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Research Article

Genetic link between Cabeza, a Drosophila homologue of Fused in Sarcoma (FUS), and the EGFR signaling pathway

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ABSTRACT

Amyotrophic Lateral Sclerosis (ALS) is a fatal neurodegenerative disease that causes progressive muscular weakness. Fused in Sarcoma (*FUS*) that has been identified in familial ALS is an RNA binding protein that is normally localized in the nucleus. However, its function in vivo is not fully understood. *Drosophila* has Cabeza (Caz) as a *FUS* homologue and specific knockdown of Caz in the eye imaginal disc and pupal retina using a *GMR-GAL4* driver was here found to induce an abnormal morphology of the adult compound eyes, a rough eye phenotype. This was partially suppressed by expression of the apoptosis inhibitor P35. Knockdown of Caz exerted no apparent effect on differentiation of photoreceptor cells. However, immunostaining with an antibody to Cut that marks cone cells revealed fusion of these and ommatidia of pupal retinae. These results indicate that Caz knockdown induces apoptosis and also inhibits differentiation of cone cells, resulting in abnormal eye morphology in adults. Mutation in EGFR pathway-related genes, such as *rhomboid-1*, *rhomboid-3* and mirror suppressed the rough eye phenotype induced by Caz

Abbreviations: Caz, Cabeza; ALS, Amyotrophic Lateral Sclerosis; *FUS*, Fused in Sarcoma; EGFR, Epidermal growth factor-receptor; SOD1, Cu/Zn superoxide dismutase; TDP-43, TAR DNA-binding protein of 43 kDa gene; CNS, central nervous system; APF, after pupal formation; ERK, Extracellular signal-related kinase.

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knockdown. Moreover, the *rhomboid-1* mutation rescued the fusion of cone cells and ommatidia observed in Caz knockdown flies. The results suggest that Caz negatively regulates the EGFR signaling pathway required for determination of cone cell fate in *Drosophila*.

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease that is characterized by degeneration of upper and lower motor neurons of the brain and the spinal cord, which leads to progressive muscle weakness and fatal paralysis [1]. Most cases of ALS are sporadic, but some patients have a familial history as a result of a mutation in the gene for Cu/Zn superoxide dismutase (SOD1) [2].

The family of MAPKs includes ERK, p38 and JNK. Each MAPK signaling pathway consists of at least three components, a MAPK kinase kinase, a MAPK kinase and a MAPK. Deviation from strict control of MAPK signaling pathways has been implicated in the development of human neurodegenerative diseases including Alzheimer's, Parkinson's and ALS [3]. Recently it was reported that aberrant expression and activation of p38 in motor neurons and microglia play important roles in ALS progression [4]. Persistent activation of p38 correlates with degeneration of motor neurons in transgenic mice expressing a mutant SOD1 [5,6]. Moreover a p38 inhibitor was demonstrated to prevent the apoptosis of motor neurons induced by a mutant SOD1 [7]. Thus a possible link between MAPK signaling and ALS has been suggested.

A substantial number of proteins linked to ALS are directly or indirectly involved in RNA processing [8]. Among RNA-binding proteins, mutations in the TAR DNA-binding protein of 43 kDa gene (*TDP-43*) and fused in sarcoma (*FUS*) gene have been identified as major genetic causes in both familial and sporadic ALS [9–18]. *TDP-43* and *FUS* are implicated in multiple aspects of RNA metabolism including transcriptional regulation, mRNA splicing and mRNA shuttling between the nucleus and the cytoplasm [19,20].

Drosophila has a single orthologue of human *FUS*, named Cabeza (*Caz*). In situ hybridization and immunohistochemistrical analyses demonstrated that *Caz* mRNA and protein are enriched in the brain and central nervous system (CNS) during embryogenesis, and the *Caz* protein has been detected in the nuclei of several larval tissues and in imaginal discs [21,22]. The full-length recombinant *Caz* protein and its RRM domain are capable of binding RNA in vitro [21]. These findings suggest that *Caz* is a nuclear RNA binding protein that may play an important role in the regulation of RNA metabolism during *Drosophila* development.

In our previous studies using neuron specific *Caz* knockdown flies, we demonstrated that *Caz* functions in neuronal cell bodies and/or axons of the CNS and is involved in elongation of synaptic branches of motoneurons [22]. However, contributions of *Caz* during development of various tissues in *Drosophila* are not fully understood. As a first step toward clarification, we investigated the effect of knockdown of *Caz* on eye development and revealed a rough eye phenotype, accompanied by apoptosis, abnormal differentiation of cone cells and defects in ommatidia rotation. In addition, a *Rhomboid-1* mutant could be shown to rescue the fusion of cone cells and mutations of *rhomboid-3* and *mirror* significantly suppressed the rough eye phenotype of the *Caz* knockdown flies. Since *rhomboid-1*, *rhomboid-3*, and *mirror* are EGFR pathway-related genes, these results indicate genetic links between *Caz* and EGFR signaling.

Materials and methods

Fly stocks

Fly stocks were maintained at 25 °C on standard food containing 0.7% agar, 5% glucose and 7% dry yeast. Canton S was used as the wild type. *w*; *UAS-Caz-IR*$_{363-399}$;+(CG3606) and *UAS-rho-IR*^{28690} was obtained from Vienna *Drosophila* RNAi Center (VDRC). The RNAi of this strain was targeted to the region corresponding to residues 363–399 of *Drosophila Caz* (*UAS-Caz-IR*$_{363-399}$). Four and seven transgenic strains carrying *UAS-Caz-IR*$_{1-167}$ and *UAS-Caz-IR*$_{180-346}$ were established [22]. Each transgenic strain showed a consistent phenotype. Alleles of the following genes were obtained from the Bloomington *Drosophila* stock center: *mirror*^{SaiD3}, *ru*^{1}, *rho*^{7M43} and *rho*^{AA69}. Enhancer trap lines carrying the lacZ markers AE127 (inserted into *seven-up*) [23] and *P82* (inserted into *deadpan*) [24] were obtained from Y. Hiromi and co- workers. These lines express the β-galactosidase marker in photoreceptor cells (R) of R3/R4/R1/R6 and R3/R4/R7. *hspFlp; +; tub*1> *FRT cd*2 *FRT*> *GAL4, UAS-GFP/ TM*3 was a kind gift from A. Plessis. Establishment of lines carrying GMR-GAL4 was as described earlier [25]. Act5C-GAL4/ TM6B was also obtained from the Bloomington *Drosophila* stock center.

Generation of RNAi clones in retinae

RNAi clones in retinae were generated with the flip-out system [26]. Female flies with *hspFlp; +; tub*1> *FRT cd*2 *FRT*> *GAL4, UAS-GFP/ TM*3 were crossed with *w; UAS-Caz-IR*$_{363-399}$;+male flies and clones were marked by the presence of GFP. Flip-out was induced 24–48 h after egg laying with a 60 min heat shock at 37 °C.

Immunostaining

For immunohistochemistory, larval eye imaginal discs and pupal retinae were dissected, and fixed in 4% paraformaldehyde/ PBS for 15 min and 30 min at 25 °C, respectively. After washing with PBS containing 0.3% Triton X-100, the samples were blocked with PBS containing 0.15% Triton X-100 and 10% normal goat serum for 30 min at 25 °C, and incubated with diluted primary antibodies in PBS containing 0.15% Triton X-100 and 10% normal goat serum for 16 h at 4 °C. The following antibodies were used; mouse anti-LacZ (1:500, Developmental Studies Hybridoma Bank [DSHB], 40-1a), mouse anti-Elav (1:200 DSHB 9F8A9), mouse anti-Cut (1:500, DSHB 2B10), mouse anti-Discs large (1:500) (DSHB) and anti-diphospho ERK (dpERK) (1: 500) (Sigma). After extensive washing