LRP4 is critical for muscle spindle development

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Background

Muscle spindles are stretch-sensitive mechanoreceptors that are embedded in skeletal muscles. They are composed of several intrafusal muscle fibers packed in capsules. Intrafusal muscle fibers are innervated by γ motor neurons at both polar ends and coiled by afferent sensory axons at the central regions. Muscle spindle provide feedback for balance and gait and their damage can alter sensorimotor function. How the development and maintenance of muscle spindles are regulated is not well understood. LRP4 is a receptor for agrin, both of which are required for MuSK activation in NMJ formation and maintenance. However, whether and how LRP4 regulates muscle spindle development remain elusive.

Methods

To examine whether LRP4 expressed in muscle spindle, we firstly constructed LRP4-CreER mice by knock-in of a 2A-CreERT2 cassette in exon 38 of the LRP4, and then crossed LRP4-CreER mice with Ai9 reporter mice. Tamoxifen was injected into the mice to induce the LRP4-Cre expression and label cells with tdTomato. Immunohistochemistry staining (IHC) of Neurofilament combined with the annulospiral endings structure were used to locate muscle spindle. To investigate the role of LRP4 in spindle development, Lrp4 null mice, all exon except the signal peptide were replaced by LacZ, were used during embryonic 15.5 to 18.5 for IHC staining. Besides, to elucidate the underlying mechanism and whether agrin-LRP4-MusK signaling involved in spindle development and maturation, we further characterized phenotype of *agrin* and *MusK* null mutant.

Results

We found that tdTomato driven by the endogenous LRP4 enriched in the muscle spindle, both intrafusal fibers and capsules. The initiation of muscle spindle, begins at E14.5-E15.5 when sensory neuron contact with myotubes, seemed normal in *Lrp4* null mutant, as well as in agrin and MuSK mutant. The induction of muscle spindle differentiation, drives by the arrival of sensory neuron, were indistinguishable in all three mutant compared to their littermate control at E18.5. Additionally, the NMJ between γ motor neurons and intrafusal fibers were disrupted in *Lrp4, agrin* and *MuSK* mutant, probably implying the same mechanism of NMJ formation both in and outside of the muscle spindle. However, the innervation of spindle by sensory neuron coiled around in the central region, as well as γ motor neurons innervated at polar ends were

reduced in *Lrp4* mutant. Intriguing, Egr3 was observed to be degenerated in some muscle spindle of this mutant at E18.5.

Conclusion

Our study finds LRP4 is enriched in the capsule and intrafusal fibers. Further study suggests that LRP4 is critical for muscle spindle development. More importantly, this function independent of NMJ formation. The regulation of Egr3 expression by LRP4 might be a novel mechanism in muscle spindle.