Biochimie xxx (2013) 1–15

Contents lists available at SciVerse ScienceDirect

Biochimie

journal homepage: www.elsevier.com/locate/biochi

Review

The stem cell secretome and its role in brain repair☆

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ARTICLE INFO

Article history:
Received 15 May 2013
Accepted 19 June 2013
Available online xxx

Keywords:
Secretome
Mesenchymal stem cells
Neural stem cells
Brain repair
Stem cell transplantation

ABSTRACT

Compelling evidence exists that non-haematopoietic stem cells, including mesenchymal (MSCs) and neural/progenitor stem cells (NPCs), exert a substantial beneficial and therapeutic effect after transplantation in experimental central nervous system (CNS) disease models through the secretion of immune modulatory or neurotrophic paracrine factors.

This *paracrine hypothesis* has inspired an alternative outlook on the use of stem cells in regenerative neurology. In this paradigm, significant repair of the injured brain may be achieved by injecting the biologics secreted by stem cells (*secretome*), rather than implanting stem cells themselves for direct cell replacement. The *stem cell secretome* (*SCS*) includes cytokines, chemokines and growth factors, and has gained increasing attention in recent years because of its multiple implications for the repair, restoration or regeneration of injured tissues.

Thanks to recent improvements in *SCS* profiling and manipulation, investigators are now inspired to harness the *SCS* as a novel alternative therapeutic option that might ensure more efficient outcomes than current stem cell-based therapies for CNS repair.

This review discusses the most recent identification of MSC- and NPC-secreted factors, including those that are trafficked within extracellular membrane vesicles (EVs), and reflects on their potential effects on brain repair. It also examines some of the most convincing advances in molecular profiling that have enabled mapping of the *SCS*.

Q2 1. Introduction

Recent advances in stem cell biology hold great promise in the development of non-haematopoietic stem cell-based therapeutics for the treatment of diseases of the central nervous system (CNS), including animal models of multiple sclerosis (MS) [1–3], Alzheimer's disease (AD) [4], spinal cord injury (SCI) [5] and stroke [6]. Growing evidence suggests that the effects orchestrated by stem cell transplants might not only be associated with the generation of new graft-derived neurons and glial cells [7,8] and that the context in which these cells are injected critically determines some of the outcomes. Thus, cell replacement is not the sole way for transplanted stem cells to foster tissue repair *in vivo* [9]. It is in fact becoming increasingly accepted that stem cells secrete a vast array of proteins – including growth factors, cytokines, chemokines, metabolites and bioactive lipids – that regulate their biology in an autocrine or paracrine manner, while orchestrating multiple interactions with the surrounding microenvironment (*therapeutic plasticity*) [9–11]. This new concept of *stem cell therapeutic plasticity* describes the various therapeutic actions of transplanted stem cells

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http://dx.doi.org/10.1016/j.biochi.2013.06.020

in vivo and their capacity to adapt fate and functions to specific microenvironments [12,13].

Among a number of promising stem cell sources, mesenchymal stromal/stem cells (MSCs; also known as *multipotent stromal cells*) and neural stem/precursor cells (NPCs) are being extensively investigated for their capacity to signal to the host upon transplantation in experimental CNS diseases. Following transplants of both MSCs and NPCs, sustained graft-to-host exchanges of signals has lead to trophic effects on endogenous brain cells and beneficial modulatory actions on innate and adaptive immune responses that have ultimately promoted the healing of the injured CNS [2,14,15]. A number of key regulatory pathways have been identified as being shared between MSCs and NPCs, thus suggesting the existence of a stem cell-like *signalling signature* that is likely to be common to other stem/precursor cell types as well [16].

Both targeted/untargeted proteomics and metabolomics are now being extensively applied to identify novel factors of potential therapeutic relevance in the *stem cell secretome* (*SCS*). Moreover, the use of gene expression approaches or culture preconditioning of modified stem cells capable of actively releasing discrete levels of a pro-regenerative secreted factor merits consideration and will be an area of intensive investigation in the near future. Finally, the development of local *vs* systemic stem cell-free therapeutics that use extracellular membrane vesicles (EVs), instead of whole parental stem cells, is emerging as an exciting new concept in regenerative medicine [17].

Here, we have reviewed the current knowledge of the *SCS* from MSCs and NPCs, and examined its potential in brain repair. We have also discussed the on-going main investigative directions aimed at both improving cellular (secretory) activities and characterizing the *SCS* and its regulation in greater detail.

1.1. The stem cell secretome and its role in brain repair

1.1.1. Mesenchymal stem cells

MSCs are self-renewing, clonal precursors of non-haema topoietic tissues that were first identified in the bone marrow (BM-MSCs) [18]. Nevertheless, intensive research efforts have suggested alternative tissue sources that include the adipose tissue (ASCs [19];), the dental pulp [20], the placenta [21], the umbilical cord blood (HUCPVCs [22];), the Wharton Jelly (WJSCs [23];), olfactory mucosa [24], deciduous teeth [25], lung and spleen [26], and even the brain [27]. MSCs can be expanded *in vitro* for some time while retaining the potential to differentiate into mesenchymal cell types closely related to the germ layer of origin, such as adipocytes, chondrocytes and osteoblasts [28]. The transplantation of MSCs has emerged as promise for the repair or restoration of several tissues, including the CNS [29]. That MSC transplants possess potential for the treatment of CNS diseases has become clear following the observation of clinical and histological recovery shown in laboratory animals with CNS disease models after the systemic injection of MSCs [30]. However, the mechanisms driving the therapeutic impact of MSC transplants remain unclear. Among a few candidate hypotheses, two main perspectives receiving attention relate to the tissue trophic and immune modulatory effects that transplanted MSCs exert on the host [31,32].

The intracerebroventricular injection of either BM- or ASC-MSCs has been shown to increase lifespan and body weight, ameliorate motor function impairments, and slow the overall deterioration of twitcher mice, as model of Krabbe's disease (KD), by inhibition of the type of inflammation associated with KD progression [33]. As such, MSC-transplanted twitcher mice showed a significant reduction in cerebral inflammation, including a significant decrease in the numbers of CNS-infiltrating macrophages, and activated microglial cells as compared to sham-treated controls [33]. Other studies also confirmed the immune modulatory properties of MSCs after systemic cell injection in rodents affected by experimental autoimmune encephalomyelitis (EAE), as a model of MS. The systemic injection of both BM-MSCs and ASC-MSCs via immune regulatory and neurotrophic mechanisms [34–36] lead to inhibition of autoreactive T cell responses as well as the stimulation of endogenous oligodendrogenesis [35–38]. Key factors responsible for some of the observed therapeutic effects have been identified as stem cell-secreted hepatocyte growth factor (HGF) [39,40], as well as fibroblast growth factor (FGF)-II, brain-derived neurotrophic factor (BDNF), and platelet-derived growth factor (PDGF)-AB [34]. The effects of both HGF and MSC-CM are mediated through the tyrosine kinase receptor cMet *in vivo*, and have led to enhanced myelin repair and immune modulation, while being fully inhibited by anti-cMet and anti-HGF antibodies [39,40]. Similar anti-inflammatory effects were observed after MSC transplantation in mice with experimental SCI, showing reduction of astro- and micro-gliosis and enhancement of sensorimotor functions [41].

On the other hand, the transplantation of BM-MSCs delivered into the lesion epicentre of rats with experimental SCI reduced the lesion volume and induced axonal regrowth. In this study, the neurite outgrowth was promoted by BM-MSC-secreted BDNF and glial cell line-derived neurotrophic factor (GDNF) [42]. WJ-MSC or ASC-MSCs are also rich in paracrine neuroprotective factors that may account for protective effects *in vivo* after transplantation. The intralesional transplantation of human WJ-MSCs in rats with experimental complete spinal cord transection led to decreased numbers of microglia and reduced astroglial scarring, and was found associated with increased levels of neutrophil-activating protein-2 (NAP-2), neurotrophin-3 (NT-3), FGF-II, glucocorticoid-induced tumour necrosis factor receptor (GITR), and vascular endothelial growth factor receptor (VEGFR)-3 [43].

In a mouse model of Huntington's disease (HD), intrastriatally transplanted BM-MSCs integrated in the host brain and exerted neurotrophic effects that correlate with increased levels of laminin, von Willebrand factor (VWF), stromal cell-derived factor-1 (SDF-1) α, and the SDF-1 receptor CXCR4, which in turn enhanced angiogenesis in the damaged striatum [44]. The intravenous (i.v.) injection of BM-MSCs in 6-hydroxydopamine (6-OHDA)-induced experimental Parkinson's disease (PD) exerted anti-apoptotic effects on host dopaminergic (DA) neurons in part via secreted SDF-1 α [45]. Rats receiving MSC transplantation showed significant behavioural recovery both in cylinder test and amphetamine-induced rotation test, compared with controls [45]. Further *in vitro* studies confirmed the relevance of MSC-secreted SDF-1 α in mediating some of the tissue trophic/protective effects of grafted MSCs. As such, MSC-conditioned media (MSC-CM) displayed anti-apoptotic effects on 6-OHDA-exposed PC12 cells *in vitro* and increased dopamine (DA) release from the cells. Incubation with anti-SDF-1α antibodies reduced the anti-apoptotic effects of the MSC-CM and confirmed a key role for MSC-secreted SDF-1 α in the observed neuroprotection. Similarly, intrastriatally transplanted ASC-MSCs protect against 6-OHDA-induced experimental PD in mice [46]. Histological, electrophysiological, neurochemical and gene expression studies suggested that the likely mechanisms by which ASC-MSCs cell grafts rescued the nigrostriatal function involved little direct differentiation of the stem cell graft into functional dopaminergic neurons, rather indirect modulation of the oxidative stress-induced neuroinflammatory environment via the secretion of GDNF, BDNF and nerve growth factor (NGF) at the level of the lesioned substantia nigra [46].

Intravenously injected human MSCs have been found to induce functional amelioration, reduce the infarct volume, and promote neuroprotection in rats with experimental middle cerebral artery occlusion (MCAo), a model of brain stroke. Increased levels of

Please cite this article in press as: D. Drago, et al., The stem cell secretome and its role in brain repair, Biochimie (2013), http://dx.doi.org/10.1016/j.biochi.2013.06.020