Roles of constitutively secreted extracellular chaperones in neuronal cell repair and regeneration

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Introduction

Local tissue inflammation and mechanical injury to neurons have been associated with the aggregation of misfolded proteins and subsequent cell death (Gidailevitz et al., 2011). Relative to mature differentiated cells, regenerating cells have higher rates of protein synthesis (Noormohammadi et al., 2018), and recent studies have highlighted the critical need for effective protein quality control in stem cells during regeneration (Yan et al., 2020). Proteomic studies of human mesenchymal stromal stem cells have also shown that during regeneration, the secretion of a number of constitutively secreted chaperones (e.g. α2-macroglobulin, A2M) is selectively enhanced (Kehl et al., 2010). Constitutively secreted extracellular chaperones (ECs) are an integral part of the systems that act to maintain protein homeostasis (proteostasis) (Yerbury et al., 2007; Wyatt et al., 2013) and are almost certain to influence the ability of an organism to repair and regenerate cells and tissues. An effective protein quality control system ensures the timely recognition, refolding, or clearance of misfolded proteins to enhance the survival and proper functioning of living organisms (den Brave et al., 2021).

Regenerating neurons need to communicate with other neurons and surrounding cells to generate an effective response to a physiological or pathological stimulus, in which both intracellular and extracellular signaling mechanisms play an important role (Liu et al., 2021). Our understanding of the roles of ECs in neuronal regeneration and repair is currently limited. ECs are multifunctional proteins expressed by many cell types in the body (including neurons and astrocytes), known to facilitate extracellular protein quality control processes. We propose that future studies of ECs in the context of neuronal damage and disease have significant potential to lead to the development of valuable new therapies. In this article, we have focussed on four ECs (transthyretin, clusterin, α2-macroglobulin, and neuroserpin) because these proteins are constitutively present in cerebrospinal fluid (CSF) and have been directly implicated in neurodegenerative disease and diseases associated with neuronal damage and repair (Satapathy and Wilson, 2022). We aim to provide a critical review of what is currently known about the functions of ECs in neuronal repair and regeneration and highlight outstanding questions and future directions for this important research area.

Database Search Strategy

The manuscript used peer-reviewed articles chosen from PubMed, PubMed Central, Google Scholar and Web of Science (Clarivate) identified using individual or combinations of the following keywords: Protein misfolding, extracellular chaperones, transthyretin, clusterin, α2-macroglobulin, neuroserpin, inflammation, cell viability. The date of the last database search was between February 20 and March 30, 2022.

Current Knowledge of the Role of Extracellular Chaperones in Neuronal Repair and Regeneration

A major role of chaperones is to protect organisms from the consequences of inappropriate protein aggregation and toxicity. As a result of age or ongoing chemical or physical stresses, proteins can misfold to form aggregates that are either amorphous or amyloid (fibrillar) in structure, some of which are cytotoxic (Gidailevitz et al., 2011; Hidalgo San Jose et al., 2020). ECs have an ATP-independent action and are best-known for their abilities to (a) inhibit the aggregation of misfolded or damaged proteins, (b) maintain aggregating proteins in a soluble state, and (c) form stable complexes with aggregating extracellular proteins to facilitate their clearance from the extracellular space and subsequent safe disposal by intracellular degradation (Wyatt et al., 2011). ECs are abundant in human body fluids such as plasma and CSF (Prikrylova Vranova et al., 2016), saliva (Pallardo-Fernández et al., 2020), urine (Musiaf et al., 2020), and semen (Saleh et al., 2020). In addition, these multifunctional proteins have roles that include suppressing inflammation, inhibiting apoptosis, promoting cell proliferation and survival, modulating ECM composition and organization, and acting as immune modulators (Satapathy and Wilson, 2022). Many of the biological functions of ECs outlined above have been proposed to play critical roles in the regeneration of mature cells, including neuronal cells (Guerin et al., 2021). Further investigation into the effects of ECs present in CSF on neuronal repair and regeneration has the potential to lead to the development of new therapies.

Transthyretin

Transthyretin (TTR) is an amyloid-specific EC (West et al., 2021) present at ~15.5 μg/mL in the CSF of healthy human adults (Maetzler et al., 2012). TTR in complex with retinol-binding protein (RBP) transports retinoic acid (RA, a growth factor) to sites of neuronal growth, thereby promoting neuronal regeneration (Vancamp et al., 2019; Eira et al., 2021), differentiation, and patterning under physiological (Wilson et al., 2004) and pathological conditions (Ikeda et al., 2005). RA carried by the TTR:RBP complex also induces the differentiation of neural stem cells into neurons and glial cells (Nonaka et al., 2004).

Abstract

Protein quality control involves many processes that jointly act to regulate the expression, localization, turnover, and degradation of proteins, and has been highlighted in recent studies as critical to the differentiation of stem cells during regeneration. The roles of constitutively secreted extracellular chaperones in neuronal injury and disease are poorly understood. Extracellular chaperones are multifunctional proteins expressed by many cell types, including those of the nervous system, known to facilitate protein quality control processes. These molecules exert pleiotropic effects and have been implicated as playing important protective roles in a variety of stress conditions, including tissue damage, infections, and local tissue inflammation. This article aims to provide a critical review of what is currently known about the functions of extracellular chaperones in neuronal repair and regeneration and highlight future directions for this important research area. We review what is known of four constitutively secreted extracellular chaperones directly implicated in processes of neuronal damage and repair, including transthyretin, clusterin, α2-macroglobulin, and neuroserpin, and propose that investigation into the effects of these and other extracellular chaperones on neuronal repair and regeneration has the potential to yield valuable new therapies.

Key Words: cell viability; clusterin; extracellular chaperones; inflammation; neuroserpin; protein misfolding; transthyretin; α2-macroglobulin

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Clusterin (CLU) is the best-studied EC found at 2–12 μg/mL in the CSF of healthy human adults (Pollock et al., 1993). It has been shown to promote the in vitro and in vivo proliferation and regeneration of damaged (a) epithelial cells of the cornea (Okada et al., 2011) and intestine (Liu and Chen, 2020), (b) renal cells (Gobe et al., 1995), and (c) hair and cochlear cells (Zhao et al., 2003). CLU is secreted by various cell types and is constitutively expressed by neurons under both physiological and pathological conditions and is secreted into the cerebrospinal fluid (CSF) at increased levels in response to neuronal injury and/or neurodegeneration (Satapathy and Wilson, 2022). By inhibiting the accumulation of misfolded proteins, inflammation, MMPs, and apoptosis, the increased expression of ECs is likely to inhibit neuronal injury and/or degeneration and promote neuronal repair and regeneration. Reduced dashed arrows indicate the biological outcomes of neuronal injury/degeneration. Green dashed lines indicate the biological outcomes of increased levels of ECs (arrowheads indicate a positive effect, the solid line indicates inhibition). ECs: Extracellular chaperones.

TTR has also been suggested to play a cytoprotective role in neurodegeneration and acute neuronal injury cases. A decrease in the level of TTR in the CSF has been associated with Alzheimer’s disease (AD; Gião et al., 2010), promote axonal and neurite growth, and facilitate cytoskeletal reorganization following sciatic nerve injury (Eira et al., 2021). Collectively, this preliminary evidence suggests that TTR has the potential to act as a therapeutic agent to reduce inflammation, and promote the survival, repair, and regeneration of neurons.

Neuroserpin (NS) is a constitutively expressed neuronal protein found at relatively low abundance in normal CSF (~7 ng/mL) (Nielsen et al., 2007). NS is a serum protease inhibitor that inhibits the activity of tissue-type plasminogen activator (tPA) (Hastings et al., 1997). Unlike TTR, NS acts as an amyloid-specific EC (West et al., 2021). Several studies have suggested that NS protects regenerating neurons from injury and neurodegeneration. For example, in a mouse model of cerebral hypoxia, a decrease in the CSF NS level has been suggested to result in an increased expression of tPA in brain neurons, ultimately resulting in neuronal cell death (Tsirka et al., 1995; D’Acuto et al., 2021). Furthermore, in a rat model of stroke, the level of NS protein in brain neurons was significantly increased as early as 6 hours post-stroke and remained high up to 28 days after the stroke (Santos et al., 2010). In the same study, direct injection of NS protein into the brain resulted in a 64% reduction in the stroke volume when compared with rats injected with a placebo (Yepes et al., 2000). These observations suggest that the cytoprotective potential of NS makes it an attractive candidate for exploration as a therapeutic agent to promote neuronal repair and regeneration.

Concluding Hypothesis and Future Directions

ECs are (i) abundantly found in CSF, and their levels increase following neuronal injury or neurodegenerative stress, and (ii) known to regulate multiple biological processes including those that are important for the repair and regeneration of cells, such as cell proliferation, apoptosis, inflammation, and interactions with the ECM. Based on these observations we propose that ECs are key players in neuronal repair and regeneration and that future studies to better characterize their effects in this specific context has the potential to lead to the development of valuable new therapies for neurodegenerative diseases. We propose that a focus on the following outstanding questions in the field would bring us closer to being able to harness the therapeutic potential of ECs to treat neuronal damage and disease:

1. In neuronal culture systems, what are the effects on receptor expression and neuronal cell viability of (i) exogenous supplementation of ECs, and (ii) silencing of the expression of one or multiple ECs using CRISPR-mediated gene editing (Bock et al., 2022)?
2. Is the level of expression of cell surface receptors known to be important in neuronal growth and regeneration (e.g., LRP1, integrins, and neurotrophic receptors) affected by the expression of ECs in injured and healthy neurons?
3. Does the level of expression of ECs affect (a) the expression of other intracellular or secreted proteins known to be important for cell repair and regeneration and/or (b) the differentiation of neural stem cells?

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Figure 1  Proposed model for the roles of ECs in the repair and regeneration of neurons.
Conflicts of interest: The authors declare that there are no competing interests associated with the manuscript.

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