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Drosophila collagen type XV/XVIII: a novel chondroitin sulfate proteoglycan and its biological functions

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During metazoan evolution, the advent of basement membranes is one of the important events, enabling their bodies to be equipped with functionally diversified tissues and to adjust to various environments on earth. Recent genomic sequencing revealed “basement membrane tool-kit”, the core components of basement membranes, are evolutionarily conserved and widely shared among metazoans (1). One of such molecules, multiplexin collagens, represented by vertebrate collagen types XV/XVIII, are structurally characterized by multiple glycosaminoglycan (GAG) side chains and flanked by N-terminal thrombospondin type I repeat and C-terminal endostatin domain.

Mutations in *COL18A1* gene cause Knobloch Syndrome 1, characterized by progressive vitreoretinal degeneration, while progressive skeletal/cardiac myopathies and neuropathies were reported in *Col15a1* knock out mice (2). In addition, defects in cell migration and axon guidance were identified in zebrafish, nematode and *Drosophila* mutants.

To further explore the biological functions of multiplexin collagen, we examined *Drosophila* orthologue Multiplexin (Mp) loss of function mutants. Adults manifested minor defects in wing veins or in abdominal cuticle plates, while mutant larvae looked apparently normal, but they responded poorly to physical stimuli, implying defects in the peripheral nervous system (PNS). We examined the PNS structure by immunostaining to find altered neurites formations in mutants, which prompted us to ask where Mp exists in the PNS. To answer this question, we prepared antibody against Mp to perform immunostaining. This antibody recognized multiple bands of expected sizes and some of them were increased after chondroitinase ABC treatment, indicating that Mp is modified with chondroitin sulfate side chains. Immunostaining with this antibody revealed Mp expressions in subsets of neuroblasts aligned in a unique segmental pattern in the central/peripheral nervous system. Moreover, Mp expression was

observed in contractile cardioblasts in the developing heart. As this expression pattern and the phenotypic manifestations strongly implicated Mp in the Wingless/Wnt signaling pathway, therefore, we compared Wingless distribution between wild types and mutants. To our surprise, mutants exhibited abnormally reduced Wingless distribution (3).

In the next exploration, to understand the pathogenesis of multiplexin-related diseases, we had asked whether or not mutant flies also suffer from progressive deterioration of muscles. To test this, we performed flight assays to compare the age-related changes of flight capacity between wild types and mutants. By one week, mutants exhibited a significantly poorer flight capacity, a reminiscent phenotype of *Col15a1* knock out mice. Ultrastructural analysis revealed alterations of mitochondrial structure in mutants, accompanied by functional deteriorations of mitochondria. These observations uncovered an unexpected role of multiplexin in mitochondrial homeostasis, which is probably shared by vertebrate as well and can be exploited for the development of therapeutic strategies to cure multiplexin-related human diseases (4). Some of our trials will be presented.

Thus, multiplexin collagens are not merely physical scaffolds. They are important for establishing the proper gradient formation of Wingless/Wnt molecules, which is likely mediated by the GAG side chains, and also for mitochondrial homeostasis.

References:

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