COMMENTARY

Glia modulate growth of the fly neurovascular unit

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The last decade has seen an explosion of research on exosomes, small (30 to 100 nm) vesicles that are trafficked to the extracellular environment by the fusion of the multivesicular body to the plasma membrane (1, 2). Exosomes arise from endosomal microdomains that, through the agency of ESCRT proteins and Alix, curve inward and bud as intraluminal vesicles (50 to 150 nm) into the lumen of the multivesicular body. The small GTPases, Ral1, Rab27a, and Rab27b, mediate the transport and fusion of multivesicular bodies to plasma membrane, resulting in the exocytic release of the exosomes into the extracellular space (3, 4). Once thought of primarily as disposal devices, tiny garbage bags, exosomes are now appreciated to act as hormone-like signals. Cells may use exosomes to exert systemic influence through the activity of exosomal microRNAs (miRNAs), messenger RNAs, and proteins. Indeed, exosomes and miRNAs have been purified from the vertebrate circulation, and shown to play important roles in health and disease (5). Likewise, exosomes have been purified from the invertebrate hemolymph (6). In PNAS, Tsai et al. (7) reveal that *Drosophila* glial exosomes stimulate growth of target motor neurons and trachea in larvae. The authors not only identify the source of a specific population of exosomal vesicles but also the identity of the cell populations targeted by the glial exosomes, and a key genetic target of the exosomal miRNA within the target cells.

Glial Regulation of Neuronal and Tracheal Growth

Glia were once considered to provide largely supportive roles in the nervous system, but are now considered to play key regulatory roles (8), including in synaptogenesis. Tsai et al. (7) show that the *Drosophila* subperineural glia serve as the key provider of miR-274 containing exosomes that stimulate growth of motor neuron synapses on muscle, and branching of tracheal terminal cells. The subperineural glia cover the entire central nervous system surface and form pleated septate junctions, thus forming a paracellular barrier that is the insect equivalent of a blood–brain barrier (hemolymph–brain barrier). At the neuromuscular junction, subperineural glia extend processes that interact with motor neuron boutons, providing a transforming growth factor β growth cue as well as recycling secreted neurotransmitters (9). In PNAS, Tsai et al. demonstrate that exosomes released by subperineural glia into the hemolymph—the fluid occupying the open insect vascular system—are taken up by motor neurons and further stimulate bouton formation by decreasing expression of the receptor tyrosine kinase pathway inhibitor, Sprouty (10–12). Exosomes released into the hemolymph are also taken up by tracheal terminal cells, and the consequent reduction in Sprouty within terminal cells induces the formation of additional terminal branches. Adult glial cells had previously been found by another group (13) to require miR-274 cell autonomously to promote circadian behavior, and Tsai et al. note a developmental requirement for miR-274 in blood–brain barrier; however, these requirements appear to be entirely distinct from the nonautonomous roles of glial-derived miR-274 exosomes.

Roles for Glia and MicroRNAs in Systemic Response to Hypoxia

In both vertebrate and invertebrate systems, the response to hypoxic stress may be significantly regulated by miRNAs and by glia. For example, in vertebrates, exosomes generated by hypoxic cells can promote an angiogenic response (14), and hypoxic astrocytes are thought to promote vascular growth via vascular endothelial growth factor secretion (15). In *Drosophila*, 4 miRNAs have previously been implicated in the response to hypoxia (16). Indeed, miR-190 expression was found to be induced by hypoxia and, in turn, to promote the hypoxic response by down-regulating HIF proly hydroxylase (Fatiga), a negative regulator of Hifα (Sima) stability. Now miR-274 is shown by Tsai et al. (7) to act through regulation of Sprouty expression levels. The roles of 2 other miRNAs are yet to be defined. Like hypoxic astrocytes in the vertebrate retina, recent work has shown that hypoxic

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perineural glia in *Drosophila* neuromuscular junctions drive responses to hypoxia—in this case, a change in bouton morphology—by Hifα-dependent secretion of a growth factor (Wingless) (17). How miR-274 expression and incorporation into exosomes is regulated and how those exosomes are targeted to specific recipient cells remain fascinating questions for future work.

1 J. Meldolesi, Exosomes and ectosomes in intercellular communication. *Curr. Biol.* **28**, R435–R444 (2018).
2 J. Zhang *et al.*, Exosome and exosomal microRNA: Trafficking, sorting, and function. *Genomics Proteomics Bioinformatics* **13**, 17–24 (2015).
3 V. Hyenne *et al.*, RAL-1 controls multivesicular body biogenesis and exosome secretion. *J. Cell Biol.* **211**, 27–37 (2015).
4 M. Ostrowski *et al.*, Rab27a and Rab27b control different steps of the exosome secretion pathway. *Nat. Cell Biol.* **12**, 19–30, 1–13 (2010).
5 W. Liu *et al.*, Role of exosomes in central nervous system diseases. *Front. Mol. Neurosci.* **12**, 240 (2019).
6 J. M. Dhahbi *et al.*, MicroRNAs circulate in the hemolymph of *Drosophila* and accumulate relative to tissue microRNAs in an age-dependent manner. *Genomics Insights* **9**, 29–39 (2016).
7 Y.-W. Tsai *et al.*, Glia-derived exosomal miR-274 targets Sprouty in trachea and synaptic boutons to modulate growth and responses to hypoxia. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 24651–24661 (2019).
8 J. B. Zuchero, B. A. Barres, Glia in mammalian development and disease. *Development* **142**, 3805–3809 (2015).
9 M. R. Freeman, *Drosophila* central nervous system glia. *Cold Spring Harb. Perspect. Biol.* **7**, a020552 (2015).
10 T. Casci, J. Vinós, M. Freeman, Sprouty, an intracellular inhibitor of Ras signaling. *Cell* **96**, 655–665 (1999).
11 N. Hacohen, S. Kramer, D. Sutherland, Y. Hiromi, M. A. Krasnow, *Sprouty* encodes a novel antagonist of FGF signaling that patterns apical branching of the *Drosophila* airways. *Cell* **92**, 253–263 (1998).
12 S. Kramer, M. Okabe, N. Hacohen, M. A. Krasnow, Y. Hiromi, Sprouty: A common antagonist of FGF and EGF signaling pathways in *Drosophila*. *Development* **126**, 2515–2525 (1999).
13 S. You, T. A. Fulga, D. Van Vactor, F. R. Jackson, Regulation of circadian behavior by astroglial microRNAs in *Drosophila*. *Genetics* **208**, 1195–1207 (2018).
14 Y. Mao *et al.*, Hypoxic exosomes facilitate angiogenesis and metastasis in esophageal squamous cell carcinoma through altering the phenotype and transcriptome of endothelial cells. *J. Exp. Clin. Cancer Res.* **38**, 389 (2019).
15 A. Rattner, J. Williams, J. Nathans, Roles of HIFs and VEGF in angiogenesis in the retina and brain. *J. Clin. Invest.* **130**, 3807–3820 (2019).
16 A. L. De Lella Ezcurra *et al.*, miR-190 enhances HIF-dependent responses to hypoxia in *Drosophila* by inhibiting the prolyl-4-hydroxylase fatiga. *PLoS Genet.* **12**, e1006073 (2016).
17 P. Y. Chen, Y. W. Tsai, Y. J. Cheng, A. Giangrande, C. T. Chien, Glial response to hypoxia in mutants of NPAS1/3 homolog Trachealess through Wg signaling to modulate synaptic bouton organization. *PLoS Genet.* **15**, e1007980 (2019).