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Roles of collagen Q in MuSK antibody-positive myasthenia gravis

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ABSTRACT

The low-density lipoprotein receptor-related protein 4 (LRP4) and the muscle-specific receptor tyrosine kinase (MuSK) form a tetrameric protein complex on the postsynaptic membrane at the neuromuscular junction (NMJ). Binding of agrin to LRP4 triggers phosphorylation of MuSK. Activated MuSK drives clustering of acetylcholine receptor (AChR). Wnt ligands also directly bind to MuSK to induce AChR clustering. MuSK anchors the acetylcholinesterase (AChE)/collagen Q (ColQ) complex to the synaptic basal lamina. In addition, an extracellular proteoglycan, biglycan, binds to MuSK.

Anti-MuSK autoantibodies (MuSK-IgG) are observed in 5–15% of autoimmune myasthenia gravis (MG) patients. MuSK-IgG blocks both ColQ-MuSK and LRP4-MuSK interactions. MuSK-IgG, LRP4, ColQ, and biglycan bind to the immunoglobulin-like domains 1 and 4 of MuSK. Lack of the effects of cholinesterase inhibitors in MuSK-MG patients is likely due to hindrance of ColQ-MuSK interaction by MuSK-IgG and subsequent deficiency of AChE observed in model mice, which, however, has not been proven in MuSK-MG patients. As ColQ enhances expression of membrane-bound MuSK, inhibition of ColQ-MuSK interaction by MuSK-IgG may account for lack of AChR clusters in MuSK-MG. We thus made passive transfer models using *Colq*+/+ and *Colq*−/− mice to dissect the effect of ColQ on AChR clustering in MuSK-MG. We found that MuSK-IgG-mediated suppression of LRP4-MuSK interaction, not of ColQ-MuSK interaction, caused defective AChR clustering. We also unexpectedly observed that both MuSK-IgG and ColQ suppressed agrin/LRP4/MuSK signaling in dose-dependent manners. Quantitative comparison revealed that MuSK-IgG blocked agrin-LRP4-MuSK signaling more than ColQ.

We propose that attenuation of AChR clustering in MuSK-MG is due to hindrance of LRP4-MuSK interaction in the presence of agrin by MuSK-IgG.

1. Introduction

The aim of this communication is to comprehensively review molecular mechanisms of anti-MuSK autoantibody in myasthenia gravis (MG). As MuSK plays a central role in pathogenesis of MuSK-MG, domains of MuSK that physiologically interact with LRP4, Wnt ligands, ColQ, and biglycan will be addressed. In addition, mechanisms underlying physiological splicing regulation of human *MUSK* exon 10, which encodes a binding-domain of Wnt ligands, will be addressed.

2. Tetrameric LRP4/MuSK complex as a receptor for agrin and Wnt ligands

The low-density lipoprotein receptor-related protein 4 (LRP4) and the muscle-specific receptor tyrosine kinase (MuSK) form a tetrameric protein complex on the postsynaptic membrane at the neuromuscular junction (NMJ). Agrin is released from the motor nerve terminal, and binding of agrin to LRP4 triggers MuSK phosphorylation [1,2].

Activated MuSK with other intracellular proteins including Dok-7 induces clustering of the acetylcholine receptor (AChR) at the postsynaptic membrane [3]. Wnt ligands also directly bind to and phosphorylate MuSK to induce AChR clustering especially at an early stage of development [4,5]. A small leucine-rich proteoglycan, biglycan, is another molecule that binds to the ectodomain of MuSK [6]. Physiological role of binding of biglycan to MuSK, however, remains unsolved.

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3. Collagen Q (ColQ) binds to perlecan and MuSK to anchor acetylcholinesterase (AChE) to the synaptic basal lamina

A catalytic subunit of AChE forms a tetramer and binds to a single molecule of ColQ [7,8]. ColQ forms a triple helix and constitutes an AChE/ColQ complex that is comprised of twelve AChE subunits and three ColQ. The AChE/ColQ complex is anchored to the synaptic basal lamina by two mechanisms. First, a pair of positively charged heparan sulfate proteoglycan-binding domains (HSPBDs) in the collagen domain of ColQ bind to perlecan [9–11]. Second, the C-terminal domain (CTD) of ColQ binds to MuSK [12]. ColQ is thus another molecule that binds to the ectodomain of MuSK. We previously reported that both HSPBDs and CTD are required for anchoring AChE/ColQ to the synaptic basal lamina [11]. Mutations in CTD do not affect formation of the AChE/ColQ complex, but compromise anchoring of AChE/ColQ complex to the NMJ [11–13]. Lack of ColQ reduces membrane-bound MuSK in myotubes [14], which is likely to a cause of compromised AChR clustering in *Colq*−/− mice [15].

4. MuSK domains that bind to Wnt ligands, biglycan, ColQ, and LRP4

Wnt ligands bind to MuSK. The ectodomain of MuSK has three immunoglobulin (Ig)-like domains (Ig1, Ig2, and Ig3) and a frizzled-like cysteine-rich domain (Fz-CRD) [16–18]. Fz-CRD carries ten cysteines forming five disulfide bonds [18], which are divided into six cysteines in the C6 box and four cysteines in the fourth Ig-like domain (Ig4) [19,20]. Ig4 was historically named for the four cysteine-containing domain [20] and is used in this communication, but there is no immunoglobulin-like motif in Ig4. The ten cysteines are a hallmark of frizzled proteins, which are receptors for Wnt-ligands [21]. Deletion of Fz-CRD of MuSK in mice causes a drastic deficit in formation of AChR clusters [22]. *MUSK* exon 10 encoding the C6 box is alternatively skipped in human, but not in mice, which produces a Wnt-insensitive MuSK. We recently reported that binding of an RNA-binding protein, hnRNP C, to a "UUUU" motif on *MUSK* exon 10 facilitates binding of two other RNA-binding proteins, YB-1 and hnRNP L, to its downstream "CAACACCU" motif on the same exon 10 [23] (Fig. 1). HnRNP C, YB-1, and hnRNP L coordinately suppress recognition of *MUSK* exon 10 in splicing, and cause skipping of *MUSK* exon 10. Physiological significance of generation of a Wnt-insensitive exon 10-skipped MuSK only in human remains unknown.

Biglycan binds to Ig1 and Ig4 of MuSK [6]. A missense mutation in the Ig1 domain of MuSK suppresses binding of MuSK to LRP4 and attenuates agrin-stimulated MuSK phosphorylation [24]. We recently reported that CTD of ColQ, as well as LRP4, bind to Ig1 and Ig4 of MuSK [25] (Fig. 2). Interestingly, among the four molecules that bind to MuSK, three (biglycan, ColQ, and LRP4) bind to Ig1 and Ig4, and one (Wnt) binds to Fz-CRD of MuSK.

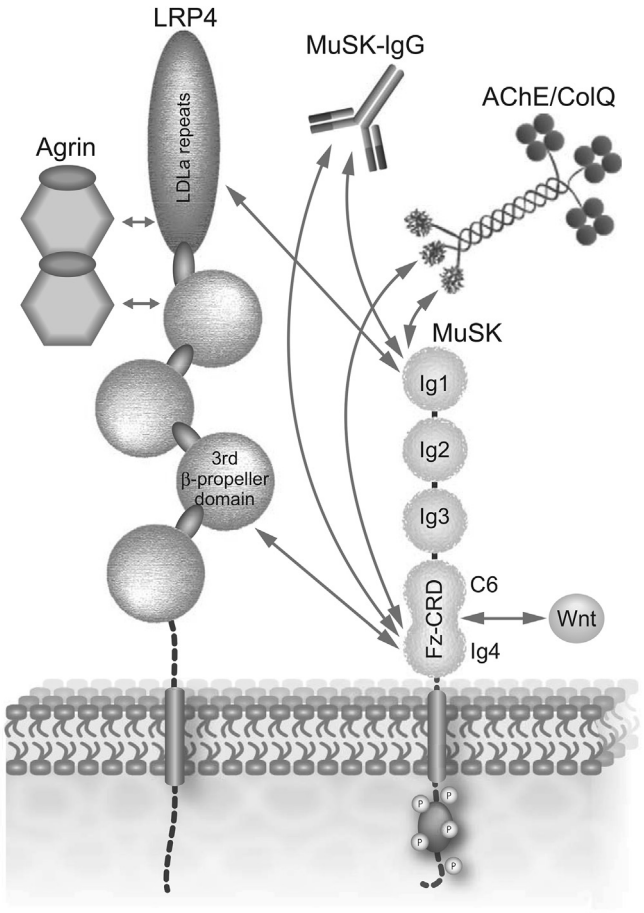

Fig. 2. Schematic interactions (double-headed arrows) of MuSK for LRP4, ColQ, MuSK-IgG, and Wnt ligands. Wnt ligands bind to the Fz-CRD domain of MuSK that is comprised of C6 box and Ig4. Ig1 and Ig4 domains of MuSK bind to the 4th and 5th LDLa repeats close to the N-terminal end and the third β-propeller domain of LRP4 [24]. Ig1 and Ig4 domains of MuSK also bind to ColQ and MuSK-IgG [25]. MuSK-IgG blocks ColQ-MuSK and LRP4-MuSK interactions, which reduces AChE and AChR at the NMJ [25]. Both ColQ and MuSK-IgG hinder MuSK-LRP4 interaction, and suppress MuSK phosphorylation [25].

In contrast to MuSK domains, binding domains of LRP4 for MuSK were previously established: the 4th and 5th LDLa repeats close to the N-terminal end, and the third β-propeller domain [24]

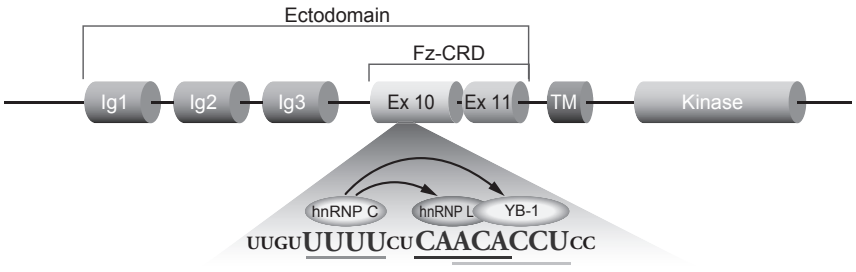

Fig. 1. Domain structure of MuSK. Fz-CRD is a Wnt-responsive domain, which is comprised of a C6 box containing 6 cysteines encoded by exon 10 and Ig4 domain containing 4 cysteines encoded by exon 11. TM, transmembrane domain. An RNA-binding protein, hnRNP C, binds to "UUUU", which stabilizes binding of two other RNA-binding proteins, hnRNP L to "CAACA" and YB-1 to "ACACCU". HnRNP C, hnRNP L, and YB-1 coordinately enhance skipping of exon 10 to generate a Wnt-insensitive MuSK isoform [23].