

Astrocyte-derived extracellular vesicles mediate intercellular communications of the neurogliovascular unit

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The cortical grey matter of mammals has a specific cyto-architecture defined by the process of “tiling” in which protoplasmic astrocytes parcellate the nervous tissue into spatially segregated territorial domains. Within these domains astrocytes integrate neuronal structures, microglial cells and neighboring capillaries into the neurogliovascular unit or neurogliovascular unit (NGVU) (Verkhratsky and Nedergaard, 2018; Sweeney et al., 2019). Signaling within NGVU is of fundamental importance for sustaining brain function, while disruptions of these signaling pathways define many aspects of neuropathology.

Extracellular vesicles (EVs) provide a potent tool for intercellular communication by acting as a miniature lipid membranous container for wide array of signaling molecules (Mathieu et al., 2019). The biological significance of EVs stands on two pillars. First, EVs are secreted by all types of cells. Second, molecular cargo content of EVs depends on the origin of producing cell, its phenotype and its physiological or pathological state. Thus, EVs from healthy cells may have therapeutic properties, whereas EVs from diseased tissues may act as promoters of pathology. The concept of EV-based signaling provides an exciting and novel dimension to the intercellular communication operating at the NGVU. Here we focus on the astrocyte-derived EVs and their potential contribution to pathological remodeling of the blood-brain barrier (BBB) in neurodegenerative diseases. The NGVU cellular elements include specialized brain capillary endothelial cells, which form the vascular barrier sealed by tight junctions, pericytes, vascular smooth muscle cells in arterioles, glial elements and neurons (Sweeney et al., 2019). The parenchymal part of the blood-brain interface is formed by astrocytic endfeet. The perivascular space between endothelial and parenchymal basement membranes at the pre- and post-capillary levels is utilized by the glymphatic system for the removal of waste products, for transport of biologically active molecules and can also be used for transport of EVs. It is well known that astrocytes support formation and integrity of BBB by secreting numerous paracrine factors including ligands of Wnt

and Hedgehog signaling pathways, retinoic acid, glial cell line-derived neurotrophic factor, vascular endothelial growth factor, fibroblast growth factor, angiopoietins and many others (Sweeney et al., 2019). These factors influence barrier properties of BBB mostly through up-regulation of expression of tight junction and adherens junction proteins, such as claudin-5, occludin, zonula occludens-1 protein, cadherins, and catenins. However, the role of astroglia-secreted EVs in regulation of the BBB integrity in the physiological and pathological settings remains largely unexplored. We have recently analysed effects of EVs derived from immortalized astrocytes prepared from hippocampi of triple transgenic Alzheimer’s disease (AD) model mouse (3xTG-AD) and wild-type controls (Rocchio et al., 2019) on monolayers of immortalized human brain endothelial cells (hCMEC/D3). It appeared that, in contrast to healthy cells, AD astrocytes fail to support the BBB integrity. Astrocytes from healthy animals up-regulated expression of tight junction proteins and increased electrical resistance of endothelial monolayers, whereas co-culturing with AD astrocytes failed to stimulate tight junction proteins expression and did not increase the electrical resistance of endothelial monolayers (Kriauciunaite et al., 2020). We further discovered that EVs derived from WT-iAstro and 3Tg-iAstro mimicked effects of their parent cells on the BBB: treatment with EVs from healthy astrocytes increased trans-endothelial electrical resistance and up-regulated expression of claudin-5, occludin, and zonula occludens-1 protein, whereas EVs from 3Tg-iAstro were ineffective (Kriauciunaite et al., 2020). These astroglial deficits may reflect global astroglial asthenia and loss of their protective capabilities in AD-affected nerve tissue (Verkhratsky et al., 2019). Further comparative “omics” analyses are needed for identification of molecules responsible for the observed effects. Of note, in our experimental setting we have used immortalized cells from different species; therefore future studies should address the effects of human astrocyte EVs in humanized disease-specific *in vitro* models of BBB.

Conceptually, many paracrine factors secreted by astrocytes and affecting

functional properties of BBB can be conveyed by EVs. For instance, ligands of Hedgehog and Wnt signaling cascades, which promote barrier integrity, are lipid-modified hydrophobic morphogens. The EVs can transport these ligands as well as retinoic acid along hydrophilic extracellular environment (Tanaka et al., 2005). It is conceivable that within the NGVU morphogens are packed into astroglia-derived EVs and thus transported to the adjacent endothelial cells, or even to the more distant sites through the Virchow-Robin space.

Several reports demonstrated that astroglial EVs can carry growth factors such as fibroblast growth factor 2, vascular endothelial growth factor, apolipoprotein-D, synapsin1, Hsp70 chaperone, and glutamate transporters EAAT-1 and EAAT-2 (Upadhyaya et al., 2020), but the effects of these proteins on the BBB are currently unknown. Cytokine and chemokine profiling of human astrocytes using proteome arrays revealed that healthy human astrocytes secrete granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, chemokine (C-X-C motif) ligand 1, interleukin-6, interleukin-8, chemokine (C-C motif) ligand 2, macrophage migration inhibitory factor, and serpin E, whereas stimulation with interleukin-1 beta and tumor necrosis factor-alpha triggers release of interleukin-1 beta, interleukin-1 receptor antagonist, tumor necrosis factor-alpha chemokine (C-X-C motif) ligand 10, chemokine (C-C motif) ligand 3, chemokine (C-C motif) ligand 5 (Choi et al., 2014). Many cytokines and chemokines, in addition to be soluble messengers, are known to be released in EV-encapsulated form and trigger biological effects after contact with target cells (Fitzgerald et al., 2018). These findings add another layer of complexity as it is becoming increasingly difficult to delineate precise mode of action of signaling molecules carried by EVs. It is tempting to speculate that signaling molecules presented to the target cells in soluble form induce different effects from the same molecules presented by the EVs. Differences in functional responses depend on many factors. Signaling molecule, for example, can be incorporated into the EV membrane, or it can be stored in the EV lumen. Furthermore, target cells may interact with EVs in different ways (employing for example clathrin-dependent endocytosis, macropinocytosis, phagocytosis, lipid raft-mediated internalization, etc.). Finally, vesicles carry many biologically active molecules that may conceal or amplify specific signals. Therefore both soluble and vesicular fractions of chemical messengers should be analyzed and compared for better understanding of astroglial secretome.

Astroglial EVs contribute to the pathogenesis of neurological disorders by carrying toxic aggregates of A β_{42} protofibrils, sAPP β and ApoE $\epsilon 4$ in Alzheimer's disease, mutant SOD1 and TDP43 proteins in amyotrophic lateral sclerosis as well as various pro-inflammatory cytokines, miRNAs and complement components (Upadhyay et al., 2020). Although much of attention is now focused on the diagnostic and prognostic value of disease-associated astrocytic EVs, in the near future their role in the pathogenesis of the disease needs to be addressed.

All cellular components of the BBB secrete multiple EVs in various physiological and pathological contexts, and hence the emerging view of multidirectional communications within NGVU is getting rather complex (Figure 1). New methods allowing precise monitoring of behavior and fate of cell-specific EVs within NGVU are of special importance. A recent study used live-cell reporter pHluorin-CD63 to visualize secreted EVs (exosomes) in 3D culture and *in vivo* (Sung et al., 2020). This approach allowed observation of pathfinding behavior of migrating cells along extracellularly deposited EVs trails. Another study used cre-dependent exosome reporter (CD63-GFP) mice by inserting a loxP-floxed stop codon upstream of human CD63 tagged with GFP (Men et al., 2019). By using AAV8-CaMKII-Cre (directing expression in neurons), AAV5-gfap-Cre (in astrocytes), or AAV-pgk-Cre (in microglia and oligodendrocytes) viruses the cell-type-specific labeling of CD63-GFP exosomes in different CNS cell types is readily achievable. In particular this approach led to identification of a new exosomal miR-mediated neuron to glial signaling pathway (Men et al., 2019). We expect that in the near future similar EV-labeling techniques will be used for probing in the BBB *in vitro* models assembled from healthy or diseased human iPSCs. This approach will enable systematic characterization of mode of action of cell-type specific EVs at the NGVU.

In conclusion, rapidly developing field of EV research is opening a new dimension of complexity for intercellular communication occurring within the brain tissue. In years to come we may witness remarkable increase in our understanding of potential role of the EVs in controlling the BBB, which may facilitate the development of novel therapeutic strategies.

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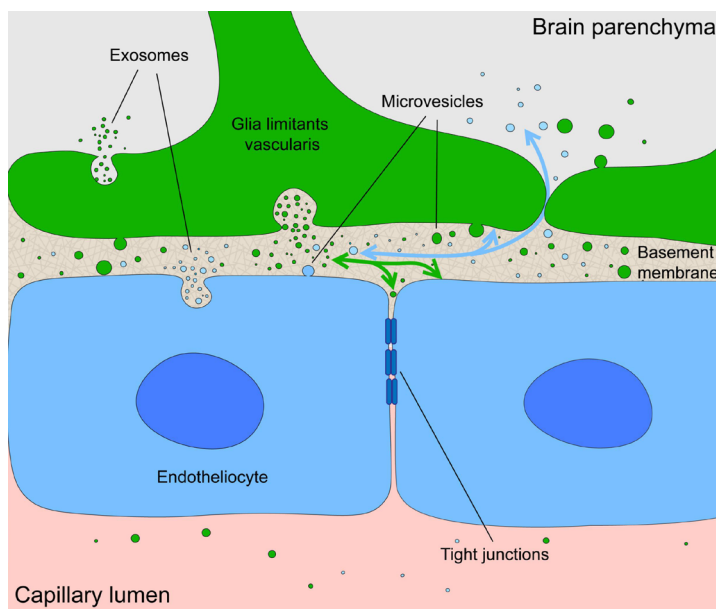


Figure 1 | Extracellular vesicles mediate intercellular communications at the neurogliovascular unit.

A simplified diagram shows intercellular communications between vascular endothelial cells and astrocytes at the level of brain capillaries. Extracellular vesicles (exosomes and microvesicles) released by astrocytes and endothelial cells can be transported to the adjacent cells, cross blood-brain barrier and enter into the blood, travel via perivascular (Virchow-Robin) space to the more distant sites or enter into the brain parenchyma.

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