Growing a thicker heart wall under *ELAC2*-linked cardiomyopathy condition in *Drosophila*

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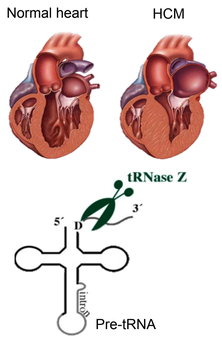
Abstract

Hypertrophic cardiomyopathy (HCM) is a pathological condition characterized by the thickening of the left ventricular heart wall. Some especially severe cases of HCM were associated with mutations of ELAC2 gene. *ELAC2* encodes the RNaseZ endonuclease which is essential for tRNA maturation. Previously, we have reported creating a Drosophila model of RNaseZ-linked HCM. Here we investigate the underlying processes leading to the heart wall thickening. We created a cardiomyocyte specific marker with strong expression at all stages of fly development. We used this marker to count the number of cardio cells in fly heart and found that RNaseZ mutant flies experience cardiomyocyte hyperplasia. Using immunostaining, we demonstrated that RNaseZ mutations cause increased deposition of extracellular matrix, a phenomenon similar to fibrosis in humans.

Background

Infantile hypertrophic cardiomyopathy (HCM) is an etiologically heterogeneous disease, characterized by thickening of heart wall which restricts the blood flow.

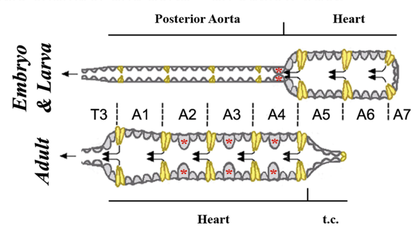
Some severe cases of HCM have been linked to recessive mutations of ELAC2. Patients diagnosed with ELAC2 linked HCM have medium life expectancy of four months and die from heart failure (1).



ELAC2 is a highly conserved gene with homologs in all eukaryotes. It encodes RNaseZL endonuclease that is essential for tRNA molecules maturation. Its function is to remove the 3' trailer of tRNA precursor molecule (2).

Due to high homology of ELAC 2 among different organisms our lab has been studying this protein using its *D. melanogaster* homolog dRNaseZ (3). Two sites of ELAC2 missense mutations that are linked to HCM, are conserved between human and fly proteins (Fig. 1), which allows to model the effect of these mutations on heart in flies.

Drosophila heart also known as dorsal vessel is a tubular structure formed by 104 contractile cardiomyocytes (CM) arranged in pairs of two opposing rows forming a luminal space between them. The number of CM is established at embryogenesis and does not change throughout larval growth. During metamorphosis the number of CM is reduced to 86 to form adult heart (4). The dorsal vessel is divided into a heart – a contractile chamber and aorta – an outflow tract.



Results

Drosophila homolog of ELAC2 used to study pathogenic mutations

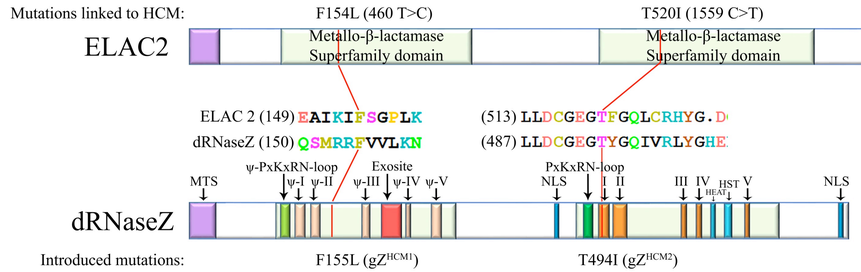


Figure 1. Comparison of human ELAC2 and Drosophila RNaseZ proteins. Domain structures of ELAC2 and dRNaseZ proteins is shown. The structural elements of both MBL domains are similar between fly and human proteins. Alignment of ELAC2 and RNaseZ was performed with ClustalW2 and the regions around the amino acids affected by HCM-linked mutations are shown. Genetic constructs of fly RNaseZ carrying a mutation homologous to either P154L or T520I were named gZ^{HCM1} and gZ^{HCM2} respectively.

Mutations of RNaseZ cause fly heart hypertrophy.

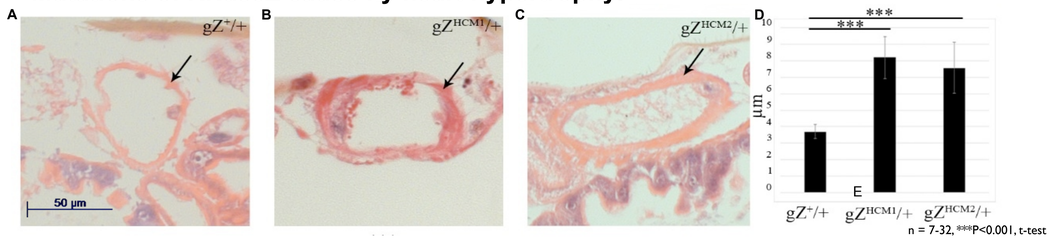
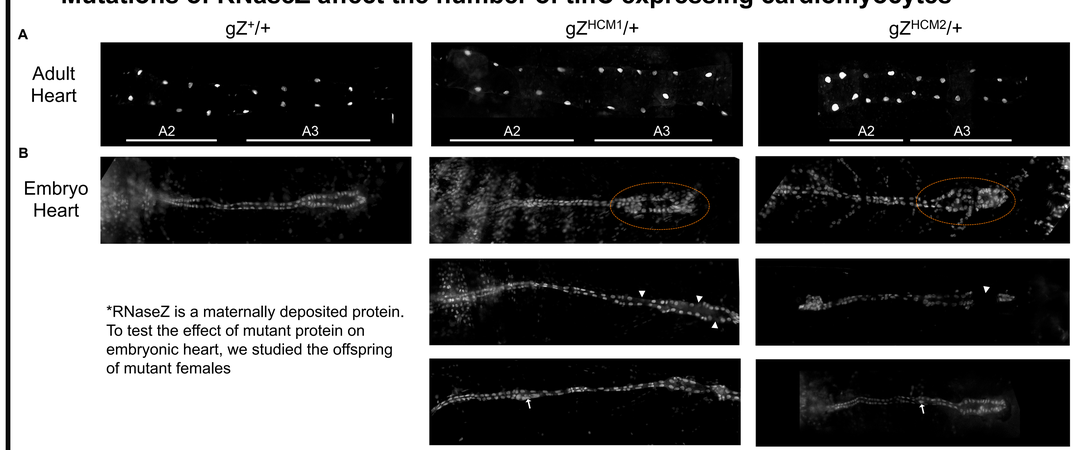


Figure 2: Histological preparation of adult flies. (A-C) Representative images of A1/A2 adult abdominal segments in transverse orientation. Arrows point to the heart. Two mutant genotypes gZ^{HCM1} (B) and gZ^{HCM2} (C) are compared to wild type control $gZ^{+}/+$ (A). (D) Summary data for heart wall thicknesses measured from serial transverse histological sections

Mutations of RNaseZ affect the number of tinC expressing cardiomyocytes



*RNaseZ is a maternally deposited protein. To test the effect of mutant protein on embryonic heart, we studied the offspring of mutant females

Figure 3: Novel construct 4x-tinC-GFP^{NLS} allowed visualizing cardiomyocyte nuclei in adult flies and embryos. (A) While wild type adult hearts have 8 CM per segment, a subset of mutant flies (25%) exhibited increased number of nuclei, up to 11 per segment. (B) There is a range of effects of mutant RNaseZ in embryonic hearts. A subset of mutant hearts display missing nuclei in the row of CM (arrowheads) while other subset of hearts display supernumerary nuclei (circled) and enlarged nuclei (arrows)

Mutations of RNaseZ cause heart fibrosis

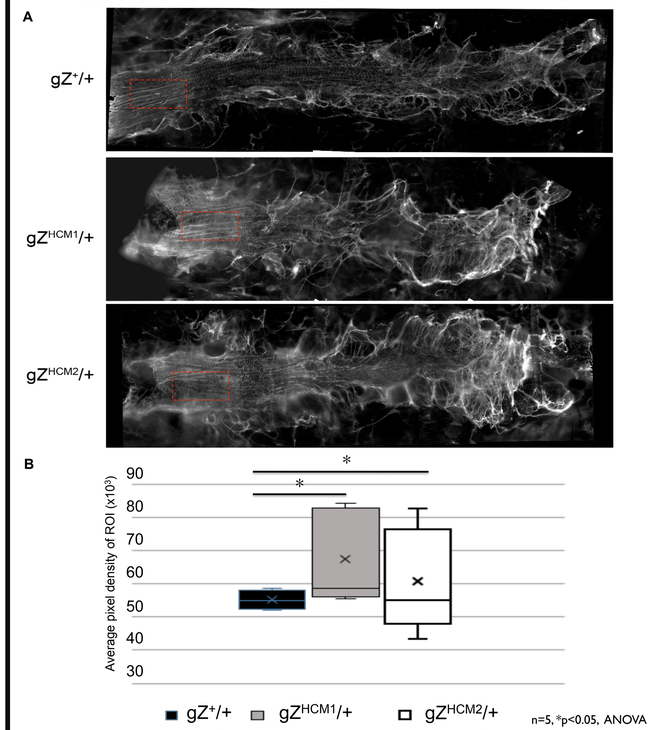


Figure 4: Immunostaining of Pericardin, a heart specific, collagen IV like protein, constituent of extracellular matrix. (A) representative images of Pericardin accumulation near adult hearts. (B) The amount of Pericardin fluorescence signal in region of interest (ROI) as shown in boxes. ROI was selected over a bulk of heart in A1-A2 segment.

Conclusions

In this study we found:

- Mutations of dRNaseZ cause heart hypertrophy in flies similar to the symptoms due to mutations of ELAC2 in humans.in this disease.
- The flies with mutant RNaseZ have increased number of CM nuclei.
- Th effect of mutant RNaseZ on number of CM nuclei is exhibited immediately post cardiogenesis with variety of possible displays.
- Mutations of RNaseZ cause increased deposition of extracellular matrix around fly heart.

Our future direction is to investigate if cardio cell hypertrophy is another factor contributing to heart hypertrophy in RNaseZ mutants.

References

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