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Circulating muscle-derived mir-206 links skeletal muscle dysfunction to cardiac autonomic denervation

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Purpose: Recent studies and our preliminary data demonstrate that muscle-specific ablation of the autophagy-related protein Atg7, leads to block of autophagy, sarcopenia and destabilization of the neuro-muscular junction (NMJ). In addition, Atg7 knock-out (Atg7 KO) muscle fibers release exosomes containing the muscle specific, miR-206, which is consistently elevated in the plasma. Interestingly, we found that miR-206 content was elevated in the heart, suggesting cardiac uptake of the miR-carrying circulating exosomes. We thus aimed at defining the effects of miR-206 on heart homeostasis.

Methods: Here, we analyzed the cardiac phenotype of adult (12mo.) and aged (24mo.) Atg7 KO mice, as well as of adult C57BL/6J mice injected, via tail vein, with scramble- or miR-206-loaded exosomes. Exosomes were isolated from EDL muscle of control and Atg7 KO mice, as well as from HEK293 cells. Heart function was assessed by echocardiography and ECG-telemetry. Confocal IF, whole-mount IF on heart blocks and multiphoton imaging were used to assess heart structure and sympathetic innervation. Bioinformatics, molecular and biochemical analyses were performed to identify novel targets of miR-206. IF, BRET assay and imaging of TrKA translocation were performed in cultured sympathetic neurons (SNs).

Results: We demonstrate that circulating exosomes, containing miR-206, are taken up by the heart leading to sympathetic dysinnervation, accompanied to reduction in the neurogenic control of cardiac rhythm and increased arrhythmogenesis. In vitro assays demonstrated that exosome-carried miR-206 targets cardiac SNs (cSNs), compromising cell structure and function. Indeed, increased miR-206 expression is accompanied by cSN atrophy, irregular axonal distribution of the active neurotransmitter release sites, and reduction in axonal sprouting. These effects are likely attributed to the miR-206-mediated down-regulation of the NGF receptor p75, as demonstrated by bioinformatics, luciferase assay, molecular and biochemical analyses in vitro and ex vivo. BRET assay, performed in cultured SNs treated with exosomes carrying miR-206, showed reduced formation of p75/TrkA complexes, which generate high-affinity binding sites for NGF and enhance neurotrophin responsiveness. Consistent with impaired NGF retrograde transport, miR-206 over-expressing SNs displayed reduced NGF protein content and decreased phosphorylation of Akt, which is an NGF downstream target, regulating neuronal survival. Interestingly, these latter results were confirmed in the stellate ganglia from Atg KO and miR-206 treated mice.

Conclusions: We identify miR-206 as a key molecular player in the “muscle-to-heart” communication. miR-206 may participate to the pathogenesis of secondary cardiac dysfunction in skeletal muscle diseases associated to increased circulating levels of miR-206, ranging from ageing to neurodegenerative disorders (i.e. ALS, DMD).
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miR-206 causes heart dysinnervation