

Atrophy of Muscle Cells Results in a Selective Packaging of Proteins into Exosomes

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Skeletal muscle atrophy occurs in a variety of conditions and can result in decreased quality of life and mortality. Previous work from our lab established that certain microRNAs in muscle cells play a role in the progression of muscle atrophy and the intracellular level of these microRNAs are altered during atrophy, at least in part, due to incorporation into small vesicles (termed exosomes) released into the extracellular environment. Currently, little information exists about muscle released exosomes. Potentially these vesicles could be taken up by other tissues and identify a mechanism by which muscle signals other tissues during chronic conditions in which atrophy is occurring. However, to know what signaling pathways these exosomes may potentially be involved in, it is important to know what potential signaling molecules are present in exosomes released from muscle cells during atrophy. **Purpose:** To identify if exosomes released from muscle cells during atrophy contain different internal cargo proteins than exosomes from healthy muscle cells. **Methods:** C2C12 cells were treated with dexamethasone (DEX; 1 μ M) for 6 hours in serum free media, media was collected, and exosomes were isolated from the media. LC-MS proteomic analysis was performed on proteins isolated from exosomes, and analyzed using Ingenuity Pathway Analysis software. Nanoparticle tracking analysis (Nanosight) was performed on a separate set of exomes measure vesicle size and number. **Results:** Compared to control cells, the exosomes released during DEX-induced muscle atrophy contained 135 proteins increased greater than two-fold and 159 proteins decreased greater than two. Nanoparticle tracking analysis revealed no change in the number of exosomes released during atrophy (6.77x10⁸ vs 7.06 x 10⁸ vesicles/mL). However, while there was no change in the total number of exosomes the size profiles of the exosomes released during atrophy was different. **Conclusion:** Skeletal muscle atrophy results in both a selective packaging of proteins into exosomes and unique size profile of exosomes released from muscles, but does not alter the total number of exosomes released. These novel findings could have broad implications for the development of biomarkers in skeletal muscle atrophy.

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