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Microenvironmental regulation of stem cells injected in the area at risk of neurodegenerative diseases

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Abstract: The complex mechanism of degenerative diseases and the non-specific modulation of regenerative targets are topics that need to be elucidated in order to advance the use of stem cells in improvement of neurodegenerative diseases. From pre-transplantation through post-transplantation, there are many changes in the conditions, both inside and outside of the stem cells that have not been carefully considered. This has hindered development in the field of cell therapy and regeneration. This viewpoint highlights the potential implications of intracellular and extracellular alterations of stem cells in transplanted areas at risk of neurodegenerative disease.

Introduction

A slow, progressive loss of structure, function, or number of neurons in the central nervous system induces neurodegenerative diseases, including Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS) (Fu *et al.*, 2018; Rahman *et al.*, 2020; Sheikh *et al.*, 2013). Despite significant efforts, recent therapeutic approaches have not been able to halt the neurodegenerative processes induced by an autonomous or inflammatory process. The complex mechanism of degenerative diseases and the non-specific modulation of regenerative targets in the brain have resulted in ineffective treatments and confusion between normal physiological and pathological processes (Kim *et al.*, 2021).

Therapies that manipulate regulatory signaling and modulatory cells, and the use of stem cells to influence trophic or immunogenic conditions, are prominently used and preferred over the use of stem cells in cell replacement in regenerative therapy. In other words, the beneficial effects of stem cells may partly modulate the environmental molecular composition of trophic factors by paracrine action and trigger resident cell responses to control neurodegenerative diseases by inducing neuroprotective or immunomodulatory roles (Raza *et al.*, 2018). Stem cell research thus far has characterized cell surface marker expression, maintenance, and proliferation (Aldrich *et al.*, 2021). Furthermore, several studies have shown promising effects as cellular therapeutics by charactering postnatal niche and by maximizing neuronal fate and multilineage differentiation potential (Luzuriaga *et al.*, 2021).

Studies that analyzed the association between transplanted 'graft' cells and adjacent 'host' cells, have provided insights which improved graft cell localization to specific injury sites and promoted their subsequent function through external regulation of the stem cell microenvironment (Wan *et al.*, 2015; Zhang *et al.*, 2019; Zhao *et al.*, 2021). Given the importance of the stem cell microenvironment, we propose that micro-regulation both inside and outside stem cells can selectively and effectively control disease (Fig. 1).

Inside Stem Cells

Stem cells undergoing *ex vivo* expansion and manipulation need to maintain a balance between self-renewal and differentiation. Stem cells culture conditions for basic research or clinical therapies are commonly designed to maintain cellular homeostasis, with nutrient rich conditions and supplements such as recombinant cytokines, growth factors, and high oxygen pressure (van der Sanden *et al.*, 2010). For example, 1) Culturing of stem cells intended for use in increasing neurotrophic growth factor or modulating the immune system *ex vivo* requires a specific 'stem cell

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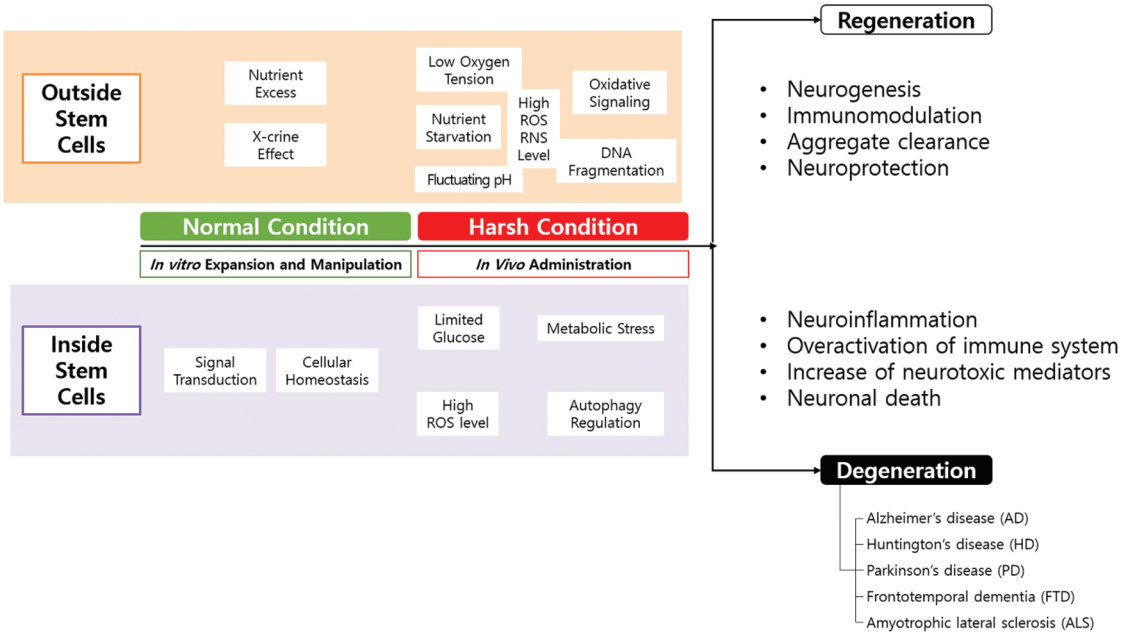

FIGURE 1. Diagram of the changing parameters inside and outside of stem cells. ROS, reactive oxygen species; RNS, reactive nitrogen species.

priming' protocol. This protocol is designed to promote stem cell characteristics, and improve cell survival, proliferation, and migration ability. These cell priming protocols alter the signals inside stem cells through the induction of cytokines or growth factors, pharmacological or chemical agents, hypoxic conditions, and 3D culture conditions. However, these protocols cannot produce cells that are continuously multipotent or capable of self-renewal (Noronha *et al.*, 2019). The cytoplasmic response to extrinsic priming components is complex, and a unique strategy for the regulation of mitochondria, the organelle at the center of stem cell homeostatic processes, should be considered. 2) When cell replacement therapy is intended for the treatment or improvement of PD, stem cell culture conditions that are optimized for the induction of midbrain dopaminergic neuron differentiation should be considered. This optimization improves neuroplasticity through intracellular signal transduction, which affects stem cell self-renewal or multipotency (Engel *et al.*, 2016).

After administration into the brain, stem cells induce a protective bioenergetic reaction to ensure their survival in response to the change from favorable *in vitro* conditions to the harsh *in vivo* environment. This is a metabolic response to a rapidly changing environment that occurs intracellularly. Previous studies have reported improvement in cell viability without impairing cell metabolism by controlling the tricarboxylic acid (TCA) cycle (Moya *et al.*, 2018). The metabolic pathway of stem cells can be considered as a gain or loss of metabolic flexibility depending on the severity of metabolic regulatory parameters, such as oxygen levels, substrate availability, and pH levels (Salazar-Noratto *et al.*, 2020). Under tolerable low glucose stress, stem cells can develop metabolic stresses on par with that of autophagy activation. Furthermore, stem cells under high glucose stress can act as a negative regulator that degrades and damages macromolecules to initiate apoptosis (Li *et al.*, 2018; Li *et al.*, 2021).

Outside Stem Cells

Prior to cell administration, stem cells are often cultured with undefined supplements, such as fetal bovine serum or recombinant proteins, under atmospheric oxygen pressure levels in excess of what *in situ* stem cells in a living body would normally experience (van der Sanden *et al.*, 2010). Under excessive nutrient and oxygen conditions, stem cells can exhibit various influences on neighboring cells by creating external signals. These signals are created by secretion molecules, extracellular matrix (ECM)-based signals, and cell-to-cell communication proteins derived from gap junctions and cadherins. Stem cells are heavily influenced by their extracellular environment due to their flexible nature. External cues such as autocrine and paracrine signals cause stem cells to undergo various processes including self-renewal, growth, and differentiation. Paracrine effects generated and regulated by the external environment have an important impact on the complex interplay between stem cell-derived mediators, including exosomes, microvesicles, and microRNAs (Asgarpour *et al.*, 2020; Rani *et al.*, 2015). Under normal or hypoxic culture conditions, artificial manipulation of paracrine mediators can enable the positive adaptation of stem cells to disease-ravaged tissue microenvironments.

Stem cells are sensitive to changes in the tissue-specific microenvironment. Brain tissue in the area at risk that is to be administered with stem cells has a microenvironment composed of secretion proteins, and biomechanical and nutritional properties created by neurons, astrocytes, and glial cells. As the majority of neurodegenerative diseases display complex etiologies, there is a different status specific to the pathological and physiological response. Despite their complex pathogenesis, it has been reported that brain tissue from neurodegenerative diseases are susceptible to oxidative damage. This may be due to the high polyunsaturated fatty acid content in neuronal membranes or weak antioxidant

defenses, leading to apoptosis and high levels of oxidative stress (Liu *et al*., 2017). In addition to the ischemic environment caused by lack of oxygen, microenvironmental factors that cause nutrient deficiency inside stem cells, especially glucose deficiency, are being touted as a major cause of cell death (Salazar-Noratto *et al*., 2020). Therefore, the addition of antioxidant substances to control reactive oxygen species (ROS) levels in the infused stem cells and the manipulation of intracellular signals to release antioxidant molecules may be used to mitigate apoptosis. Substances with antioxidant properties include glutathione (GSH), vitamin C, vitamin E, and coenzyme Q10. Similarly, treatment with N-acetyl-L-cysteine (NAC), a free radical scavenger, can have a positive impact on the focal adhesion and the oxidative stress by improving connectivity between neighboring cells (Li *et al*., 2015; Song *et al*., 2010). Furthermore, the induction of antioxidant defense mechanisms via the control of redox homeostasis in mitochondria, one of the major producers of ROS, can also improve the areas of risk in neurodegenerative diseases (Lee *et al*., 2021; Lisowski et al., 2018; Rahman *et al*., 2021).

Conclusions and Future Perspectives

More often than not, the conditions inside and outside of stem cells, before and after transplantation, change drastically from normal and tolerable to hostile and intolerable. This negatively affects the survival, function, and fate of the cells used in cell therapy for the treatment and regeneration of degenerative brain diseases. Furthermore, the relationship between stem cells and the disease-specific microenvironment operates in both directions: the microenvironment is able to affect the regulation of stem cell homeostasis, and stem cells can also positively affect the injected area that is at risk of neurodegenerative disease.

Stem cells endure a drastic change in the microenvironment from excessive nutrients *in vitro* to harsh conditions *in vivo*. A rational step forward in the development of stem cell therapeutics is to prioritize the development of methods controlling oxidative stress to cell organelles, including control of ROS levels, and processes that contribute to oxidative stress, such as mitochondria and autophagy. Further research is required to enable the utilization of exogenous modulators, such as microRNA, exosomes, and specific gene targets, as a therapeutic strategy. Such a strategy would not only ensure the survival of transplanted stem cells, but also promote activation of their stem cell functions (paracrine or transdifferentiation), and ultimately, enable stem cell regulation of degeneration and regeneration in the brain.

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