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# Stem cell secretome derived from human amniotic fluid affords neuroprotection in an ischemic model

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## Abstract

Human amniotic fluid stem cells (hAFSCs) are growing in interest; yet, little is understood about their secretome and neuroprotective actions in different diseases, including stroke. When stem cells are grown *in vitro*, they release an array of cytokines and growth factors that can stimulate neuroprotective processes. Furthermore, administering secretome rather than cells may be a safer route for patients who are at risk for rejection, promoting innate restorative processes. Current literature implicates that the miRNA contents of such secretome, more specifically exosomes, may regulate the effectiveness of secretome administration. In this review, we explore what factors may promote pro-survival and pro-apoptotic pathways after the administration of hAFSCs-derived secretome in ischemic models.

## Keywords:

miRNAs, oxygen-glucose deprivation, secretome, stroke

## Introduction: Update on Stroke

Stroke remains a significant cause of death and chronic disability across the world. The two major subcategories of stroke are ischemic and hemorrhagic. Ischemic stroke results from occlusion of an artery that supplies blood to the brain,<sup>[1]</sup> while hemorrhagic stroke is caused by a ruptured artery or an unusual vascular design.<sup>[2,3]</sup> Recent data have shown that the majority of stroke incidences are ischemic in nature.<sup>[4]</sup> A disruption in the cerebral blood supply has devastating effects, such as oxidative stress, tissue damage, inflammation, and eventually death of resident neurons.<sup>[5,6]</sup> Currently, stroke treatment is limited to tissue plasminogen activator (tPA), which has a narrow therapeutic window.<sup>[5-7]</sup> Furthermore, tPA has other limitations such as cerebral ischemia/reperfusion (I/R) injury, which can cause severe disability and even mortality.<sup>[8,9,10]</sup> Due to the unfavorable

outcomes of current treatment options like I/R injury, treatments that target secondary inflammation pathways, warrant the need to be investigated.

## *In vitro* Methods Provide Insight into Molecular Actions

*In vitro* mechanisms are useful in understanding the cellular and molecular actions of pathologies like stroke. Although not complete, *in vitro* models are critical for the translation of a treatment to a clinical setting. Furthermore, they allow for a lost-cost solution to develop *in vivo* models, which further improve the clinical translation.

Oxygen and glucose deprivation models (OGD) are an accurate way to simulate cerebral ischemia and cell damage that occur during stroke *in vitro*.<sup>[11-13]</sup> This model is accomplished by placing cells in a glucose-free medium while simultaneously prohibiting oxygen.<sup>[13,14]</sup> Further, the OGD model undergoes reperfusion in normal oxygen conditions.<sup>[15]</sup>

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## Amniotic Fluid: A Source of Regenerative Properties

Current research in regenerative medicine suggests that stem cells from amniotic fluid may be promising. In adult stem cells, epigenetic changes may be preserved, thus limiting their use for specific applications.<sup>[16]</sup> However, fetal stem cells are less differentiated when collected, and could be used more broadly. Furthermore, there are limited ethical concerns since the amniotic fluid is collected during amniocentesis and cesarean section.<sup>[17]</sup>

To further elucidate the therapeutic potential of amniotic fluid cells, signal transduction pathways triggered by human amniotic fluid stem cells (hAFSCs)-derived secretome in an I/R *in vitro* model were studied by Western blot analysis. In addition, the expression of miRNA within the exosomes of the conditioned medium was also studied. hAFSCs-derived secretome was observed to initiate pro-survival and anti-apoptotic mechanisms.<sup>[18]</sup> After microRNA analysis in the exosomes, it was found that 16 miRNAs were overexpressed and involved in the management of signaling pathways.<sup>[18]</sup> The pathways relating to I/R, including neurotrophin signaling, and those associated with neuroprotection and neuronal death, were of particular interest.<sup>[18]</sup> The evidence compiled proposes that hAFSCs-conditioned medium commences neuroprotective actions within *in vitro* ischemia models. These observed effects can be achieved through adjusting and initiated pro-survival processes, partially, due to the secreted miRNAs.

### The Role of Secretome and Exosomes in Neuroprotection

The amniotic fluid serves as a protective liquid that surrounds the fetus providing support and critical nutrients to aid in the development of the embryo.<sup>[19]</sup> The fluid consists of main water, cells, and other chemical elements.<sup>[20]</sup> The cells are primarily fetus-derived (respiratory, epithelial, urinary, and intestinal tract), as well as connective tissues and amniotic membranes. In addition, amniotic fluid possesses other cell subtypes such as amniotic, fibroblastic, and epithelioid, differing in prevalence depending on the age of gestation.<sup>[21]</sup> Amniotic fluid mesenchymal stem cells (AFMSCs) are of particular interest given their potential for therapeutic applications. AFMSCs have shown the ability to differentiated broadly toward chondrogenic, osteogenic, and adipogenic lineages, consequently presenting as a viable candidate for regenerative and therapeutic methods.<sup>[22]</sup>

However, there remains a concern on the ability of engrafted stem cells to develop into the specific cell type of the damaged area. The mechanism of action has been

reported broadly,<sup>[23-26]</sup> yet some of the exogenous cells that persist into the regenerated tissue do not completely explain the regenerative effects. Potentially, the insufficient integration may be due to a degree of differentiation that took place *in vitro* before transplant. Furthermore, it is worth noting that only some of the cell populations may undergo differentiation. This group of cells may elicit poor immune-activating responses but may recruit nearby progenitor cells to repair the injured tissue. Several studies support this theory, highlighting the protective ability of the conditioned medium in the regeneration of damaged areas where chronic inflammation persists.<sup>[27-30]</sup>

Arising from previously described theories, efforts have been targeted towards stem cells secretome, and the effects it can have on stimulating regenerative pathways in damaged areas.<sup>[31-33]</sup> Within conditioned medium, there exists extracellular vesicles (EV) and other soluble factors. More recently, EV have been implicated in upregulating mesenchymal stem cells (MSCs) regenerative effects,<sup>[34-37]</sup> however, this mechanism is not fully understood.

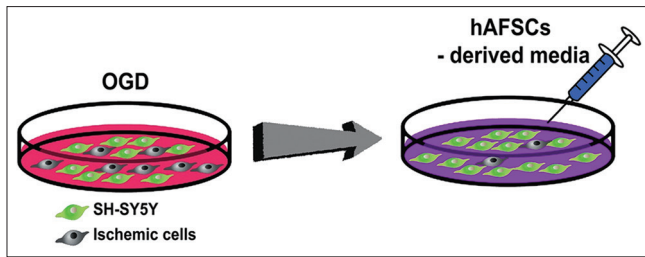
Exosomes are a subcategory of microvesicles, that have gained interests due to their ability to communicate from cell-to-cell, store biological information, serve as biomarkers, and their potential for regeneration and protection of the nervous system.<sup>[38-40]</sup> Exosomes are the result of fusing with the plasma membrane and releasing its contents outside of the cell.<sup>[38]</sup> The contents of an exosome usually consist of miDNA, DNA, proteins, carbohydrates, and lipids.<sup>[41,42]</sup> Since exosomes are relatively simple in structure and small in size, they can cross the blood-brain barrier and present novel approaches for treatment delivery in cerebral injuries. Therefore, exosomes provide another therapeutic route besides cell transplanatation.<sup>[43]</sup>

### Mitochondria Direct Apoptosis

Deficits relating to I/R injuries can be traced back to the mitochondria. For example, when mitochondria are unable to produce a strong proton gradient, their ability to produce energy suffers and thus affect other organelles like the endoplasmic reticulum in calcium uptake. Furthermore, oxidative stress can result from changes in the mitochondria leading to a building up of reactive oxygen species.<sup>[44]</sup> As a result, irregular mitochondria often initiate pro-apoptotic factors in the cytosol or nucleus leading to apoptosis.<sup>[45]</sup>

### Human Amniotic Fluid Stem Cells-Secretome Initiates Pro-Survival and Pro-Apoptotic Pathways

Following MSCs or MSCs-CM administration, a decrease in apoptosis around the injury site, upregulation of



**Figure 1:** A graphical abstract demonstrating the capacity of Human amniotic fluid stem cells-derived media to promote neuronal survival after exposed to ischemic conditions

pro-inflammatory factors, enhanced axonal growth, and neurogenesis and amelioration of neurological deficits have been shown [Figure 1].<sup>[30,46,47]</sup> Yet, it is the contents of the secretome that determine the therapeutic capacity.<sup>[48]</sup> In the CM, a myriad of regenerative properties and growth-stimulating factors exist, which originate from stem cells. Furthermore, proteomic studies demonstrate the presence of several cytokines and growth factors in CM.<sup>[49,50]</sup> In fact, MSCs have an unparalleled ability to produce effective exosome-producing cells.<sup>[51]</sup>

Given such evidence, paired with the paracrine hypothesis, which poses that stem cells elicit a positive effect by stimulating resident cells to deliver bioactive molecules and EV, the use of secretome may be more advantageous than MSCs, especially in regard to safety. Recent studies have shown that the release of the secretome from MSCs could attenuate neurological impairments observed in several neurodegenerative diseases such as traumatic brain injury, Parkinson's disease, stroke, and Alzheimer's disease.<sup>[22,52-55]</sup> Conditioned media and secretome demonstrate the capacity to upregulate neurotrophins like BDNF, a vascular endothelial growth factor. Neurorestoration is initiated under the release of nerve growth factors that improve neuronal projections.<sup>[47,56]</sup>

Several factors support the use of hAFSCs for safe and effective treatment in stroke including highly proliferative, low tumorigenicity, immunogenicity, and anti-inflammatory activity.<sup>[57]</sup> It was found that CM and more specifically, the exosomal component served an important role in fostering cell survival.<sup>[18]</sup> Increased survival was accompanied by suppressing cell-death pathways (p75/JNK) and upregulating pro-growth pathways (BDNF/TrkB).<sup>[18]</sup> In addition, pro-survival pathways PI3K/Akt and Erk5 were increased.<sup>[18]</sup>

### miRNA Contents Determines Therapeutic Capacity

Within exosomes are proteins, lipids, and regulatory RNAs that can be transferred and alter surrounding cell metabolism.<sup>[48]</sup> Interestingly, among MSC-derived

exosome proteins, higher levels of mBDNF were observed. Previously, several studies demonstrated that exosomes contained various neuronal proteins, all capable of passing the blood-brain barrier.<sup>[58]</sup> Furthermore, it has been shown that high levels of mBDNF were found in both exosomal fractions and in soluble CM.<sup>[18]</sup>

In addition to previously discussed exosomal contents, numerous miRNAs have been found that can initiate neurorestoration. miRNAs that have been shown to have neuroprotective effects (miR-146a-5p, miR-154-5p, miR-22-3p, miR-23a-3p, miR-27a-3p, miR-29a-3p, and miR-31-5p) were also found in the hAFSC-derived exosomes studied. In particular, miR-146a was previously demonstrated to dampen inflammation<sup>[59,60]</sup> and promote new formations of oligodendrocytes<sup>[61]</sup> within the injured perinatal brain. In promoting the growth of nervous tissue, miR-154 was observed in EV derived from astrocytes.<sup>[62]</sup> In addition, miR-154 has demonstrated to seek out DKK2, causing Wnt signaling activation and  $\beta$ -catenin upregulation,<sup>[63]</sup> both important pathways in synaptic maintenance and neuronal survival.<sup>[64]</sup> MiR-22-3p, 23a, and 27a all have roles in inhibiting apoptosis, thus providing neuroprotection.<sup>[65-69]</sup>

Recent studies suggest that miRNAs are dynamic in their response to ischemic conditions and are present in neuroprotection, such as TNF, Hippo, and PI3K.<sup>[70-72]</sup> From the miRNAs that afford neuroprotection, several genes were identified that are involved in modulating apoptosis and inflammation.<sup>[18]</sup>

In the HIF-1 pathway, a systemic deactivating mechanism can be seen from the experimentally based miRNAs and target genes. However, apoptosis, neurotrophins, and PI3K-Akt pathways, all expected to be mostly inactive, were observed taking part in upregulating or downregulating various processes such as apoptosis, proliferation, and inflammation. However, this response is undeviating with the pleiotropic and adaptive nature of miRNAs, since they can regulate the expression of many genes at the epigenetic level.<sup>[73]</sup> Despite the evidence, more investigation is needed to validate the role miRNAs have in regulating neurogenic pathways following ischemic injury. Furthermore, elucidating possible molecular mechanisms will aid in explaining the protective role miRNAs play in the central nervous system. Finally, it is certainly important to study how hAFSC-derived conditioned media behaves *in vivo*, as there are many other cells in the central nervous system besides neurons.

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## Conflicts of interest

There are no conflicts of interest.

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