



● PERSPECTIVE

Fortuitous benefits of activity-based rehabilitation in stem cell-based therapy for spinal cord repair: enhancing graft survival

Traumatic injuries to spinal cord elicit diverse signaling pathways leading to unselective and complex pathological outcomes: death of multiple classes of neural cells, formation of cystic cavities and glial scars, disruption of axonal connections, and demyelination of spared axons, all of which can contribute more or less to debilitating functional impairments found in patients with spinal cord injury. The multitude of pathobiological processes involved in spinal cord trauma may make it highly challenging to develop a clinically meaningful therapeutic approach targeting only a specific molecule or signaling pathway. A hopeful alternative might be a cell therapy, especially a transplantation approach using neural stem cells (NSC) with a clear potential to differentiate into various neural cell types. Provision of NSCs with capacity to differentiate into mature neural cells can ideally replace lost segmental neurons and dying oligodendrocytes around surviving axons. Furthermore, NSCs secrete various growth factors that provide protective or pro-regenerative effects. It has been also demonstrated that NSCs can exert powerful modulatory effects on immune cells ameliorating secondary degenerative processes.

The basic premise for successful stem cell-based therapy would be a good extent of survival of NSC grafts. Survival of transplanted NSCs would be particularly critical when attempting to replace lost neural cells. In the authors' opinion, the issue of graft survival has been underestimated partly because of a frequent use of genetically immune-compromised animals as hosts for NSC grafts (for example SCID mice or athymic rats). Complete avoidance of immunological interactions between host and grafts would be unimaginable in clinical situations, and there was a report that even induced pluripotent stem cells derived from the same host animal can evoke some degree of inflammatory reactions (Zhao et al., 2011). Therefore, designing strategies to enhance survival of NSC grafts should be considered as a prerequisite for the successful translation of stem cell-based transplantation therapy.

We have recently reported that treadmill locomotor training (TMT), which is routinely prescribed for patients with paraplegia, substantially enhances survival of grafted NSCs in a rat spinal cord injury model (Hwang et al., 2014). In our model where rat NSCs are transplanted into rat spinal cord (allograft), a majority of grafted stem cells disappear within several days after transplantation. This is consistent with the previous studies showing that

more than 90% transplanted NSCs die within several days in injured CNS (Okada et al., 2005; Nakagomi et al., 2009). When a group of animals with NSC grafts were subjected to intensive TMT (three sessions per day with each lasting 20 minutes and 6 days per week), they showed three times and five times higher number of surviving NSCs at 3 and 9 weeks after injury, respectively. We also found that the markers of cellular stresses related to reactive oxygen or nitrogen species were substantially attenuated in grafted NSCs by TMT. Importantly, the increase in the number of surviving NSCs was significantly correlated with the degree of behavioral improvement in the same group of animals. What is the molecular mechanism of the TMT-induced enhancement of NSC graft survival? It has been shown that exercise increases peripheral production of insulin-like growth factor-1 (IGF-1) and the IGF-1 can be delivered to the CNS through the blood-cerebrospinal fluid (CSF) barrier (Fernandez and Torres-Aleman, 2012). We found that the level of IGF-1 was increased in serum and CSF in injured animals with TMT. To establish a causative role of IGF-1, neutralizing antibodies against IGF-1 were intrathecally delivered. Neutralization of IGF-1 almost completely abrogated the pro-survival effect of TMT on grafted NSCs. Intriguingly, neutralization of either brain-derived neurotrophic factor (BDNF) or neurotrophin-3 (NT-3), which have been implicated in TMT-induced neuroplasticity and whose levels were increased within the spinal cord tissue, but not in the CSF compartment, by TMT, did not affect the effect of TMT in enhancing NSC survival.

Our data suggest an interesting scenario in which IGF-1 produced in peripheral organs by TMT enters into the CNS *via* the blood-CSF barrier and provides beneficial effects on the survival of grafted NSCs in patients with spinal cord trauma (Figure 1). Rehabilitative exercise or training mobilizes intense muscular activities accompanied by metabolic challenges in various peripheral organs. These changes in turn are probably responsible for the production of IGF-1 and other insulin-like peptide molecules. These insulin-like peptide molecules are traditionally known as important regulators of glucose and lipid metabolism in peripheral organs. More recent studies, however, have uncovered various beneficial influences of the insulin-like peptide molecules on CNS involving brain development, neural cell differentiation, and even cognitive functions (Fernandez and Torres-Aleman, 2012). Our findings of IGF-1 regulating NSC survival add to the growing evidence that insulin and IGF receptor signaling play critical roles in NSC homeostasis (Ziegler et al., 2015). Therefore, it is conceivable that intensive rehabilitative training will be able to potentiate the therapeutic effects of NSC transplantation by not only promoting NSC survival but also enhancing multiple functionalities of NSCs. Indeed, we found that TMT increased the percentage of NSCs differentiating into neurons or oligodendrocytes. A previous study reported that IGF-1 promotes migration of newly generated neuroblasts. In consistence with the report, the rostrocaudal distribution of grafted NSCs was also greatly extended in animals with

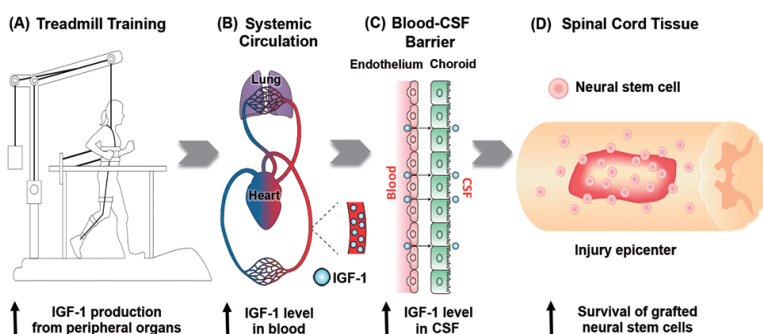


Figure 1 A diagram illustrating our model for potential mechanisms of treadmill training-induced enhancement of grafted neural stem cell survival following spinal cord injury.

(A) Physical exercise by treadmill training increases production of insulin-like growth factor-1 (IGF-1) from peripheral organs such as the liver and muscles. (B) Increase of IGF-1 production raises the concentration of IGF-1 in systemic blood circulation. (C) As the level of IGF-1 in the cerebrospinal fluid (CSF) compartment is dependent on its level in the blood, the CSF IGF-1 level increases accordingly. The IGF-1 in the CSF compartment is known to be transported to the parenchymal tissue by way of IGF binding proteins. (D) Since neutralization of IGF-1 in the CSF space largely attenuates the effects of treadmill training on the survival of grafted neural stem cells, we propose that IGF-1 mediates the beneficial effects of IGF-1 in neural stem cell survival following treadmill training.



TMT in our study.

What is the potential signaling pathway by which IGF-1 promoted survival of NSCs? Our study provided evidence that TMT activated mitogen-activated protein kinase (MAPK or ERK) signaling within grafted NSCs and the proportion of ERK positive NSCs was largely attenuated by IGF-1 neutralization, suggesting that ERK signaling activated IGF-1 may contribute to the enhancement of NSC survival. ERK signaling is involved in the regulation of not only neural cell survival but also axonal sprouting and plasticity. This raises an interesting hypothesis that activity-based rehabilitative intervention could also activate programs in NSCs to promote and establish neural connections in the host tissue. A recent study showed that enhancement of stem cell graft survival by a mixture growth factors and a calpain inhibitor led to an unforeseen extent of axonal growth derived from grafted stem cells (Lu et al., 2012). The astonishing graft-derived axonal growth was attenuated by mTOR inhibitor, rapamycin. IGF-1 is one of the most potent natural activators of the PI3K/AKT pathway, an upstream activator of mTOR signaling. Therefore, it is conceivable that IGF-1 can stimulate NSCs to activate programs for axonal growth *via* both ERK and mTOR pathways. Our lab is now delving into detailed molecular mechanisms of IGF-1-mediated NSC survival and NSC-derived axonal growth. We hope that information gained from these studies can lead to a development of small molecules promoting functionalities of NSCs and thereby improving therapeutic efficacy of NSC-based transplantation approaches.

What are functional and structural consequences of combining NSC transplantation and activity-based rehabilitation? We found that the extent of functional recovery in animals with the combination of NSC transplantation and TMT was superior to that in animals with either treatment alone. Moreover, tissue protection and sparing of residual white matter were synergistically, or at least additively, enhanced by the combination treatment. We assume that enhanced survival of grafted NSCs by TMT could entail more powerful neurotrophic and/or immune-modulatory effects in the epicenter region of the spinal cord. Enhanced provision of new oligodendrocytes could contribute to the white matter sparing with higher myelin content. These beneficial effects of the combination treatment at the epicenter region may be complemented by those of TMT at the region caudal to the epicenter. It has been well known that TMT promotes plasticity of the neural pathways involved in the locomotor network which is known as the central pattern generator. Our microarray study also found that TMT restored the expression of neuroplasticity genes in the spinal cord tissue caudal to the epicenter (Shin et al., 2014). Expression of angiogenesis-related genes was also increased in the same region, suggesting that TMT may promote neurovascular remodeling in the neural circuitry controlling locomotion. In addition, we found that TMT enhanced serotonergic fiber innervation in the lumbar spinal cord (Hwang et al., 2014). Therefore, it is highly likely that the multimodal effects of TMT on the locomotor neural circuit in the lumbar spinal cord cooperated with the beneficial influence of NSCs with enhanced survival at the epicenter, leading to the improvement in functional recovery. We also tested a consequence of the combination (NSC transplantation + TMT) on neurotrophic factor levels in the lesioned spinal cord. In this aspect, glial cell line-derived neurotrophic factor (GDNF), of which level was severely depleted after injury, was of considerable interest. We found that TMT alone almost completely restored the GDNF level at the both epicenter and caudal regions, and the combination (NSC transplantation + TMT) even further increased the GDNF levels. Further studies are required to clarify roles of interaction between NSC and TMT in the production of GDNF and its potential effects on function recovery, structural repair, and neuroplasticity after spinal cord injury.

In summary, our study demonstrated that TMT can support survival of grafted NSCs *via* IGF-1 signaling and that combination of NSC transplantation and TMT provides synergistic benefits in functional locomotor recovery. We believe that these

findings possess important clinical implications. Presurgical and postsurgical intensive activity-based rehabilitative programs are already being incorporated into various cell therapy paradigms in ongoing clinical trials. It has been shown that the graft survival deteriorates more severely in chronically than acutely or subacutely injured spinal cord. Therefore, the benefits of intensive training for graft survival would be greater for patients with chronic injury. In addition to the well-known roles of TMT in neuroplasticity at the caudal level, our study proposes a novel neurobiological basis for complementing NSC-based spinal cord repair with the activity-based neurorehabilitative approaches. Another ramification of our results is that a strategy to promote survival of NSC grafts should be a crucial component of future therapeutic approaches based on NSC transplantation. Dissecting detailed signaling pathways by which IGF-1 regulates NSC survival may lead to an identification of novel targets for developing small molecules to potentiate therapeutic efficacy and functionality of grafted NSCs.

This work was supported by a National Research Foundation of Korea grant funded by the Korean Government (NRF-2014R1A1A2056452 to D.H.H. and NRF-2014M3A9B6034224 to BGK).

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Accepted: 2015-08-20

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doi: 10.4103/1673-5374.167750 <http://www.nrronline.org/>

Hwang DH, Shin HY, Kim BG (2015) Fortuitous benefits of activity-based rehabilitation in stem cell-based therapy for spinal cord repair: enhancing graft survival. *Neural Regen Res* 10(10):1589-1590.

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