through lentiviral transduction. *In vitro*, BDNF was shown not to be a necessary factor for the observed protective effect on spinal motoneurons. BMSCs with silenced BDNF production had a similar beneficial effect on neural survival as control BMSCs, possibly due to compensatory effects of other secreted trophic factors. BMSCs overexpressing BDNF resulted in an increase in motoneuron survival that was not seen with control BMSCs or with BMSCs with silenced BDNF production. *In vivo*, this beneficial effect of BDNF-overexpressing BMSCs was confirmed; more motoneurons survived in the spinal cord after transplantation of BDNF-hypersecreting BMSCs, compared to unmodified BMSCs or BMSCs with silenced BDNF production. Rats that received BDNF-hypersecreting BMSCs were found to have a higher density of grey matter blood vessels, which could have been a mediating factor in the improved motoneuron survival. In addition, we found BDNF to be a necessary factor for BMSC survival *in vivo*. BMSCs with silenced BDNF production did not survive the first week of transplantation, which may explain the lack of tissue sparing in these rats.

BMSC SURVIVAL

Survival of BMSCs in the damaged spinal cord is poor and limits their repair efficacy. One week after transplantation, about twenty percent of the transplanted cells survive. Different factors contribute to this poor BMSC survival, including phagocytosis by macrophages, lack of oxygen and nutrients by ruptured blood vessels, and the presence of reactive oxygen species and other cytotoxic molecules at the injury site. In **chapter 4** we investigated whether BMSC survival could be improved by suppressing the inflammatory response. Three clinically used anti-inflammatory drugs, Minocycline, Methylprednisolone, and Cyclosporine were tested for their ability to suppress the number of activated macrophages in the injured spinal cord and thereby increase BMSC survival. All three drugs were effective in decreasing the macrophage response, but this did not improve transplanted BMSC survival.

In **Chapter 5** we investigated whether BMSC survival could be improved by transplanting the cells in the reverse thermal gel, poly(ethylene glycol) -poly(serinol hexamethylene

urethane), or ESHU, which has anti-oxidative properties. We showed that BMSCs survived the first week of transplantation better when transplanted in ESHU and that this improved survival was associated with increased tissue sparing and improved motor and sensorimotor function recovery. A likely contributor to the improved BMSC survival is the anti-oxidative ability of the poly-urethane group of ESHU. This antioxidant effect was confirmed *in vitro*.

CONCLUSION

BSMC transplantation has beneficial anatomical effects associated with improved motor, sensorimotor, and sensory function recovery in a rat model of spinal cord contusion. Some of these effects can be further enhanced by overexpressing BDNF in the transplanted BMSCs. Short term BMSC survival can be improved by transplanting the cells in ESHU and this leads to increased tissue sparing and functional improvement, indicating that survival is a determinant in the therapeutic efficacy of BMSC transplants. Future research will need to focus on combinations of neuroprotective BMSC transplants with axonal regenerating promoting therapies to further optimize BMSC-based therapy for spinal cord injury.

