Genotype - phenotype relationship in Drosophila type IV collagen mutants

Ph.D. THESIS Booklet

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Publications directly related to the subject of thesis

I. **Drosophila basement membrane collagen col4a1 mutations cause severe myopathy**
Kelemen-Valkony I[a], Kiss M[a], Csiha J[a], **Kiss A[a]**, Bircher U[a], Szidonya J[a], Maróy P[a], Juhász G[c], Komonyi O[a], Csiszár K[b], Mink M[a, *]
Matrix Biology (2012)
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II. **Drosophila type IV collagen mutation associates with immune system activation and intestinal dysfunction**
Márton Kiss[a], **András A. Kiss[a]**, Monika Radics[a], Nikoletta Popovics[a], Edit Hermesz[b], Katalin Csiszár[c] and Mátyás Mink[a]
Matrix Biology *(2016)*
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III. **Altered stress fibres and integrin expression in the Malpighian epithelium of Drosophila type IV collagen mutants**
András A. Kiss[a], NikolettaPopovics[a], GáborSzabó[a], Katalin Csiszár[b], Mátyás Mink[a],*
Data in Brief *(2016)*
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IV. **4-Hydroxy-2-nonenal Alkylated and Peroxynitrite Nitrated Proteins Localize to the Fused Mitochondria in Malpighian Epithelial Cells of Type IV Collagen Drosophila Mutants**
András A. Kiss[1], Nikoletta Popovics[1], Zsolt Boldogkői[1], Katalin Csiszár[2] and Mátyás Mink[1]
BioMed Research International *(2018)*
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V. **Novel Phenotypic Elements of Type IV Collagenopathy Revealed by the Drosophila Model**
András A. Kiss[1], Nikoletta Somlyai-Popovics[1], Vilmos Tubak[2], Zsolt Boldogkői[1], Katalin Csiszár[3] and Mátyás Mink[1*]
Applied Sciences *(2019)*
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VI. **Type IV Collagen Is Essential for Proper Function of Integrin-Mediated Adhesion in *Drosophila* Muscle Fibers**
András A. Kiss[1], Nikoletta Somlyai-Popovics[1], Márton Kiss[1], Zsolt Boldogkői[1], Katalin Csiszár[2] and Mátyás Mink[1]
International Journal of Molecular Sciences *(2019)*
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Publications indirectly related to the subject of thesis

VII. **Muscle dystrophy is triggered by type IVcollagen alleles affecting integrin binding sites directly or indirectly in Drosophila**
Márton Kiss[1], Ildikó Kelemen-Valkony[1], **András Kiss[1]**, Brigitta Kiss[2], Katalin Csiszár[3], Mátyás Mink[1]
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Introduction

Mammals carry three pairs of head-to-head type IV collagen genes, whereas *Drosophila* has one, the *col4a1* and *col4a2* loci in the same genomic organization [1]. Mutations in the *COL4A3*, *COL4A4* and *COL4A5* genes associate with Alport Syndrome [2,3]. Deletions within the *COL4A5* and *COL4A6* genes are also reported to cause diffuse leiomyomatosis [4]. Heterotrimers of the composition $[COL4A1]_2COL4A2$ are the stoichiometrically most significant molar ratios of mammalian basement membranes. *Col4a1* or *Col4a2* mouse mutants develop the most complex systemic phenotypes that affect the central nervous, ocular, renal, pulmonary, vascular, reproductive, and musculoskeletal systems [5–7] as in humans [8]. Severe muscle phenotypes have been shown in patients with the *COL4A1* mutation as part of a multi-system disorder referred to as hereditary angiopathy with nephropathy, aneurysms, and muscle spasms (HANAC) [9,10].

In *Drosophila col4a1* mutants, a conditional, temperature-sensitive allele series were identified. The $col4a1^{-/-}$ homozygotes are embryonic lethal, whereas $col4a1^{+/-}$ heterozygotes are viable and fertile at the

permissive temperature of 20 °C, but perish under restrictive conditions of 29 °C. In these mutants, we have presented severe myopathy [1], irregular and thickened BM, detachment of the gut epithelial and visceral muscle cells from the BM [11], intestinal dysfunction, antimicrobial peptides overexpression, hydrogen peroxide and peroxynitrite overload [12]. We have demonstrated fused mitochondria, membrane peroxidation [13], actin stress fibers, and irregular integrin expression in the epithelial cells of the Malpighian tubule [14]. Our results suggested that muscular dystrophy may also be present in the *Drosophila col4a1* mutant [15].

To characterize the muscle phenotype, in the *col4a1* allelic mutant series, we have performed in immunohistochemical experiments. Abnormal sarcomeres, altered integrin expression and localization, Z-disk disorganization, streaming, and atrophy were detected by focusing on striated oviduct muscle. Collectively, the results indicate that the dystrophic muscle phenotype in mutants is derived from compromised integrin interactions with abnormal COL4A1 and supports

the role of type IV collagen as part of integrin-mediated muscle cell adhesion.

The Collagens

The key to the diversity of multicellular animals is the ability of cells to connect and form organs and tissues. This process required a molecular "glue" to hold them together. These molecular "adhesives" are collagens. The collagen proteins that make up the basement membrane are triple-helical and are capable of a binding [16]. As a result of the interconnection in a defined way, a regular network structure is obtained. The primary function of this mesh is to provide a support structure and to provide considerable flexibility and resistance to organs and tissues. They also play a crucial role in cell migration, cell adhesion, regeneration, and signaling. The collagen superfamily is a very complex family, including the 28 known collagen and collagen-like proteins, into 9 major families [17]. All collagen proteins share a common structural feature but have at least a triple-helical domain. Collagen is found throughout our bodies. Different types of organs and tissues display their characteristic collagen network. They

make up the cartilage, the vitreous body in the eyes, and can also be found in the vascular walls, the lungs, kidneys, and the basement membrane [18]. In my dissertation, I want to deal more with type IV collagen.

Aims

Immunohistochemical images were taken of the common oviduct of Drosophila to demonstrate the loss of the sarcomere structure and the Z-disks streaming due to the *col4a1* mutation. Some structural proteins have been selected to follow phenotypic changes (actin, COL4A1, integrin, kettin).

The determination of the amount of CO4A1 protein, in control, and the mutant animals by Western blot. For a more comprehensive picture, samples were taken from two different stages of development, incubated at different temperatures (larva and adult). For specific measurements, we made our own antibody for the experiment.

Detection of differences in the expression of extracellular matrix proteins at different stages and times

of development in the midgut (integrin PSI and PSII alpha subunits, Laminin Gamma-1 and COL4A1 protein).

To confirm the adverse systemic character of the mutation throughout the body, immunohistochemical staining was performed on proteins involved in the formation of the cytoskeletal-extracellular axis (actin, COL4A1, integrin) in the Malpighian tubules.

Detection of quantitative changes in protein alkylation, lipid peroxidation, and mitochondrial aggregation caused by temperature increase in *col4a1* mutants was induced from Malpighi tubules by immunohistochemical methods using confocal microscopy.

Methods

Eight dominant temperature-sensitive (DTS) mutant Drosophila melanogaster strains were available in our lab. The mutations are on the chromosome 2, and each is a point mutation affecting the glycine amino acid. The strains were maintained using a balancer chromosome (chromosome 2). Wild-type *Oregon* flies and *col4a1*

mutant stocks were maintained at 20 °C and 29 °C on yeast-cornmeal- sucrose-agar food, consisting of nipagin to prevent fungal infection. Malpighian tubules, gut and oviduct were removed under carbon dioxide anaesthesia from adults that were grown at both the permissive and restrictive temperature for tissue dissection. Immunostaining was then performed with various antibodies. Photomicrographs of the Malpighian tubules, intestinal tract and common oviduct were generated by confocal laser scanning fluorescence microscopy (Olympus Life Science Europa GmbH, Hamburg, Germany). The statistical calculations were performed with Microsoft Office Excel and free R-3.5.2 and RStudio-1.1.463. The western blot experiments were performed from whole flies.

Results and conclusions

Because the phenotype of the oviduct and the larval body wall muscle [1], threatened the organization and deposition of actin, loss of sarcomere structure was observed in all the alleles tested, with features characteristic of myopathic or dystrophic states, with disintegrating muscle sarcomeres together with disintegration and streaming of Z-discs [19].

The Western blot was performed because our hypothesis was that the expression of COL4A1 protein was lower in the mutant flies. In my opinion, the gene carrying the point mutation adversely affects expression at the permissive temperature. No difference was found between adult wild-type and mutant animals at 20 °C. In mutant adult animals, however, the amount of COL4A1 protein was apparently reduced at the restrictive temperature (29 °C). Earlier results from our group indicate that there was no decrease in the transcriptional level of *col4a1* mRNA. These data suggest that decreasing levels of COL4A1 protein in the mutants at 29 °C are caused by inadequate assembly and deposition.

We wanted to validate and visualize these results by immunohistochemistry and were also interested in whether other proteins involved in the formation of the cytoskeletal-extracellular axis with COL4A1 exhibit a similar phenotype. The selected proteins were COL4A1, alpha subunits of integrin PS I and PS II, and Laminin gamma 1. We found that integrin staining and reduction in the midgut of mutant L3 larvae were at the permissive temperature. This phenomenon is exacerbated at restrictive temperatures, its distribution is irregular, and can be observed in adult animals. Laminin expression was also reduced at restrictive temperature in the midgut of mutant L3 larvae. The COL4A1 protein accumulates in the midgut of mutant animals and shows a further reduction, diffuse, and uneven staining at restrictive temperatures.

Following the common oviduct and the midgut, another organ was included in the study, the Malpighian tubule, which is analogous to the mammalian kidney. Immunohistochemical staining was performed on the organs of adult animals at different ages (3 and 18 days old) and at different temperatures. The investigated

proteins were the actin, COL4A1, and integrin alpha PS I and PSII subunits. Our recordings clearly showed that col4a1 mutants increase the number of actin stress fibrils as a result of restrictive temperature, decrease the amount of COL4A1 protein level, and the integrin distribution is uneven.

As part of the stress response, *Drosophila col4a1* mutants synthesize peroxynitrite above physiological concentrations. Excessive production of peroxynitrite initiates severe protein tyrosine nitration and protein alkylation, which adversely affects protein function, and also induces membrane lipid peroxidation and mitochondrial fusion. Wild-type control animals did not show these posttranslational modifications under physiological conditions because the nitrosoperoxycarbonate pathway was used to eliminate peroxynitrite. We suggest that in the mutant *col4a1 Drosophila* model, posttranslational protein modifications are an integral part of the col4a1-associated pathology and represent post mechanical details that have not yet been detected in humans or mice COL4A1 mutants.

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