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PERSPECTIVE

Cell-free therapy: a neuroregenerative approach to sensory neuropathy?

Stem cells are considered as key therapeutic tools in the field of regenerative medicine. In the last decade, based on a growing body of knowledge about their mechanisms of action, a new way of exploring the therapeutic potential of stem cells without the need for cell transplantation has been evidenced. This approach, called cell-free therapy, uses stem cells as a source of therapeutic molecules rather than the therapeutic agent per se, and several studies have shown relevant therapeutic effects under a variety of experimental conditions, including nervous system disorders.

Sensory neuropathy (SN) is a chronic syndrome resulting from injury or dysfunction of the nervous system, frequently associated with different clinical conditions and diseases, such as cancer, diabetes, chemotherapy, trauma, infection or autoimmune diseases. This syndrome is characterized by the presence of persistent sensory symptoms, such as paresthesia and neuropathic pain, which may ultimate lead to sensory loss. The pharmacological approaches available for the management of SN are palliative, poorly efficacious and devoid of disease-modifying profile. Sensory neuropathies are closely associated with poor quality of life, disability and comorbidities, such as depression and anxiety, thus demanding rehabilitation and psychological support for SN patients. In view of this devastating scenario, the development of new approaches effective in attenuating sensory symptoms and promoting the functional reestablishment of nervous tissue represents a fundamental advance in SN therapeutics.

Based on the analgesic, neuroprotective and pro-regenerative potential of stem cells, cellular therapies have been considered as promising alternatives for the treatment of sensory neuropathies. The initial evidence of the therapeutic effects of stem cells in chronic neuropathies were reported in the first decade of the 2000s, using mesenchymal stem/stromal cells (MSC), an adult multipotent stem cell type. Coronel et al. (2006) showed that MSC selectively migrated to the dorsal root ganglion ipsilateral to the sciatic nerve constriction in a classical animal model of neuropathic pain. One year later, this same group demonstrated that MSC transplantation was able to reduce the behavioral painful neuropathy associated with the sciatic nerve constriction (Musolino et al., 2007), highlighting the analgesic potential of MSC. Since then, several preclinical and clinical studies have demonstrated the consistent and long-lasting analgesic effects of MSC in different types of sensory neuropathies (Huh et al., 2017).

The use of MSC for the treatment of sensory neuropathies represents a new therapeutic concept – the neurorestorative therapy for pain – that can be achieved by associating the analgesic effect of stem cells with their pro-regenerative properties. In this proposal, MSC represents not only an analgesic treatment, but also a way to repair the damaged nervous system. In fact, the regenerative effects of MSC on peripheral nerves during states of chronic neuropathy have been demonstrated (Zhou et al., 2016). In line with this concept, we have recently demonstrated, in a mouse diabetes model, that MSC transplantation reverses the behavioral signs of SN, *i.e.* mechanical allodynia and thermal hyperalgesia. Importantly, the therapy promoted the reduction of sciatic nerve ultrastructural alterations, such as degenerative changes in axons and myelin sheath, considered hallmarks of diabetic neuropathy (Evangelista et al., 2018).

It is important to note that both the analgesic and neuroregenerative effects of MSC during neuropathic conditions appear to be associated with their paracrine action. While the analgesic properties of MSC are associated with the endogenous release of opioid peptides in the pain modulatory circuits (Guo et al., 2011), their pro-regenerative effects are related to immunomodulatory, anti-inflammatory, antioxidant, angiogenic and neurotrophic factors, released or induced by the MSC. Shibata et al. (2008) showed that MSC transplantation was effective in normalizing axonal circularity and improving the conduction velocity and blood flow in the sciatic nerve of rats with diabetic neuropathy. These effects were attributed the MSC-induced beneficial effects to locally released angiogenic factors, such as vascular endothelial growth factor and basic fibroblast growth factor. In agreement with this seminal study, the contribution of neurotrophic and angiogenic factors to the therapeutic effects of MSC in chronic neuropathy has been extensively demonstrated (Zhou et al., 2016).

Despite the unequivocal role of primary sensory neurons for the induction and maintenance of neuropathic conditions, numerous biochemical, cellular and functional alterations in the central nervous system have been shown to participate in the pathophysiology of these syndromes (Ji et al., 2013). Nerve injury and the resulting oxidative stress affect the local microenvironment in the spinal cord, promoting activation of glial cells, which are key elements in the development and maintenance of neuropathic pain. The activation signaling pathways involved in the production of inflammatory and hyperalgesic mediators, such as cytokines, chemokines, eicosanoids, glutamate and ATP, can modulate the activity of both glial cells and sensory neurons, triggering a cross-talking phenomenon between these cells which induces and maintains the spinal neuroinflammatory cascade (Ji et al., 2013). In line with this idea, in the chronic diabetic neuropathy model we found the reversion of SN to correlate with a reduced production of neuroinflammatory parameters in the spinal cord of MSC-transplanted mice (Evangelista et al., 2018). Moreover, we observed a reduction in glial fibrillary acidic protein and ionized calcium bindingadaptor molecule-1expression after cell therapy, indicating a suppressive effect of MSC on the spinal activation of astrocytes and microglia. Importantly, transplantation of MSC promoted the reestablishment of redox homeostasis in the spinal cord of neuropathic mice, as shown by the upregulation of antioxidant factors, such as catalase, superoxide dismutase, glutathione peroxidase and nuclear factor erythoid2-related factor2, and reduction of oxidative stress markers. MSC also modified the pattern of spinal cytokine production in diabetic mice, reduced the pro-inflammatory cytokines interleukin-1β and tumor necrosis factor-α, as well as increased the anti-inflammatory interleukin-10 and tumor growth factor-β levels, indicating a shift on the balance towards anti-inflammatory cytokines caused by MSC. Taken together, this work presented consistent evidence that inhibition of the spinal neuroinflammatory cascade is an important mechanism by which MSC induce therapeutic effects in SN.

Interestingly, we found the therapeutic effects induced by MSC during the diabetic neuropathy to be independent of the presence of transplanted cells at the lesion site, since RT-qPCR analysis showed minimal retention of transplant-

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ed MSC in the sciatic nerve, dorsal root ganglion and spinal cord of transplanted mice (Evangelista et al, 2018). This result indicates a secretory action, rather than cell replacement and differentiation, as the mechanism of action of MSC in neuropathic conditions. This hypothesis was reinforced by the demonstration that MSC secretome, *i.e.* soluble factors and extracellular vesicles secreted by MSC, induced a similar effect to MSC transplantation in SN (Evangelista et al., 2018). In fact, in the last decade many of the therapeutic effects of MSC have been shown to arise from the soluble factors and extracellular vesicles secreted by these cells (Kim et al., 2013). This concept has opened new perspectives for the use of MSC as a source of therapeutic molecules rather than as the therapeutic agent per se. This cell-free therapy approach has been validated as a promising concept to extend the therapeutic applicability of stem cells (Kim et al., 2013; Pawitan, 2014).

The secretome of MSC consists of soluble factors and extracellular vesicles that contain biologically active molecules, such as growth factors, cytokines and functional RNAs. When released by MSC, these factors can exert effects on cells of the cell millieu or reach distant organs via the bloodstream, including the central nervous system, depending on the permeability of the blood-brain barrier. Therapeutic effects of cell-free approaches for nervous system disorders have been demonstrated, and are probably due to the control of neuroinflammation (Kim et al., 2013). Thus, the efficacy of cell-free approaches for the control of chronic sensory neuropathies can be predicted. In agreement with this idea, the injection of MSC secretome reduced the SN and reestablished the Th1/Th2 balance in the peripheral nerve, spinal cord and dorsal root ganglion of neuropathic animals (Brini et al., 2017). Data from our laboratory also corroborate the potential of cell-free therapies for the control of chronic sensory neuropathies. Using a well-characterized neuropathy model induced by partial sciatic nerve ligation in mice, we have demonstrated that MSC secretome induces a potent and long-lasting antinociceptive effect, which was equivalent to that obtained by MSC transplantation (Gama et al., 2018). Similarly, a single administration of secretome shifted the cytokine balance towards an anti-inflammatory profile at both the peripheral and central nervous systems. The characterization of MSC secretome by protein array evidenced the presence of growth factors and cytokines relevant to antinociception, neuroregeneration, cellular growth and inflammation. Among the proteins identified in the secretome, hepatocyte growth factor, vascular endothelial growth factor and angiopoietin-1 are good candidates as key therapeutic factors secreted by MSC, due to their powerful angiogenic and neurotrophic actions (Gama et al., 2018).

In conclusion, the application of cell-free therapy for neurorestorative therapy of pain may represent an innovative strategy that provides pain control, avoiding the inherent limitations of cell transplantation, such as immune rejection and tumorigenic potential. Neuroregenerative effects on peripheral nerves are likely to be achieved by the action, in concert, of angiogenic and neurotrophic molecules present in MSC's secretome. In addition, cell-free products may cross the blood-brain barrier to act on the central nervous system where, by modulating pain and neuroinflammation, they may induce long-lasting analgesia and exhibit a disease modifying profile. Additional studies, however, should be carried out aiming to validate this hypothesis, as well as characterizing the mechanisms involved in the beneficial effects of cell-free therapy for neuropathy.

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