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Novel intercellular transportation system may have potential for delivering RNAi and other gene-based therapeutics

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This illustration by Vivian Budnik, PhD, was featured on the cover of the journal Neuron, which published her team's research on the role of exosomes in cell signaling mechanisms.

(Medical Xpress)—Important new research from UMass Medical School

demonstrates how exosomes shuttle proteins from neurons to muscle cells where they take part in critical signaling mechanisms, an exciting discovery that means these tiny vehicles could one day be loaded with therapeutic agents, such as RNA interference (RNAi), and directly target disease-carrying cells. The study, published this month in the journal *Neuron*, is the first evidence that exosomes can transfer membrane proteins that play an important role in cell-to-cell signaling in the nervous system.

"There has been a long-held belief that certain cellular materials, such as integral membrane proteins, are unable to pass from one cell to another, essentially trapping them in the cell where they are made," said Vivian Budnik, PhD, professor of neurobiology and lead author of the study. "What we've shown in this study is that these cellular materials can actually move between different cell types by riding in the membrane of exosomes.

"What is so exciting about this discovery is that these exosomes can deliver materials from one cell, over a distance, to a very specific and different cell," said Dr. Budnik. "Once inside the recipient cell, the materials contained in the exosome can influence or perform processes in the new cell. This raises the enticing possibility that exosomes can be packed with gene therapies, such as RNAi, and delivered to diseased cells where they could have a therapeutic effect for people."

Discovered in the mid-80s, exosomes have only recently attracted the attention of scientists at large, according to Budnik. Exosomes are small vesicles containing cellular materials such as microRNA, messenger RNAs (mRNAs) and proteins, packaged inside larger, membrane-bound bodies called multivesicular bodies (MVBs) inside cells. When MVBs containing exosomes fuse with the cell plasma membrane, they release these exosome vesicles into the extracellular space. Once outside the cell, exosomes can then travel to other cells, where they are taken up.

The recipient cells can then use the materials contained within exosomes, influencing cellular function and allowing the recipient cell to carry out certain processes that it might not be able to complete otherwise.

Budnik and colleagues made this startling discovery while investigating how the synapses at the end of neurons and nearby muscle cells communicate in the developing Drosophlia fruit fly to form the neuromuscular junction (NMJ). The NMJ is essential for transmitting electrical signals between neurons and muscles, allowing the organism to move and control important physiological processes. Alterations of the NMJ can lead to devastating diseases, such as muscular dystrophy and Amyotrophic lateral sclerosis (ALS). Understanding how the NMJ develops and is maintained is important for human health.

As organisms develop, the synapse and muscle cell need to grow in concert. If one or the other grows too quickly or not quickly enough, it could have dire consequences for the ability of the organism to move and survive. To coordinate development, signals are sent from the neuron to the muscle cell (anterograde signals) and from the muscle cell to the neuron (retrograde signals). However, the identity of these signals and how their release is coordinated is poorly understood.

Normally, the vesicle protein Synaptotagmin 4 (Syt4) is found in both the synapse and the muscle cells. Previous knockout experiments eliminating the Syt4 protein from Drosophlia have resulted in stunted NMJs. Suspecting that Syt4 played an important role in retrograde signaling at the developing NMJ, Budnik and colleagues used knockdown experiments to decrease Syt4 protein levels in either the neurons or the muscle cells. Surprisingly, when RNAi was used to knockdown Syt4 in the neurons alone, Syt4 protein was eliminated in both neurons and muscles. The opposite was not the case. When Syt4 was knocked down in muscle cells only, there was no change in the levels of Syt4 in either muscles or neurons.

To confirm this, Budnik and colleagues inserted a Syt4 gene into the neurons of a Drosophlia mutant completely lacking the normal protein. This restored Syt4 in both neurons and muscle cells. Further experiments suggested that the only source of Syt4 is the neuron. These observations were consistent with the model that Syt4 is actually transferred from neurons to muscle cells. As a transmembrane protein, however, Syt4 was thought to be unable to move from one cell to another through traditional avenues. How the Syt4 protein was moving from neuron to muscle cell was unclear.

Knowing that exosomes had been observed to carry transmembrane proteins in other systems and from their own work on the Drosophlia NMJ, Budnik and colleagues began testing to see if exosomes could be the vehicle responsible for carrying Syt4 form neurons to muscles. "We had previously observed that it was possible to transfer transmembrane proteins across the NMJ through exosomes, a process also observed in the immune system," said Budnik. "We suspect this was how Syt4 was making its way from the neuron to the muscle."

When exosomes were purified from cultured cells containing Syt4, they found that exosomes indeed contained Syt4. In addition, when these purified exosomes were applied to cultured muscle cells from fly embryos, these cells were able to take up the purified Syt4 exosomes. Taken together, these findings indicate that Syt4 plays a critical role in the signaling process between synapse and muscle cell that allows for coordinated development of the NMJ. While Syt4 is required to release a retrograde signal from muscle to neuron, a component of this retrograde signal must be supplied from the neuron to the muscle. This establishes a positive feedback loop that ensures coordinated growth of the NMJ. Equally important is the finding that this feedback mechanism is enabled by the use of exosomes, which can shuttle transmembrane proteins across cells.

"While this discovery greatly enhances our understanding of how the neural muscular junction develops and works, it also has tremendous promise as a potential vector for targeted genetic therapies," said Budnik. "More work needs to be done, but this study significantly supports the possibility that exosomes could be loaded with therapeutic agents and delivered to specific cells in patients."

Provided by University of Massachusetts Medical School

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