Neuroprotective properties of extracellular vesicles derived from mesenchymal stem cells

Extracellular vesicles (EVs) provide a novel mechanism of intercellular communication via the transfer of proteins, lipids, and miRNAs between cells. It is now widely accepted that cargo content of EVs depends on cell type and its physiological state. Accordingly, EVs derived from healthy cells may have a comparable therapeutic potential as cells themselves. Indeed, several studies confirmed this notion and demonstrated therapeutic potential of EVs in different clinical settings. Exosomes represent a class of EVs, that can cross blood-brain barrier (Alvarez-Erviti et al., 2011), therefore they can be delivered into the CNS using intravenous, or intranasal routes avoiding the need for neurosurgical interventions. This property makes them particularly attractive as a new tool for the neuroregenerative therapies. However, new protocols require large amounts of EVs which can be obtained only from cells expanded in vitro. In this respect human mesenchymal stem cells (MSCs) represent one of the most promising cellular sources of EVs.

In this perspective, we briefly summarize the current state of knowledge about neuroprotective properties of MSC-derived EVs and also discuss challenges that lie ahead.

Despite extensive experimental and clinical studies, the true nature and function of the MSCs in vivo remain elusive (Bianco, 2014). However, from a practical standpoint, considering therapeutic applications, several key properties of MSCs should be taken into account. The same considerations may also apply for the EVs secreted by MSCs.

First, MSC cultures comprise functionally different heterogeneous subpopulations. In other words, only a small fraction of freshly isolated MSCs will contribute to the formation of a new tissue when transplanted in vivo, assuming that, cellular heterogeneity is also reflected in cargo content and functional properties of secreted EVs. Since currently accepted sets of surface markers characterize the whole stromal cell cultures of MSCs and do not reflect their heterogeneous nature, generation of functionally homogeneous EV populations from these cultures is problematic.

It is also important to note that MSCs isolated from different tissues are not equivalent and display distinct tissue-specific differentiation capacities (Bianco, 2014), implying they also produce EVs with different properties. Here we will summarize current data about neuroprotective properties of EVs produced by MSCs derived from different tissues (Table 1).

Genetic lineage tracing revealed that MSCs isolated from dental pulp, also known as dental pulp stem cells (DPSCs), or stem cells derived from the dental pulp of human exfoliated deciduous teeth (SHEDs) originate from the peripheral nerve-associated glia (Kaakua et al., 2014). Therefore, in contrast to the MSCs derived from other mesodermal tissues, DPSCs and SHEDs might be particularly useful for studies of neuronal and glial differentiation. Indeed, several studies demonstrated that DPSCs and SHEDs can be efficiently differentiated into neuronal and Schwann cells in vitro and even more importantly, these cells displayed neuroprotective properties in vivo. Several lines of evidence demonstrated the importance of paracrine signalling during neuroregeneration induced by DPSCs and SHEDs. For instance, DPSCs produced neurotrophic factors in culture, promoted survival of trigeminal neurons in vitro and also rescued motor neurons after spinal cord injury (Nosrat et al., 2001). Another recent study demonstrated that dopaminergic neuron-like cells derived from SHEDs contributed to neuroprotection against 6-OHDA-induced neurodegeneration by using paracrine mechanisms (Fuji et al., 2015). These findings suggest the importance of paracrine mechanisms in the neuroprotective action of DPSCs and SHEDs. However, much less is known about the role of EVs in this process. We recently asked, whether EVs (exosomes and microvesicles) derived from SHEDs display neuroprotective properties during 6-OHDA-induced apoptosis in human dopaminergic neurons. Our results indicate that exosomes, but not microvesicles derived from SHEDs grown on the laminin-coated alginate microcarriers, suppressed 6-OHDA-induced apoptosis in dopaminergic neurons (Fujimia et al., 2015). Importantly, no such effects were observed for the exosomes derived from SHEDs grown under standard culture conditions, showing that culture conditions have a profound influence on functional properties and cargo content (unpublished data) of exosomes. Future studies will identify unique proteins and (or) microRNAs responsible for the neuroprotective action of SHED-derived exosomes. In conclusion, MSCs derived from dental pulp have unique neurogenic properties and therefore represent useful source of EVs for the neurotherapeutic applications. The main disadvantage of dental pulp as a source of MSCs is relatively low availability limiting collection of large amounts of EVs necessary for therapeutic applications.

In contrast to dental pulp, adipose tissue (AT) represents an abundant and easily accessible source of MSC-like cells. It must be noted, however, that therapeutic potential of AT-MSCs depends on different factors like age, disease condition, anatomical harvest site, or body mass index. It is therefore easy to predict that all these factors may also affect therapeutic properties of EVs. Several studies demonstrated neuroprotective properties of EVs derived from AT-MSCs. For instance, AT-MSCs secreted functional neprilysin-bound exosomes and contributed to decrease of both secreted and intracellular levels of β-amyloid peptide in N2a neuroblastoma cells (Katsuda et al., 2013). Importantly, exosomes from AT-MSCs expressed significantly higher levels of neprilysin than MSCs derived from bone marrow (BM-MSCs), highlighting the differences between functional properties of exosomes derived from different tissues (Katsuda et al., 2013). Another study demonstrated that EVs (exosomes and microvesicles) derived from the murine AT-MSCs rescued human neuroblastoma cells SH-SY5Y and primary murine hippocampal neurons exposed to oxidative damage with H2O2 (Farinazzo et al., 2015). Interestingly, authors observed an inverse dose-dependent effects of EVs on cell viability. In addition, EVs derived from murine AT-MSCs increased the process of renylationlation and activated nestin-positive oligodendroglial progenitors in cerebellar slice cultures demyelinated with lysophosphatidylcholine (Farinazzo et al., 2015). More recently, the same group presented evidence for neuroprotective effects of exosomes derived from murine AT-MSCs using in vitro model of amyotrophic lateral sclerosis (Bonafe et al., 2016). Exosomes were able to protect motor neuron-like cell line NSC-34 overexpressing different mutants of human superoxide dismutase 1 from oxidative damage showing potential for future therapeutic applications in motor neuron disease (Bonafe et al., 2016).

Bone marrow, represents the most common source of MSCs, but relatively few studies have focused on the neuroprotective properties of BM-MSC-derived EVs. Interestingly, human BM-MSCs and BM-MSC-derived EVs similarly improved post-stroke neuroregeneration in C57BL6 mice (Doepner et al., 2015). EVs promoted neuroregeneration and neurological recovery and also modulated systemic immune responses as evidenced by attenuated post-ischemic immunosuppression. These findings demonstrate the importance of EVs as modulators of systemic immune responses for neurological recovery. Thus, EVs may promote neuroregeneration by acting simultaneously on local (at the site of injury) and systemic (modulation of immune response) levels. EVs derived from rat BM-MSCs also promoted functional recovery and neurovascular plasticity after traumatic brain injury.

Human umbilical cord (UC) tissue is another excellent alternative source for MSCs. Thus, MSCs isolated from the Wharton’s jelly of the UC provided better neuroprotection efficacy than BM-MSCs in an oxygen-glucose deprivation culture model (Hsieh et al., 2013). These neuroprotective effects were related to unique...
secretion patterns of paracrine factors involved in angiogenesis and neurogenesis (Hsieh et al., 2013). However, currently there is no reliable data about neuroprotective properties of EVs derived from UC-MSCs.

All these studies clearly demonstrated therapeutic potential of MSC-derived EVs. However, there are a number of key challenges that need to be addressed before EVs can enter clinical development. First of all, we still have limited knowledge about the molecular mechanisms underlying neuroprotective actions of EVs. Since EVs carry complex and variable cargo, it is likely that neuroprotection is achieved by simultaneous action of several miRNAs and (or) proteins making the identification of these mechanisms a difficult task. Another problem is related to the heterogeneous nature of in vitro MSC cultures complicating isolation of functionally homogeneous EV populations. Therefore, systematic and comprehensive studies are required to compare proteomic and RNAsomic profiles of EVs produced by MSCs derived from different tissues and grown under standard conditions (preferably using animal component-free cell culture medium). In addition, MSCs polarized into pro-inflammatory and anti-inflammatory phenotypes should be included into these studies. Most recently, similar approach has been used to compare proteomic profiles and angiogenic properties of exosomes derived from BM-MSCs cultured under expansion conditions and under ischemic tissue-simulated conditions (Anderson et al., 2016). At the next stage, neuroprotective properties of EVs could be systematically explored using validated in vitro models (for example, human neural cell lines ReN cell VM and SH-SY5Y, or different types of neurons derived from human iPSCs) and then extended using in vivo experimental models. This strategy may help to establish specific screening tests for different lots of EVs ensuring better reproducibility and therapeutic efficacy. Last but not least, it should be kept in mind that the whole field of EV research is still in its infancy and that there are many unresolved issues regarding nomenclature, isolation, characterization and quantification that need to be addressed to ensure better reproducibility. Nevertheless, despite all these challenges, EVs have great potential as a novel therapeutic tool against neurodegenerative diseases.

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Table 1 Comparison of extracellular vesicles produced by mesenchymal stem cells derived from different tissues

<table>
<thead>
<tr>
<th>Tissue Source</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Dental pulp</td>
<td>- Originate from peripheral nerve-associated glia (Kaukua et al., 2014). - In contrast to MSCs from mesodermal tissues could be particularly suitable for the induction of neural (Jarmalavičiūtė et al., 2013) and glial differentiation. - Promote neuroregeneration by producing neurotrophic factors (Fujii et al., 2015; Nosrat et al., 2001) and EVs (Jarmalavičiūtė et al., 2015).</td>
<td>- Relatively low availability of dental pulp tissue. - Difficult to obtain large amounts of EVs for therapeutic applications. - Potential therapeutic use of EVs derived from autologous cells is limited.</td>
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<tr>
<td>Adipose tissue</td>
<td>- Abundant and easily accessible source of MSCs. Allows generation of large amounts of EVs necessary for therapeutic applications. Potential use for autologous therapies. - EVs derived from AT-MSCs displayed neuroprotective properties under several experimental conditions (Katsuda et al., 2013; Farinazzo et al., 2015; Bonafede et al., 2016).</td>
<td>- Therapeutic potential of AT-MSCs depends on different factors like age, disease condition, anatomical harvest site, or body mass index. This may also affect therapeutic properties of EVs derived from AT-MSCs.</td>
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<tr>
<td>Bone marrow</td>
<td>- The best-characterized source of MSCs in humans. Allows generation of large amounts of EVs necessary for therapeutic applications. Potential use for autologous therapies. - BM-MSC-derived EVs promoted neuroregeneration and neurological recovery after ischemic stroke (Doepchner et al., 2015) and traumatic brain injury.</td>
<td>- Aspiration of BM from donor is an invasive and painful procedure. - The number of BM-MSCs significantly decreases with age. - The possible effects of BM-MSC-derived EVs as modulators of systemic immune response must be carefully evaluated.</td>
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