

New insights into regeneration of intervertebral disc and spinal cord

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INTRODUCTION: The intervertebral disc (IVD) and the spinal cord are the two major components of the spinal column. Various medical problems such as degenerative disease to high-energy trauma induce alteration of function and organ structure. While the bone elements can be reconstructed using various bone grafts and tissue engineering techniques, biological regeneration of IVD and spinal cord has not yet been achieved. In this lecture, 2 studies will be presented assessing the possibility of utilizing intrinsic cells for regeneration of these organs.

METHODS:

Study 1. Can bone marrow mesenchymal cells be recruited for regeneration of IVD? IVD degeneration was applied to mice whose BM had been replaced with BM cells from green fluorescent protein (GFP)-transgenic mice. Histological and immunohistochemical analyses were performed in 3 groups: Group D: degeneration, Group R: regeneration and Group C: control vehicle.

Study 2. Mobilization and recruitment of intrinsic cells from bone marrow for spinal cord regeneration. Spinal cord injury was applied at Th10 level by a static load (25g, 5min) in mice whose BM had been replaced with BM cells from GFP-transgenic mice. Injured mice were separated into different 4 groups (Group A: Combination of SCF and G-CSF, Group B: SCF alone, Group C: G-CSF alone, Group D: PBS). G-CSF (300µg/kg/day) and SCF (100µg/kg/day) were subcutaneously injected in subacute phase (days 11 to 20). BrdU injections were performed to assess cell proliferation in peri-injured area. Neural markers were evaluated 4 weeks after injury and hind limb locomotor using BBB Scale was analyzed until 12 weeks after injury.

RESULTS&DISCUSSION:

Study 1. Histologically, severe degeneration was observed in Group D and moderate degeneration was observed in Group R. GFP+ cells were detected in end plate and annular/endplate junction in Group D and R, while no GFP+ cells were detected in Group C. GFP+ cells were also seen in nucleus pulposus in Groups D and R, but were few. Immunofluorescent analysis revealed that these GFP+ cells did not co-stain with

hematopoietic markers; CD34, CD45 or Mac1, but co-stained with keratan sulfate. These findings suggest that induction of degeneration and sequential regeneration induces mesenchymal cells from the bone marrow to regenerate IVD structure. **Study 2.** No significant difference between groups was seen in GFP+ cells. The result showed that these cytokines were not effective for mobilization of BM-derived cells to injured spinal cord. Most of GFP+ cells were CD45+ and F4/80+ activated macrophage. Small portion of GFP+ cells with treatment were NG2+, indicating that they were oligodendrocyte progenitor cells (OPCs). However, differentiation of BM-derived cells to astrocyte and neuronal cells were not detected. The number of GFP- F4/80+ cells was significantly increased in Group A and most of them were negative for BrdU suggesting combined cytokine treatment was effective to recruit activated microglia to injured area from peri-injured area but not to proliferate the microglia in injured center. The number of GFP-NG2+ BrdU+ cells significantly increased in Group A which also demonstrated GFP- GSTy π + BrdU+ cells. These findings suggest that intrinsic OPCs were actively proliferating and some of them differentiated to mature oligodendrocyte. Locomotor of hind limb showed significant recovery after 6 weeks in combination group.

CONCLUSIONS:

Results of the current study demonstrate that intrinsic cells mobilize to the degenerated or injured site possibly to promote regeneration in IVD and spinal cord. These findings are informative in finding out the involvement of intrinsic stem cell niche in control of homeostasis of IVD and spinal cord. Modulation of these cells combined with growth factor treatment may be useful in IVD and spinal cord regeneration.

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