A genetic screen for modifiers of Drosophila caspase Dcp-1 reveals caspase involvement in autophagy and novel caspase-related genes

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Abstract

Background: Caspases are cysteine proteases with essential functions in the apoptotic pathway; their proteolytic activity toward various substrates is associated with the morphological changes of cells. Recent reports have described non-apoptotic functions of caspases, including autophagy. In this report, we searched for novel modifiers of the phenotype of Dcp-1 gain-of-function (GF) animals by screening promoter element-inserted Drosophila melanogaster lines (EP lines).

Results: We screened ~15,000 EP lines and identified 72 Dcp-1-interacting genes that were classified into 10 groups based on their functions and pathways: 4 apoptosis signaling genes, 10 autophagy genes, 5 insulin/IGF and TOR signaling pathway genes, 6 MAP kinase and JNK signaling pathway genes, 4 ecdysone signaling genes, 6 ubiquitination genes, 11 various developmental signaling genes, 12 transcription factors, 3 translation factors, and 11 other unclassified genes including 5 functionally undefined genes. Among them, insulin/IGF and TOR signaling pathway, MAP kinase and JNK signaling pathway, and ecdysone signaling are known to be involved in autophagy. Together with the identification of autophagy genes, the results of our screen suggest that autophagy counteracts Dcp-1-induced apoptosis. Consistent with this idea, we show that expression of eGFP-Atg5 rescued the eye phenotype caused by Dcp-1 GF. Paradoxically, we found that over-expression of full-length Dcp-1 induced autophagy, as Atg8b-GFP, an indicator of autophagy, was increased in the eye imaginal discs and in the S2 cell line. Taken together, these data suggest that autophagy suppresses Dcp-1-mediated apoptotic cell death, whereas Dcp-1 positively regulates autophagy, possibly through feedback regulation.

Conclusions: We identified a number of Dcp-1 modifiers that genetically interact with Dcp-1-induced cell death. Our results showing that Dcp-1 and autophagy-related genes influence each other will aid future investigations of the complicated relationships between apoptosis and autophagy.

Background

Apoptosis, or programmed cell death, is an evolutionarily conserved, genetically regulated process, whereby cells that are no longer needed undergo self-destruction through the activation of a cell suicide program [1,2]. This cell death program is associated with characteristic morphological alterations, such as condensation of the nucleus and cytoplasm, fragmentation of nuclear DNA, reorganization of the cytoskeleton, and reduction of the cell into apoptotic bodies that can be phagocytosed by neighbouring epithelial cells or phagocytes [1,3].

Autophagy is also an evolutionarily conserved mechanism that degrades unnecessary long-lived proteins and organelles. During autophagy, cellular components are sequestered by double-membrane structures called autophagosomes. These autophagosomes then fuse with lysosomes to form autolysosomes, where degradation occurs [4]. The autophagy acts as a cellular response against extracellular stresses, such as nutrient starvation, hypoxia, and overcrowding and against intracellular stresses, such as formation of damaged or redundant organelles and cytoplasmic components [4]. Even though autophagy can induce a cell-survival response to some conditions, autophagic structures, especially the autophagic vacuoles, are associated with cell death. This
cell death phenomenon is classified as type II cell death and called autophagic cell death. In *Drosophila*, loss of function of the *Atg* genes leads to lethality in the transition from the larval to pupal stages, because autophagic cell death is essential for puparium formation [4].

The caspases are a family of ubiquitously expressed cysteine proteases whose prototypic member is the *Cae-
norhabditis elegans* death effector, CED-3 [5]. Activation of caspases typically leads to the selective cleavage of a restricted set of target proteins, generally resulting in inactivation of the target proteins. Normally present in cells as inactive precursors, caspases are proteolytically activated following upstream pro-apoptotic signals. Activated caspases cleave their substrates at an aspartic acid residue, and substrate specificity is determined by a four-residue motif N-terminal to the cleavage site [6,7]. The “initiator” caspases primarily activate the down-stream “effector” caspases whose proteolytic activity is directed toward the deconstruction of the cellular machinery during apoptosis.

In *Drosophila*, a normally functioning apoptotic pathway depends critically on caspases, seven of which have been identified in the *Drosophila melanogaster* genome [8,9]. These *Drosophila* caspase genes include three initiators and four effectors. The initiators are *Dronc* (*Drosophila* Nedd-2-like caspase), *Dredd* (Death-related ced-3/Nedd2-like), and *strica/dream*. The effectors are *Dcp-1* (Death caspase-1), *Drice* (*Drosophila* ice), *Damm*, and *decay* (Death executioner caspase related to Apop-
pain/Yama) [10-13]. These caspases are expected to have functions that lead to apoptosis. Many recent reports have described non-apoptotic functions of caspases, such as the cell proliferation function of *Dronc* [14,15], spermatid individualization by *Drice* [14,16], and activation of the *Drosophila* immune system, toll receptor signaling, by *Dredd* [14].

*Dcp-1* proteins cleave cysteine protease substrates and are important for development and oogenesis [13,17]. *Dcp-1* deletion mutants display a lack of germ-line cell death phenotype during mid-stage oogenesis in response to nutrient deprivation [18], whereas in normal flies, cell death occurs during mid-stage oogenesis under nutrient-deprived conditions (stage 7 to 8) [3,19-21]. In contrast, over-expression of a single copy of the truncated N-terminal region of *Dcp-1* (constitutively active *Dcp-1*), specifically in the eye using the *Glass* Multimer Reporter (GMR) promoter, results in a slightly rough and reduced pigment eye phenotype [22]. In addition to the critical roles of *Dcp-1* and caspase 3 in apoptosis, recent studies in mammals and flies suggest that these caspases have many important non-apoptotic roles [13,23], although how these caspases act in these non-apoptotic responses are incompletely understood.

We hypothesized the existence of unknown effectors for *Dcp-1*. Identification and characterization of such proteins would allow us to better understand apoptotic pathways and *Dcp-1*-related non-apoptotic pathways. Since a large-scale genetic screen to identify compo-
nents of the *Dcp-1* pathway had not been preformed, either *in vivo* or *in vitro*, we screened ~15,000 GenExel EP fly lines. Interestingly, we noticed that autophagy-related genes specifically suppressed the rough eye phe-
notype caused by *Dcp-1* expression. In addition to eight autophagy genes and two genes reported to be related to autopha,
y, we identified five Insulin/IGF and TOR signaling genes, six MAP kinase and Jun N-terminal kinase (JNK) signaling, four Ecdysone genes, and others (Additional file 1). There were several interesting novel genes among the 72 *Dcp-1* genetic interactors. The identification of many new *Dcp-1*-interacting genes will help clarify the molecular mechanisms by which *Dcp-1* regulates apoptosis and other non-apoptotic cellular processes. In addition, our findings that autophagy genes influence the roles of the *Dcp-1* caspase in autopha,
y suggest a relationship between autophagy and apoptosis. Finally, our findings that signaling pathways such as MAP kinase, JNK, and ecdysone signaling regulate *Dcp-1* indicate that there are other regulatory path-
ways for caspase functions.

**Results**

**Phenotypes caused by ectopic expression of *Drosophila* caspases**

To identify genes that interact with fly *Dcp-1*, we first generated transgenic flies that over-express full-length *Dcp-1* using the Upstream Activation Sequence (UAS)/GAL4 system. We obtained 49 stable lines by microinj ecting the UAS-*Dcp-1* construct in *w*1118 embryos. Forty out of 49 fly lines carrying this construct showed a small, slightly rough and reduced eye pigment pheno-
type when expressed with the eye-specific GMR-GAL4 drivers (Figure 1A-F). Among the UAS-*Dcp-1* lines we generated, we chose to use UAS-*Dcp-1*19-2 flies in this study for several reasons: First, we found that these flies did not exhibit a lethal phenotype. Second, the UAS-
*Dcp-1* was inserted between *GstS1* and CG30456 without disrupting any flanking genes (Figure 1H). We found that in some other lines the UAS-*Dcp-1* construct was inserted into exon regions of genes (Figure 1I and J). In addition, some transgenic flies showed a male pupal lethal phenotype when induced by GMR-GAL4 (Figure 1G). Lastly, since the GMR-GAL4/UAS-*Dcp-1*19-
2 flies displayed a modest rough eye phenotype, we rea-
soned that this fly line was suitable for identifying both suppressors and enhancers of *Dcp-1* from our modifier screen. The eye phenotype that we observed for the GMR-GAL4, UAS-*Dcp-1* flies was similar to a previous...
study in which flies that express one copy each of GMR-ΔN-Dcp-1 and GMR-fl-Dcp-1 (full-length Dcp-1) showed a faintly-colored and ablated eye phenotype [22]. The intermediate phenotype caused by one copy of GMR-GAL4 and one copy of UAS-Dcp-1 makes these flies very useful for screening for modifiers (Figure 2B and 2F). Flies carrying two copies of GMR-GAL4 and UAS-Dcp-1 displayed a much more severe eye phenotype, in general agreement with the phenotype from a previous study using two copies of GMR-ΔN-Dcp-1 (Figure 2C and 2G). These flies were semi-lethal and had a short life span. As positive controls for the suppressors of Dcp-1 over-expressing flies, we expressed caspase inhibitor p35 and Drosophila inhibitor of apoptosis (Diap1; thread, th). The eye phenotype caused by GMR-GAL4, UAS-Dcp-1-19-2 (hereafter abbreviated as Dcp-1 GF (gain of function); GMR-GAL4 and UAS-Dcp-1-19-2 are linked on one chromosome) was completely rescued by the expression of the caspase inhibitors (Figure 2D and 2H).

**Dcp-1 modifier screen**

We screened ~15,000 EP lines obtained from the Szeged Stock Center, GenExel, and cell signaling pathway gene lines from the Indiana University Bloomington Stock Center and Dr. J. Chung’s laboratory (KAIST, South Korea). Among them, 414 transgenic flies showed suppression or enhancement of the rough eye phenotype caused by the Dcp-1 GF. False positives were excluded by comparing these lines with the lines induced by GMR-GAL4 lines without UAS-Dcp-1. We confirmed that 85 alleles corresponding to 72 genes showed specific genetic interaction with Dcp-1 (Additional file 1). In addition, we verified that the GAL4 mRNA expression was constant even when Dcp-1 GF was crossed with representative alleles, Atg1EP (G13748) and Atg6EP (G6854) (Additional file 2). We also confirmed that EP insertions induced the genes placed down-stream by the GMR-GAL4 while not affecting the expression of flanking genes placed at a distance: the expression level of CG10969, down-stream gene of Atg1, was not affected.

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**Figure 1** *Dcp-1* fly lines used for screening

(A) GMR-GAL4/UAS-Dcp-1-19-2 eyes exhibited a disordered rough eye phenotype with decreased pigmentation.

(B, C, D, E, F) GMR-GAL4/UAS-Dcp-1-19-2, GMR-GAL4/UAS-Dcp-1-19-2, GMR-GAL4/UAS-Dcp-1-19-2, GMR-GAL4/UAS-Dcp-1-19-2, and GMR-GAL4/UAS-Dcp-1-19-2 eyes exhibited a disordered rough eye phenotype and pigment loss.

(G) Lines derived from UAS-Dcp-1, UAS-Dcp-1, and UAS-Dcp-1 showed a male pupal lethality phenotype when crossed with GMR-GAL4.

(H) UAS-Dcp-1-19-2 was inserted in 2R at position 12987659 between GstS1 and CG30456.

(I) UAS-Dcp-1 was inserted in 2R at position 3283377 in the 5’ region of CG1707.

(J) UAS-Dcp-1 was inserted in X at position 9029070 in the 5’ region of Id14.
by EP inserted up-stream of Atg1 gene (Additional file 3) and the expression level of Tango5, down-stream gene of Atg8a, was not affected by EP inserted up-stream of Atg8a gene (Additional file 4).

**Apoptotic cell death pathway**

We identified three genes that are known to regulate apoptotic cell death. Expression of Diap1 suppressed the eye phenotype, whereas EP lines of effete (eff) and faf enhanced the rough eye phenotype (Additional file 1A). These results are consistent with the previous reports that Diap1 is a caspase inhibitor and that eff and faf enhance apoptotic cell death [24,25]. The fact that we recovered apoptosis-modulating genes with the expected direction shows the efficacy of our genetic screen.

**Autophagy-related genes**

Three autophagy-related genes, Aut1, SNF4/AMP-activated protein kinase γ subunit (SNF4Aγ), and Blue cheese (Bchs), were identified as genetic modifiers from unbiased screening: expression of Aut1 and SNF4Aγ suppressed the rough eye phenotype caused by Dcp-1 expression, whereas Bchs expression enhanced this phenotype, (Figure 3E, H and 4A and 4B, respectively). Aut1 is an important regulator of autophagy that is required for modification of Atg8 (Autophagy-specific gene 8) in a ubiquitin conjugation-like manner [26,27]. Aut1 loss of function mutant larvae fail to induce autophagy in the fat body before puparium stage and die during metamorphosis [26]. Recently, SNF4Aγ function in autophagosome formation during larval metamorphosis was revealed through a combination of Drosophila mutational and RNAi studies [28]. We found that eyespecific expression of SNF4Aγ suppressed the rough eye phenotype in the Dcp-1 GF flies (Figure 3H). It has been shown that SNF4Aγ induces developmental and stress-mediated autophagy [28]. Thus, our data suggest that autophagy induced by SNF4Aγ over-expression negatively modulates the Dcp-1-mediated cell death phenotype. Bchs is a Drosophila homolog of human Alfy (autophagy-linked FYVE protein), which may serve as a scaffold protein to promote autophagosome-related vesicle trafficking to lysosomes [29-31]. Since we identified three autophagy-related genes from our primary screen and since EP lines of Aut1, SNF4Aγ, and Bchs resulted in opposite phenotypes, we examined the EP lines for other autophagy-specific genes, including Atg1, Atg2, Atg4, Atg6, Atg7, Atg8a, and Atg18 (Additional file 1B). We found that 10 of 40 EP alleles for autophagy-specific genes partially recovered the disordered ommatidia and
red...t was caused by Dcp-1 GF, as shown by introduction of Atg2\textsuperscript{EP(G6691)}, Atg6\textsuperscript{EP(G665A and G13772)}, and Atg1\textsuperscript{EP(G13748)} (Figure 3BD). Atg1, whose activity is regulated by Tor (Target of rapamycin), initiates the induction of autophagy. Atg2 is important for retrieval of autophagic proteins and vesicles by interacting with membrane protein Atg9 during autophagosome formation. Atg6 plays a role in nucleation of the autophagic vesicles by formation of Class III PI3K complexes [4,32,33]. The general process of autophagy is considered to be a survival mechanism against cell death [34].

These data suggest that important components of autophagy generally counteract the cell death caused by Dcp-1 over-expression. Consistent with this idea, we found that a Bchs\textsuperscript{EP(G2362)} line can suppress the rough eye phenotype in an allele-specific manner (see Additional file 1B and Discussion).

**Genetic interaction between the insulin-IGF pathway or TOR pathway and Dcp-1**

In our screen, we identified a set of genes in the insulin-IGF signaling pathway, including InR (insulin receptor), P3k, Akt1, S6k (ribosomal subunit S6 kinase), Tor (Target of Rapamycin), and Pten (Additional file 1C). Insulin-IGF signaling is initiated by the activation of InR, which leads to the activation of a downstream kinase cascade including P3k and Akt1. On the other hand, PTEN phosphatase inhibits this signaling by dephosphorylating 3-phosphoinositides (PI(3)Ps), the product of P3k [35]. Tor is an important regulator of nutrient responses, cellular growth, and protein synthesis [36]. Tor regulates cellular nutrients by insulin or the AMP:ATP ratio. Tor is activated by the insulin/IGF signaling through the IGF, Akt1, TSC1/2 and Rheb or by the LKB1 and AMPK signaling pathway through LKB1, AMPK, TSC1/2 and Rheb. The signals from these two pathways converge on TSC1/2, Rheb, and Tor [37]. One of the important downstream targets of Tor is S6k, which is an important regulator of nutrient translation [38-40]. We found that the rough eye phenotype caused by Dcp-1 GF was exacerbated by over-expression of InR, P3k, Akt1, Pten, or Tor (Figure 3C-H). The Tor hypomorphic mutant Tor\textsuperscript{K1700E}, which shows a more moderate phenotype than Tor null mutant Tor\textsuperscript{AP} [41], recovered the Dcp-1 GF phenotype (Figure 3G).

We also identified several genes in the TOR pathway from our screen (Additional file 1C). In addition to the main role of S6k in the regulation of cell size [42], recent reports showed that S6k mediates many other physiological processes, such as larval feeding behaviour [43], adult lifespan [44], and autophagy [45]. The progeny from the cross of Dcp-1 GF with the UAS-dS6k showed a suppressed rough eye phenotype (Figure 3F and Additional file 1C). On the other hand, Pten is known to antagonize P3k signaling. However, our data showed that the eye phenotype of Dcp-1 GF was exacerbated by co-expression of UAS-Pten\textsuperscript{20.2}, the line that over-expresses wild type Pten. Although this result seems to contradict the enhancement of the rough eye phenotype caused by P3k expression, recent studies have shown that Pten over-expression induces apoptosis in a cell context-dependent manner even though Pten functions to alleviate the effect of Insulin/IGF signaling [46]. Thus, it is possible that Pten may reinforce the apoptotic effect of Dcp-1 by acting in some other signaling pathways that are distinct from the insulin P3k signaling and may worsen the rough eye phenotype caused by Dcp-1 GF.

Overall, nine autophagy-specific genes as well as Tor, which is a well-known regulator of autophagy, modified Dcp-1 GF. The identification of these genes as rescuers of the Dcp-1 phenotype raised questions about the effect of autophagy genes on Dcp-1 caspase function. Thus, we performed caspase assays using the autophagy strains that over-expressed Dcp-1. We confirmed that over-expression of Dcp-1 strongly increased caspase activity in Dcp-1 GF animals (Figure 3M). The increased caspase activity induced by Dcp-1 over-expression was reduced by the expression of autophagy genes in the co-heterozygotic lines with Atg1, Tor\textsuperscript{K1700E}, Atg6, dS6k, and Atg4 (Figure 3M).

**The MAPK and JNK pathway**

The JNK pathway is a well known signaling cascade that regulates apoptosis. We found that the rough eye phenotype of Dcp-1 GF was also severely affected by the expression of many components in the MAPK and JNK pathways, such as Tak1 (TGF-β activated kinase 1), Mekk1 (MAP kinase kinase kinase), hep, aop (anterior open), dominant negative form of bsk (Drosophila Jnk), and mkp (MAP kinase phosphatase). The progeny of the co-heterozygotic lines of the MAPK and JNK pathway genes with Dcp-1 GF showed extensive, although not complete, lethality. In rare cases, a few progeny reached adulthood although they displayed severe rough eye phenotypes and shortened life span (Additional file 1D). Our finding that expression of Tak1, Mekk1, hep, or aop enhanced the rough eye phenotype is consistent with the notion that the JNK pathway positively regulates apoptosis. Paradoxically, we found that expression of the mkp, a negative regulator of JNK, or the dominant negative form of bsk (jnk) enhanced apoptosis in the eye, as well. These data suggest that the interaction between Dcp-1-induced apoptosis and the JNK pathway is not simple but complicated (see Discussion).

**The ecdysone pathway**

We identified the EP lines of four ecdysone-regulated genes, Eip74EF (ecdysone induced protein 74EF), Eip78C (ecdysone induced protein 78C), broad (br), or Eip55E (ecdysone induced protein 55E) from our screen.
Ecdysone is a hormone that regulates the metamorphosis of insects and the ecdysone hormonal signaling is known to induce apoptotic and autophagic cell death during metamorphosis [47]. The co-heterozygote of Eip74EFEP\(^{G15347}\) in the Dcp-1 background enhanced the Dcp-1 GF eye phenotype (Figure 4M and 4N). The Eip78CEP\(^{G14526}\) progeny showed a phenotype similar to Eip74EF when they were crossed with Dcp-1 GF (Additional file 1E). In contrast, when crossed to one of the Eip55E alleles, Eip55EEP\(^{G13564}\), Dcp-1 GF phenotype was suppressed (Figure 3I). The co-heterozygote of one of the br alleles, brEP\(^{G10174}\), with Dcp-1 GF rescued the eye phenotype almost completely (Figure 3J), while another allele, brEP\(^{G1972}\), showed an enhanced phenotype (Data not shown). We noticed that Eip55EEP\(^{G13564}\) and brEP\(^{G10174}\) significantly suppressed the rough eye phenotype. One possibility is that the insertion positions of EP lines may have caused opposite phenotypes. Consistent with this idea, we found that Eip55EEP\(^{G13564}\) and brEP\(^{G10174}\) have insertions in the exon region of Eip55E and the
first intron of br, respectively (data not shown) suggesting that these EP elements may reduce the function of the corresponding genes and suppress the cell death phenotype.

**Relationship between autophagy and Dcp-1-induced apoptosis**

Our initial characterization of the Dcp-1-modifier screen suggested that autophagy specifically interacts with Dcp-1-induced apoptosis. First, we identified many genes directly involved in autophagy. Second, we identified several components in the insulin/IGF-1/TOR pathway, which has been shown to regulate autophagy. Third, the involvement of ecdysone signaling in autophagic cell death is well documented and we identified several ecdysone-induced proteins in our screen. These findings led us to contemplate the relationship between autophagy and Dcp-1.

To verify our finding that autophagy suppresses Dcp-1, we over-expressed eGFP fused to Atg5 (eGFP-Atg5) and the Dcp-1 in the eye imaginal disc using the UAS/GAL4 system.
system. Indeed, we found that the eye phenotype was suppressed by eGFP-Atg5 over-expression (Figure 5A). Intriguingly, we noticed that the eGFP-Atg5 level was increased in the Dcp-1 GF animals (Figure 5C and 5D) compared with control animals (Figure 5B), suggesting that Dcp-1 over-expression may induce autophagic genes and autophagy. We tested this possibility using LysoTracker Red staining, which is an autophagy marker. The level of LysoTracker Red was higher in the UAS-eGFP-Atg5/Dcp-1 GF animals (Figure 5E and 5F) than in the UAS-eGFP-Atg5 control animals (Additional file 5E and F). Together, these data suggest that there exists an interaction between autophagy and Dcp-1-induced apoptosis: autophagy suppresses Dcp-1-induced cell death, whereas Dcp-1 induces autophagy. To further demonstrate the Dcp-1-regulated autophagic gene induction, we used UAS-Atg8b-GFP as an autophagy marker in the Dcp-1 GF animals. Similar to Atg5, Atg8b-GFP was induced in these animals (Figure 5I), even though Atg8b-GFP over-expression did not suppress the rough eye phenotype (Figure 5G). These data suggest that the Dcp-1 may be able to increase autophagic gene expression even when autophagy does not suppress Dcp-1-induced apoptosis.

Next, we confirmed these results using the Drosophila S2 cell culture system. We transfected S2 cells with the Atg8b-GFP construct with or without full-length Dcp-1 and monitored the pattern of GFP fluorescence to observe the induction of autophagy. As a positive control, we starved S2 cells and found that starvation, which is a known cause of autophagy, induced Atg8b-GFP punctate expression (Figure 6B). In cells co-transfected with Atg8b-GFP and full-length Dcp-1, punctate GFP expression appeared even under replete conditions (Figure 6C), suggesting that Dcp-1 increased autophagy in S2 cells. Taken together, our data imply that increased autophagy suppresses Dcp-1-induced apoptosis and that Dcp-1 in turn positively regulates autophagy through a feedback regulation.

Discussion

In this study, we identified potentially important genes that interact with fly caspase Dcp-1 through a large-scale in vivo screen. We used a GAL4-induced Dcp-1 over-expressing strain to screen for modifiers of the caspase pathway in Drosophila. Recently, various caspase substrates or effectors were identified through a genome-wide microarray analysis using Diap1 knockdown, a caspase inhibitor, and anti-caspase RNAi in Drosophila embryo-derived Kc cells [48]. To the best of our knowledge, the present study represents the first large-scale in vivo screen with a Drosophila caspase. Moreover, we used a full-length form of Dcp-1, which has the advantage of mimicking the pre-apoptotic state. As expected, we identified both enhancer and suppressor genes that exacerbated and ameliorated apoptosis, respectively. Moreover, our genetic screen revealed that the phenotype caused by caspase Dcp-1 is suppressed by autophagy gene expression and influenced by genes from other pathways. We showed that Dcp-1 GF was rescued by the expression of autophagy genes, S6k, ecdysone-inducible genes, such as Eip55e and broad, and hypomorphic allele of Tor. Our data indicate that Dcp-1 activity is negatively controlled by autophagy.

Relationship between Dcp-1 and autophagy

While we found that over-expression of autophagy genes suppressed Dcp-1-induced apoptosis, we also observed that expression of full-length Dcp-1 induced autophagy in eye imaginal disc and S2 cell. This autophagy induction caused by full-length Dcp-1 expression was very similar to the one induced by starvation in S2 cell. It has been shown that RNAi knock down of Dcp-1 reduces autophagy in tumor larval hemocytes-derived I(2)mbn cells during starvation, indicating that Dcp-1 is required for autophagy regulation [49]. In addition, the Dcp-1

\[Dcp-1_{\text{pre}}\] reduction-of-function mutation has been shown to reduce autophagy in region two germaria and stage 8 degenerating egg chambers during Drosophila oogenesis [49]. Autophagic cell death has also been shown to be associated with the transcriptional up-regulation of pro-apoptotic genes, including Dcp-1, in salivary glands and the mid-gut [47,50,51]. These results are consistent with our observations that autophagy signals were found in the Dcp-1-expressing eye discs and in the full-length Dcp-1 transfected S2 cells.

Our findings showed that the Dcp-1 GF phenotype was rescued by autophagy and that autophagy was in turn induced by Dcp-1 expression. This seemingly paradoxical relationship between apoptosis and autophagy is not unprecedented. It has been shown that the autophagy gene, Atg5, also plays important roles in the interplay between autophagy and apoptosis. The full length Atg5 protein is an essential component for autophagy induction. However, when Atg5 is cleaved by calpain, it loses the ability to induce autophagy. Instead, the truncated Atg5 interacts with BCL-XL, is translocated to mitochondria, and causes apoptosis [52,53]. Interestingly, we found that full-length Dcp-1 could also induce autophagy, whereas the truncated active Dcp-1 resulted in apoptosis. Thus, our results suggest that Dcp-1 may act as a key protein in the regulation of not only apoptosis but also autophagy, similar to Atg5.

It remains to be determined how full-length Dcp-1 induces autophagy. It is tempting to speculate that full-length Dcp-1, which is a less active form, causes autophagy as a defensive mechanism at a very early stage in response to cellular damages. When the cellular damages reach the threshold level for apoptosis, Dcp-1
Figure 5 eGFP-Atg5 and Atg8b-GFP were expressed near condensed chromatin in Dcp-1 GF imaginal discs. (A) Adult eye phenotype of Dcp-1 GF;UAS-eGFP-Atg5. (B) GFP expression pattern in eye imaginal disc of a GMR-GAL4;UAS-eGFP-Atg5. (C) Imaginal discs of late third instar larva of Dcp-1 GF;UAS-eGFP-Atg5. (D) eGFP-Atg5 spots were observed near the morphogenetic furrow. A punctate nucleus was observed adjacent to the GFP spots. (E) LysoTracker Red spots. (F) LysoTracker Red spots and eGFP-Atg5 spots overlapped (white arrows indicate autophagosome). (D, E and F) higher-magnification images of (C). (G) Adult eye phenotype of Hs-Atg8b-GFP, Dcp-1 GF. (H) GFP expression pattern in eye imaginal disc of Hs-Atg8b-GFP larva after heat shock. (I and J) Eye imaginal discs of third instar larva of Hs-Atg8b-GFP, Dcp-1 GF. (J) Condensed chromatin was detected in the inner part (near the morphogenetic furrow) of the eye imaginal discs. Atg8b-GFP spots were observed near condensed chromatin (12 hour after five 1 hour heat shocks).
is cleaved to its active form and facilitates full-fledged apoptosis. Consistent with this idea, previous studies have shown that autophagy delays apoptosis, perhaps by eliminating unwanted or damaged molecules, to establish cellular homeostasis at the initial stages of cisplatin injury [54]. Further studies are needed to test the interaction between Dcp-1 and autophagy proteins and to elucidate the molecular mechanisms linking Dcp-1 and autophagy.

**Other signaling pathways that interact with Dcp-1**

Our genetic screening of EP lines and their interactions with other candidate lines allowed us to identify genetic pathways other than the classical apoptotic pathway. The major signal that triggers entry into metamorphosis and the activation of autophagic cell death in *Drosophila melanogaster* is a high-titer pulse of ecdysone (20-hydroxyecdysone) that occurs during puparium formation [55-57]. The ecdysone receptor complex and the nuclear receptor competence factor ftz-f1 lead to salivary gland death through the transcriptional activation of a set of “early” gene transcription factors, *br, Eip74EF,* and *Eip93F,* which in turn regulate the expression of “late” effector genes that appear to function more directly in apoptosis [56]. Mutations in each of *ftz-f1, br, Eip74EF,* and *Eip93F* impair salivary gland degeneration during metamorphosis at different stages in the cell death process. *Eip93F* seems important in autophagic induction because mutations in this gene produce an early block
in the formation of autophagic vacuoles in the salivary gland and midgut cells [47,58]. In conclusion, apoptotic and autophagic cell deaths during Drosophila metamorphosis seem to be either combined or sequential, depending on various factors. Serial analysis of gene expression (SAGE) and microarray analyses have revealed that Eip93F is required for the transcriptional up-regulation of genes that are activated in dying salivary glands, including genes involved in autophagy, apoptosis, non-caspase proteolysis, and cytoskeletal remodelling [50].

A GMR-Hid modifier screen and Hs-Hid suppression assay showed that Hid is inactivated by the ERK/MAPK pathway [59]. Even though our data differ from previous findings that Dcp-1 is down-regulated by reaper and grim but not by Hid [22], our screen data uncover a relationship between Dcp-1 and Hid, as well as between Dcp-1 and MAPK. Co-expression of GMR-Hid and GMR-full-length Dcp-1 line leads to a slight increase in eye roughness and pigment loss [22]. A similar phenomenon was observed in the present study with our Dcp-1 GF/CyO line (Additional file 6). These results suggest that Hid affects Dcp-1, albeit not as strongly as grim and reaper. The pro-apoptotic protein Hid may also have a role in autophagic induction [60]. Dcp-1 activity was shown to be regulated by Diap1 and Hid [61]. These reports suggest that autophagic cell death in organs, which undergo metamorphosis, is regulated by ecdysone, Eip93F, and pro-apoptotic proteins. Thus, we propose that Dcp-1 and Hid regulate autophagy and that Dcp-1 may regulate autophagy by receiving a signal from Hid through a non-apoptotic pathway.

**Opposite phenotypes and allelic differences**

Some of the transgenic lines that we tested in this study showed somewhat unexpected phenotypes. For example, while the enhancement of the rough eye phenotype by co-expression of Taki, Mek1, hep, or aop is consistent with the notion that the JNK pathway positively regulates apoptosis, the expression of mkp, a negative regulator of JNK, or the dominant negative form of bsk (jnk) also enhanced the eye phenotype. Likewise, we found that various reduction-of-function mutations of Tor either enhanced or suppressed the rough eye phenotype (Figure 3G and Additional file 1). Both the JNK pathway and the TOR pathway have been shown to affect apoptosis and autophagy [62-65]. Therefore, we speculate that the different expression levels of these signaling pathway genes may lead to survival or death of the cells by differentially affecting autophagy and apoptosis. For example, a mild increase in autophagy may lead to cell survival by suppressing the apoptosis caused by Dcp-1 over-expression, whereas strong induction of autophagy may worsen the cell death phenotype through the combination of apoptotic and autophagic cell deaths.

Consistent with this idea, whereas Atg1 is essential for autophagy, strong induction of Atg1 has been shown to result in cell death [68]. Thus, it is possible that different levels of autophagic induction in the flies mentioned above may explain the seemingly unexpected phenotypes.

Also, we noticed that the Bchs alleles (EP(2)2299, G12113, and G13044) enhanced the Dcp-1 GF severely. However, the Bchs EP(G2362) line can suppress the rough eye phenotype (Additional file 6). Therefore, we further examined the insertion positions of these EP lines. Bchs EP(G2362), Bchs EP(G12113), Bchs EP(G13044) were inserted at 1182, 985, and 727 base pairs upstream from the start codon, respectively (data not shown). We speculate that perhaps the insertion positions determined the expression level of Bchs and affected the rough eye phenotype differently.

**Conclusions**

In this study, we aimed to identify genes that modify Dcp-1 function in a large-scale genetic screen. We demonstrate that the effector caspase Dcp-1 regulates and/or is regulated by autophagy, ecdysone signals, ubiquitination signals, JNK, MAPK, and various transcription factors and cell death signals. Our genetic screen provides a wealth of information on various genes and pathways that may regulate caspase functions for future research. Furthermore, our findings on the integrated regulation between autophagy and Dcp-1 could aid the elucidation of molecular mechanisms connecting autophagy and apoptosis.

**Methods**

**Fly stocks**

w 1118, BL11218 Tor K 17004, BL5368 UAS-Egfr, BL6292 UAS-Rac1N17, BL5844 UAS-Hsc70-4.D206S, and BL5613 UAS-DiP N were obtained from the Indiana University Bloomington Stock Center. For screening, ~1,500 EP lines were obtained from the Szeged Drosophila Stock Center, and ~13,000 EP lines were obtained from GenExel Inc. Eye-specific GMR-GAL4, wing-specific MS1096-GAL4, AP-GAL4, and other signal transduction-related lines used for screening were kind gifts from Dr. J. Chung (KAIST, South Korea). To make transgenic flies carrying Dcp-1, pUAST-Dcp-1 was injected into Drosophila embryos (w 1118) prior to the time of pole cell formation using a microinjector model IM300 (Narishige, Japan) and an Axiovert25 micromanipulator (Carl Zeiss, Germany). UAS-Dredd, UAS-Dark, and UAS-Dronc lines were generated using the same method that was used for creating the UAS-Dcp-1 line. Fly cultures and crosses were maintained at 23.5°C.

**Generation of caspase constructs**

To make template cDNA, total RNA from all developmental stages (embryo to adult) was extracted using...
Trizol reagent (MRC, Inc). Reverse transcription was carried out using Sensiscript RT kit (Qiagen) and Oligo (dT) 12-18 primer (Invitrogen) following manufacturer’s instruction. To generate the UAS-Dcp-1 vector, an upstream primer with a BglII site and a downstream primer with an XbaI site were designed as follows: forward (5’-AAC AGA TCT ATG ACC GAC GAG TGC GTA AC-3’), reverse (5’-AGT TCT AGA CTA GCC AGC CTT ATT GCC GT-3’). To generate UAS-Dredd, UAS-Dark, and UAS-Dronc, primers were designed as follows: UAS-Dredd, forward (5’-CTCGA ATTCA TGGCC GGATC AAACC TGTT-3’) and reverse (5’-GCGCT CGAGT CACAG ACGAG GTTGA AAG-3’); UAS-dark, forward (5’-ATAGC GGCCG CATGG ATTTT GAAAC TG-3’) and reverse (5’-GCTCT CGAGT CATGA ACTGG CCTCC TCC-3’); UAS-Dronc, forward (5’-AAAGA ATCTA TGCAG CCTGC CCGCC GAGC T-3’) and reverse (5’-GCGTC TAGAC TATCC CCTGA AAAAC CCGGG A-3’). Cloning was performed using standard methods.

Electron microscopy of fly eyes
The surfaces of eyes from wild-type Drosophila or from flies with single or double copies of Dcp-1 GF were examined using a Scanning Electron Microscope (SEM; Leo 1455VP, Leo Electron Microscopy. Ltd., Korea Basic Science Institute).

Genetic screening with UAS-Dcp-1 and EP lines
We first mapped several UAS-Dcp-1 lines and selected the line UAS-Dcp-119-2, which has an insertion in chromosome 2R at position 12987659 between GstS1 and CG30456. Male flies from this line were crossed with GMR-GAL4 virgin female flies. Progeny virgin flies showing the unique Dcp-1 GF phenotype were crossed with Bc/CyO males. Among those offspring, flies having the same phenotype as their mother as well as curled wings were isolated. These flies possess GMR-GAL4 and UAS-Dcp-1 on the second chromosome as a result of homologous recombination. To conduct the screen, we performed the crossing of Dcp-1 GF/CyO virgin females with males from the EP lines. Flies showing any phenotype that differed from that of the Dcp-1 GF flies were selected. The eye phenotypes from the selected flies were photographed using a Carl Zeiss Stemi 2000C microscope with Axio Vision AC software. Inverse polymerase chain reaction (PCR) and the sequencing of the selected EP lines were both performed according to the descriptions found at http://www.fruitfly.org/about/methods/inverse.pcr.html.

Induction of Hs-Atg8b-GFP flies
yw, Hs-Atg8b-GFP/yw, Hs-Atg8b-GFP (Hs-Atg8b-GFP) flies were crossed with Dcp-1 GF/CyO. Then, yw, Hs-Atg8b-GFP/+; Dcp-1 GF/+ and yw, Hs-Atg8b-GFP/+; CyO/+ progenies were collected and crossed to each other. yw, Hs-Atg8b-GFP/yw, Hs-Atg8b-GFP, Dcp-1 GF/CyO (Hs-Atg8b-GFP; Dcp-1 GF) flies were selected by GFP positives with Dcp-1 GF adult eye phenotype. Hs-Atg8b-GFP, Dcp-1 GF and Hs-Atg8b-GFP control flies were allowed to lay eggs, which developed to the early third instar larval stage. Then, these larvae were heat shocked five times at 37°C for 1 hour with a 30-min break interval. About 6 hour after the last heat shock, when the flies had reached the climbing late third instar larval stage, larvae were collected and dissected.

UAS-eGFP-Atg5 flies
wg/CyO; UAS-eGFP-Atg5 flies were crossed with Dcp-1 GF/CyO or GMR-GAL4 flies. Then, Dcp-1 GF/UAS-eGFP-Atg5 and GMR-GAL4; UAS-eGFP-Atg5 flies were collected and dissected separately.

Eye imaginal disc preparation and staining
Eye imaginal discs were dissected from third instar larval in PBS. Discs were incubated for 1 min in 100 μM LysoTracker Red (Molecular Probes) and 1 μM Hoechst 33342 in PBS. Collected discs were washed twice with PBS, mounted on slide glasses with 80% glycerol in PBS on glass slides with cover slips, and immediately photographed using a Leica DM6000B microscope.

S2 cell culture and transfections
S2 cell culture and transfections were performed as previously reported [69]. Full-length and truncated Dcp-1 coding regions were subcloned into the pENTR directional TOPO vector (Invitrogen) and then cloned into the Drosophila gateway vector pHWF by the LR recombination reaction with the LR clonase enzyme mix (Invitrogen) following the provided protocol. The UAS-Dcp-1 construct was used for the PCR template. To make Atg8b-GFP construct, the Atg8b coding region was subcloned into the pENTR directional TOPO vector (Invitrogen) and the relevant construct region was cloned into the Drosophila gateway vector pHGW by performing the LR recombination. The pHS-48A construct from the Neufeld laboratory was used as a PCR template. Full-length Dcp-1 primers were designed as follows: forward (5’-CACCAG ACC GAG TGC GTA ACC AGA-3’), reverse (5’-GCC AGC CTT ATT GCC GTT CCG GT-3’). Truncated Dcp-1 primers were designed as follows: forward (5’-CACCAG GCC AAC AAG GCC TTG ACG CCG GAG-3’), reverse (5’-GCC AGC CTT ATT GCC GTT CCG GT-3’). Full-length and truncated primer sequences were identical to those used previously [22], except for the CACCAG in the upstream primer for TOPO cloning. Atg8b-GFP primers were designed as follows: forward (5’-CACCAG GAT ATG AAC TAC CAG TA-3’), reverse (5’-CTA CGG TCC ATG GAC GT-3’). Cells were plated on round cover glasses inserted into 6-well plates (NUNC).

Starvation induction
48 hours after transfection of S2 cells, the culture medium was replaced with complete Schneider’s medium.
(GIBCO) containing FBS. After 12 hours stabilization, starvation was induced by changing the complete medium to Hank’s balanced salt solution with CaCl₂ and MgCl₂ and without phenol red (HBSS; Welgene Inc.). After 3 hours, the HBSS medium was replaced with Schneider’s medium without FBS.

**Observation and quantification of GFP fluorescence**

The transfected cells attached on the cover glasses were fixed with 2% final concentration of paraformaldehyde solution for 10 min and washed three times with PBS and mounted on glass slides. The cells on the cover glasses were mounted on the slide glasses with nail polish and photographed using a Leica DM6000B microscope. The GFP-positive spots in the cells were counted from top to bottom and again from left to right under the 1000× magnification. Transfected cell populations were then counted three times with three different batches of transfection and the results were normalized. The tests were done blindly and the statistical analysis was performed by using Student’s t-test.

**Quantitative RT-PCR and real-time PCR**

Fly lines containing UAS-Dep-1 alleles were crossed to GMR-GAL4 flies, and their progeny were grown to adulthood. Heads from virgin progeny were collected. For each sample, the same number of collected heads was ground in Trizol reagent (Invitrogen) using a hand pestle. Total RNA was purified using the provided protocol. Reverse transcription was carried out with the Sensiscript RT kit (Qiagen) and Oligo (dT)12-18 primer (Invitrogen) using the provided protocols [70]. The resulting cDNA was used for PCR. QRT-Dep-1 primers were designed as follows: forward (5'-TCG ACG AGC TAC AAG ATA-3'), reverse (5'-GCT GGT TAA CGA TCC GC-3'). PCR was performed as follows: 5-min denaturation at 95°C followed by 30 cycles with 94°C for 30 sec, 52°C for 30 sec, and 72°C for 30 sec. mRNA expression level was constant in every GAL4 mediated line. (A) The level of GAL4 expression was not detected in the w^1118 expression level of reaction sets No template/GG: GMR-GF and crossed progeny of Atg1EP(G13748). (Black arrow indicates the P-element insertion site of G13748). (B) The expression level of GAL4 was compared with that of RP49 by using the real-time PCR experiment. NT: No template/GG: GMR-GAL4, homozygote/GD: Dcp-1 GF/GyO/A1D: Atg1GF (G13748)/Dcp-1 GF/A6D: Atg1GF/GyO/A1D/Dcp-1 GF. Soon after the real-time PCR was performed using the IQ5 real-time PCR detection system (Bio-Rad). Amplification was performed using IQ5 SYBR green supermix (Bio-Rad). Template cDNAs were prepared as previously described [70]. At least three independent experiments were performed for each sample. PCR conditions and primers used in each reaction were designed as follows: 3-min denaturation at 95°C followed by 40 cycles with 95°C for 30 sec, 52°C for 30 sec. Elongation was then performed at 72°C for 30 sec followed by 72°C for 1 min and followed by 81 cycles of Melt curve analysis from 55°C to 95°C. QRT-Dep-1 primers were designed as previously described. QRT-Atg6b primers were designed as follows: forward (5’-AGT TCT ACT TTC TCA TCC GC-3’), reverse (5’-CAT AGA CGT TCT CAT CGG TAT-3’). QRT-Tor primers were designed as follows: forward (5’-ACC ACA AAC GAA CTA CGA-3’), reverse (5’-TAC CTT GTG AGC AGA CCT-3’). QRT-RP49 primers were designed as follows: forward (5’-AGA TCG TGA AGA AGC GCA CC-3’), reverse (5’-CGA TCC GTA ACC GAT GTT GG-3’). Real-time PCR results were analyzed using IQ5-2.0 software (Bio-Rad). Each of individual expression level was normalized with w^1118 expression level of reaction sets of same primers and with RP49 expression level of reaction sets of same templates.

**Caspase activity test**

Caspase activity was determined using the Caspase-Glo® 3/7 Assay (Promega) and measured using a Wallac Vitor 1420 multilabel counter (Perkin-Elmer). Each lysate was prepared by grinding ~20 heads of virgin progeny with 1 mM phenylmethylsulfonyl fluoride (PMSF: Serine specific protease inhibitor). The lysates were centrifuged to remove debris-like cuticles and cell membranes. The lysates were immediately mixed with Caspase Glo3/7 reagent as 1:1 ratio individually. Samples were incubated for 30 min at room temperature. Luminescence of each sample was then measured by using Wallac Vitor 1420 multilabel counter (Perkin-Elmer). At least three independent experiments were performed for each genotype. Statistical analysis was performed by using Student’s t-test.

Additional file 1: Table S1 Enhancers and suppressors of the eye phenotype caused by Dcp-1 over-expression. Click here for file

Additional file 2: GAL4 mRNA expression level was constant in every GAL4 mediated line. (A) The level of GAL4 expression was not detected in the w^1118 expression level of reaction sets. (B) The expression level of GAL4 was compared with that of RP49 by using the real-time PCR experiment. NT: No template/GG: GMR-GAL4, homozygous/GD: Dcp-1 GF/GyO/A1D: Atg1GF (G13748)/Dcp-1 GF/A6D: Atg1GF/GyO/A1D/Dcp-1 GF. Click here for file

Additional file 3: Expression level of CG10969, down-stream gene of Atg1, was not affected by EP inserted up-stream of Atg1. (A) The surrounding genes of Atg1. CG10969, CG17666, CG17667 and CG11006 are located next to the Atg1. However, the directions of CG17666, CG17667 and CG11006 are opposite to the direction of EP (G13748) insertion. CG10969 is placed in down-stream site of Atg1 and EP (G13748). (Black arrow indicates the P-element insertion site of Atg1EP (G13748)). (B) The genomic DNA of w^1118 was used as a template for the lane 1, while appropriate cDNAs were used for the lanes 2, 3, 4 and 5. The lane 1 indicates that all the primer sets worked properly. The lane 2 indicates that Atg1 and CG10969 were not expressed in the w^1118. The lane 3 indicates that Atg1 was slightly up-regulated by the expression of Dcp-1. The lane 4 and 5 indicates that Atg1 expression level was increased in GMR-GAL4/Atg1GF(G13748) and GMR-GAL4, Dcp-1/Atg1GF(G13748). No expression was detected by the CG10969 primer set. Click here for file
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Authors’ contributions
YIK managed overall experiments and performed S2 cell experiments, molecular biology experiments and Drosophila experiments, and wrote the manuscript. TJL performed primary screen with random selected EP lines. YIK and TJL confirmed the selected lines from the screening. YSH carried out the RNA experiments including the quantitative RT-PCR. JA participated in the design of the study and helped writing the manuscript. SJL participated in experimental designs and coordination, and helped writing the manuscript during the revision. OJY conceived the study, and participated in experimental designs and coordination and helped writing the manuscript. All authors read and approved the final manuscript.

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References
1. Kerr JF, Wyllie AH, Currie AR: Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972, 26(4):239-257.
2. Steller H: Mechanisms and genes of cellular suicide. Science 1995, 267(5203):1445-1449.
3. Nezis IP, Stravopodis DJ, Papassideri I, Robert-Nicoud M, Margaritis LH: Stage-specific apoptotic patterns during Drosophila oogenesis. Eur J Cell Biol 2000, 79(9):610-620.
4. Levine B, Krohn KI: Development by self-digestion: molecular mechanisms and biological functions of autophagy. Dev Cell 2004, 6(4):463-477.
5. Nicholson DW: Caspase structure, proteolytic substrates, and function during apoptotic cell death. Cell Death Differ 1999, 6(11):1026-1042.
6. Heimgartner MO: The biochemistry of apoptosis. Nature 2000, 407(6805):770-776.
7. Thornberry NA: The caspase family of cysteine proteases. Br Med Bull 1997, 53(3):478-490.
8. Kumar S, Dournamis J: The fly caspases. Cell Death Differ 2000, 7(11):1039-1044.
9. Vernosy SY, Copeland J, Ghaborosi N, Griffin EE, Yoo SJ, Hay BA: Cell death regulation in Drosophila: conservation of mechanism and unique insights. J Cell Biol 2000, 150(2):F69-76.
10. Hay BA, Guo M: Caspase-dependent cell death in Drosophila. Annu Rev Cell Dev Biol 2006, 22:623-650.
11. Abrams JM: An emerging blueprint for apoptosis in Drosophila. Trends Cell Biol 1999, 9(11):435-440.
12. Song Q, Lu H, Zhang N, Luckow B, Shah G, Poirier G, Lavin M: Specific cleavage of the large subunit of replication factor C in apoptosis is mediated by CPP32-like protease. Biochem Biophys Res Commun 1997, 233(2):343-348.
13. Song Z, McCall K, Steller H: DCP-1, a Drosophila cell death protease essential for development. Science 1997, 275(5299):536-540.
14. Kuranaga E, Miura M: Nonapoptotic functions of caspases: caspases as regulatory molecules for immunity and cell-fate determination. Trends Cell Biol 2007, 17(3):135-144.
15. Kondo S, Semio-Matsuura N, Hirono Y, Miura M: DRONC coordinates cell death and compensatory proliferation. Mol Cell Biol 2006, 26(19):7258-7268.
16. Huh JR, Vernosy SY, Yu H, Yan N, Shi Y, Guo M, Hay BA: Multiple apoptotic caspase cascades are required in nonapoptotic roles for Drosophila spermatid individualization. PLoS Biol 2004, 2(1):E15.
17. McCall K, Steller H: Requirement for DCP-1 caspase during Drosophila oogenesis. Science 1998, 279(5348):230-234.
18. Laundrie B, Peterson JS, Baum JS, Chang JC, Filippone D, Thompson SR, McCall K: Germline cell death is inhibited by F-element insertions disrupting the dcp-1/pita nested gene pair in Drosophila. Genetics 2003, 164(4):1881-1888.
19. Gorgi F, Den P: Cell death in ovarian chambers of Drosophila melanogaster. J Embryol Exp Morphol 1976, 35(3):521-533.
20. Chao S, Nagoshi RN: Induction of apoptosis in the germline and follicle layer of Drosophila egg chambers. Meth Dev 1999, 88(2):159-172.
21. Drummond-Barbosa D, Spalding AC: Stem cells and their progeny respond to nutritional changes during Drosophila oogenesis. Dev Biol 2001, 231(1):265-278.
22. Song Z, Guan B, Bergman A, Nicholson DW, Thornberry NA, Peterson EP, Steller H: Biochemical and genetic interactions between Drosophila caspases and the proapoptotic genes rp, hid, and grim. Mol Cell Biol 2000, 20(8):2907-2914.
23. Leulier F, Rodriguez A, Khush RS, Abrams JM, Lemaire B: The Drosophila caspase Dredd is required to resist gram-negative bacterial infection. EMBO Rep 2000, 1(4):353-358.
24. Cadavid AL, Gerez A, Fischer JA: The function of the Drosophila fat facets deubiquitinating enzyme in limiting photoreceptor cell number is intimately associated with endocytosis. Development 2000, 127(8):1737-1746.
25. Ryoo HD, Bergmann A, Gonen H, Ciechanover A, Steller H: Regulation of Drosophila IAP1 degradation and apoptosis by reaper and ubcD1. Nat Cell Biol 2002, 4(6):432-438.
26. Juhasz G, Csinko G, Srinia R, Erdelyi M, Sass M: The Drosophila homolog of Atu1 is essential for autophagy and development. FEBS Lett 2003, 543(1-3):154-158.
27. Ichimura Y, Kirschho T, Takao T, Satomi Y, Shimomishi Y, Ishihara N, Mizushima N, Tanida I, Kominami E, Ohsumi M, et al: A ubiquitin-like system mediates protein lipidation. Nature 2000, 408(6811):488-492.
28. Lippar C, Csinko G, Maricy P, Lukacs-Morvaj T, Juhasz G, Sass M: SNI-Fagamma, the Drosophila AMPK gamma subunit is required for regulation of developmental and stress-induced autophagy. Autophagy 2008, 4(4):476-486.
29. Finley KD, Edeen PT, Cumming RC, Marzahl-Dumesnil MD, Taylor BJ, Mardahl-Dumesnil MD, Taylor BJ: Genetic modifiers of the Drosophila blue cheese gene link apoptosis and autophagy enables necrosis. Autophagy 2009, 1(4):741-752.
30. Chung J, Kuo CJ, Crabtree GR, Blenis J: Rapamycin-FKB: specifically blocks growth-dependent activation of and signaling by the 70 kd S6 protein kinases. Cell 1992, 69(7):1227-1236.
31. Kamada Y, Funakoshi T, Shintani T, Nagano K, Ohsumi M, Ohsumi Y: Tor-mediated induction of autophagy via a Apr1 protein kinase complex. J Cell Biol 2000, 150(6):1507-1513.
32. Maiuri MC, Zalckvar E, Kimchi A, Kroemer G: Alfy, a novel FYVE-domain-containing protein associated with protein granules and autophagic membranes. J Neurosci 2004, 24(4):1254-1264.
33. Pyo JO, Jang MH, Kwon YK, Lee HJ, Jun JI, Woo HN, Cho DH, Chai B, Lee H, Kim JH, et al: Essential roles of Atg5 and FADD in autophagic cell death: dissection of autophagic cell death into vacuole formation and cell death. J Biol Chem 2005, 280(21):20722-20729.
34. Yang C, Kaushal V, Shah SV, Kaushal GP, Brunner T, Simon HU: Calpain-mediated cleavage of Atg6 switches autophagy to apoptosis. Nat Cell Biol 2006, 8(10):1124-1132.
35. Lee CT, Baehrecke EH: Steroid regulation of autophagic programmed cell death during development. Development 2001, 128(8):1443-1453.
36. Scott RC, Schulman N, Neufeld TP: Role and regulation of starvation-induced autophagy in the Drosophila fat body. Dev Cell 2004, 7(2):167-178.
37. Wullschleger S, Loewith R, Hall MN: 70-kilodalton S6 protein kinase regulates the 70-kilodalton S6 protein kinase. Genes Dev 2000, 14(21):2712-2724.
38. Gao X, Zhang Y, Arnaola P, Hino O, Kobayashi T, Yeung RS, Ru B, Pan D: Tsc tumour suppressor proteins antagonize amino-acid-TOR signalling. Nat Cell Biol 2002, 4(9):699-704.
39. Wu Q, Zhang Y, Xu J, Shen P: Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in Drosophila. Proc Natl Acad Sci USA 2005, 102(37):13289-13294.
40. Price DJ, Grove JR, Calvo V, Avruch J, Beere BE: Rapamycin-induced inhibition of the 70-kilodalton S6 protein kinase. Science 1992, 257(5072):973-977.
41. Zhang H, Stielack JP, Ng JC, Reinhard C, Neufeld TP: Regulation of cellular growth by the Drosophila target of rapamycin dTOR. Genes Dev 2000, 14(21):2712-2724.
42. Capai P, Zid BM, Harper T, Koslover D, Sapin V, Benzer S: Regulation of lifespan in Drosophila by modulation of genes in the TOR signaling pathway. Curr Biol 2004, 14(10):R85-890.
43. Wu Q, Zhang Y, Xu J, Shen P: Regulation of hunger-driven behaviors by neural ribosomal S6 kinase in Drosophila. Proc Natl Acad Sci USA 2005, 102(37):13289-13294.
44. Kim H, Cho DH, Choi B, Jeong H, et al: Essential roles of Atg5 and FADD in autophagic cell death: dissection of autophagic cell death into vacuole formation and cell death. J Biol Chem 2005, 280(21):20722-20729.
45. Yousef S, Perozzo R, Schmid I, Ziemiecki A, Schaffner T, Scapozza L, Brunner T, Simon HU: Calpain-mediated cleavage of Atg6 switches autophagy to apoptosis. Nat Cell Biol 2006, 8(10):1124-1132.
46. Lee CT, Baehrecke EH: Steroid regulation of autophagic programmed cell death during development. Dev Growth Differ 2001, 43(3):271-279.
47. Abraham MC, Shaham S: The Drosophila gene hid is a member of the TNF family. J Cell Biol 2000, 150(1):185-195.
48. Park J, Wullschleger S: Disrupting the dcp-1/pita nested gene pair in Drosophila melanogaster. J Cell Biol 2000, 150(1):185-195.
49. Abraham MC, Shaham S: The Drosophila gene hid is a member of the TNF family. J Cell Biol 2000, 150(1):185-195.
50. Abraham MC, Shaham S: The Drosophila gene hid is a member of the TNF family. J Cell Biol 2000, 150(1):185-195.
64. Wu H, Wang MC, Bohmann D. JNK protects Drosophila from oxidative stress by transcriptionally activating autophagy. Mech Dev 2009, 126(8-9):624-637.
65. Wang T, Lao U, Edgar BA. TOR-mediated autophagy regulates cell death in Drosophila neurodegenerative disease. J Cell Biol 2009, 186(5):703-711.
66. Blommaart EF, Luiken JJ, Meijer AJ. Autophagic proteolytic control and specificity. Histochem J 1997, 29(5):365-385.
67. Elmore SP, Qian T, Grissom SF, Lemasters JJ. The mitochondrial permeability transition initiates autophagy in rat hepatocytes. Faseb J 2001, 15(12):2286-2287.
68. Scott RC, Juhasz G, Neufeld TP. Direct induction of autophagy by Atg1 inhibits cell growth and induces apoptotic cell death. Curr Biol 2007, 17(1):1-11.
69. Han K. An efficient DDAB-mediated transfection of Drosophila S2 cells. Nucleic Acids Res 1996, 24(21):4362-4363.
70. Kim YI, Cho JH, Yoo OJ, Ahn J. Transcriptional regulation and life-span modulation of cytosolic aconitase and ferritin genes in C. J Mol Biol 2004, 342(2):421-433.

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