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***Drosophila* Chk2 and p53 proteins induce stage -specific cell death independently during oogenesis**

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Abstract

In *Drosophila*, the checkpoint protein-2 kinase (DmChk2) and its downstream effector protein, Dmp53, are required for DNA damage-mediated cell cycle arrest, DNA repair and apoptosis. In this study we focus on understanding the function of these two apoptosis inducing factors during ovarian development. We found that expression of Dmp53, but not DmChk2, led to loss of ovarian stem cells. We demonstrate that expression of DmChk2, but not Dmp53, induced mid-oogenesis cell death. DmChk2 induced cell death was not suppressed by *Dmp53* mutant, revealing for the first time that in *Drosophila*, overexpression of DmChk2 can induce cell death which is independent of Dmp53. We found that over-expression of caspase inhibitors such as DIAP1, p35 and p49 did not suppress DmChk2- and Dmp53-induced cell death. Thus, our study reveals stage -specific effects of Dmp53 and DmChk2 in oogenesis. Moreover, our results demonstrate that although DmChk2 and Dmp53 affect different stages of ovarian development, loss of ovarian stem cells by p53 expression and mid-oogenesis cell death induced by DmChk2 do not require caspase activity.

Keywords

Drosophila melanogaster; ovary; Chk2; p53; caspase; cell death

Introduction

The DNA damage response is a signal transduction network which is activated upon recognition of DNA lesions to elicit appropriate cellular responses to these assaults (such as DNA repair, cell cycle arrest or apoptosis). Chk2, a checkpoint effector kinase, is activated mainly by Ataxia-telangiectasia mutated (ATM) protein in response to double strand DNA breaks (DSB's). Upon activation by ATM, Chk2 phosphorylates several proteins which in turn affect cell cycle checkpoint regulation, DNA repair, replication fork maintenance and apoptosis (1). During DNA damage-induced apoptosis, Chk2 promotes p53-dependent transcriptional responses mainly by phosphorylation of p53 (2–3) or by phosphorylation of other substrates that affect p53 stability (4–6). In mammals, it has also been shown that Chk2 may regulate DNA damage-induced apoptosis independently of p53, in part through the modification of the promyelocytic leukemia protein (7).

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The *Drosophila* p53 homologue (Dmp53) is required for the induction of apoptosis following DNA damage events (8–10). Recently, it was shown that *Dmp53* is also required for cellular differentiation in the *Drosophila* eye (11) and for compensatory proliferation due to cellular damage (12). During development, loss of *Dmp53* activity affects fly longevity (13) and also programmed cell death of primordial germ cells (PGC's) (14). Recently, it was shown that Dmp53 is required for meiotic recombination in *Drosophila* (15) which is highly conserved. The *Drosophila* Chk2 homologue, *DmChk2*, is required for DNA damage-mediated cell cycle arrest and apoptosis (16–21). As shown for mammalian cells, the role of *DmChk2* in mediating irradiation-induced apoptosis is dependent on *Drosophila* Dmp53 protein (17–18). During meiosis, *DmChk2*, but not *Dmp53*, was found to be essential for activation of a meiotic checkpoint, which occurs in double strand DNA repair enzyme mutants (22–23). *DmChk2* was also essential for activation of a second meiotic checkpoint due to mutations in genes in the repeat associated small interfering RNA (RasiRNA) pathway (24–26).

As described above, both *DmChk2* and *Dmp53* had an important role during oogenesis, thus in this study we focused on analyzing the role of *DmChk2* and *Dmp53* in apoptosis processes during *Drosophila* ovarian development. We found that *DmChk2* and *Dmp53* could independently induce cell death in a stage and tissue-specific manner. Whereas expression of *Dmp53* led to loss of ovarian stem cells, expression of *DmChk2* led to cell death during mid-oogenesis. Moreover, expression of *Dmp53* but not of *DmChk2* in the somatic follicle cells affected egg chamber survival. Interestingly, we show that inhibition of caspase activity is not sufficient to suppress loss of germ cell by *Dmp53* or mid-oogenesis cell death induced by *DmChk2*.

Materials and Methods

Drosophila stocks

The following mutant and transgenic flies were used: *p53* (27), *DmChk2* (*lok^{p6}*) (22), *Atg7^{d14}*, *Atg7^{d77}* (28), *pUASp-DIAP1* (29). The *UASp-p35* line was obtained from Andreas Bergmann (Houston, TX, USA). The *GMR-Gal4*, *P{matalpha4-GAL-VP16}V2H*, *UASp-GFP-LC3*; *nanos-GAL4* and *nanos-Gal4VP16* lines were all obtained from the Bloomington Stock Center. For expression in follicle cells *CY2-Gal4* (30) and *GRI-Gal4* (kindly provided by T. Schüpbach) were used. For LysoTracker and TUNEL experiments the driver was *NGT40*; *nosGAL4VP16* (31).

Transgenic flies

To create *HA-tagged DmChk2*, the entire coding sequence of *DmChk2* was amplified by PCR using modified primers to create a *XbaI* restriction site at the 5' end and a *NotI* site at the 3' end. The resulting PCR product was cut using *XbaI* and *NotI* and was cloned into HA-pBlueScript. The resulting pBlueScript vectors were cut using *KpnI* and *NotI* and the inserts were cloned into pUASp vectors. To make the *pUASp-p53* or *p49* fusion construct the entire coding sequence of either *Dmp53* or *p49* was amplified by PCR using modified primers to create a *KpnI* restriction site at the 5' end and a *NotI* site at the 3' end. The resulting PCR products of each gene were cut using *KpnI* and *NotI* and were cloned into pUASp.

P-element-mediated germ-line transformation of this construct was carried out according to standard protocols (32). Ten independent lines from each construct were established.

Ovary staining

Ovaries were dissected in phosphate-buffered saline (PBS), fixed in 200 μ l 4% formaldehyde in PBS combined with 600 μ l heptane for 20 minutes, and washed in PBST (PBS + 0.3% Triton X-100). The ovaries were incubated with primary antibodies overnight at 4°C, and then with secondary antibodies for one hour. The ovaries were separated onto slides in 50% glycerol. The following antibodies were used at the designated dilutions: rabbit anti-vasa was used at 1:1000, mouse anti Adducin-like (1B1) was used at 1:20 (Hybridoma bank), mouse anti-HA monoclonal (Sigma) were used at a 1:10 dilution, mouse anti-p53 (17) were used at a 1:1000 dilution and rabbit anti-PH3 antibody (Upstate Biotech) was used at 1:1000 dilution. Cy2-, Cy3- and Cy5- conjugated secondary antibodies (Jackson Laboratories) were each used in 1:100 dilutions. For DNA staining, Hoechst stain (Molecular Probes) was used at a 1 μ g/ml. Larval ovaries from GFP-LC3 flies were stained with rabbit anti-GFP (Santa-Cruz) at 1:100 dilution. Egg chambers were photographed using a Zeiss LSM510 laser-scanning confocal microscope. The TUNEL assay was performed as described (33) using the ApopTag Fluorescein Direct In Situ Apoptosis Detection Kit (Chemicon International). LysoTracker (Invitrogen) labeling was carried out as described (34). TUNEL and LysoTracker stained egg chambers were mounted in Vectashield with DAPI (Vector Labs) and observed on an Olympus DSU spinning disc microscope or Olympus FluoView® FV10i.

Scanning Electron Microscopy

Adult *Drosophila* were fixed and dehydrated by immersion in increasing concentrations of alcohol (25%, 50%, 75%, 2x100% each for 10 minutes). The samples were then completely dehydrated using increasing concentrations of hexamethyldisilazane (HMDS) in alcohol (50%, 75%, 2x100%, each for 2 hours), air dried overnight, placed upon stubs, coated with gold, and examined with a scanning electron microscope (JEOL model JSM-5610LV).

Results

Over-expression of Dmp53, but not DmChk2, leads to ovarian stem cell loss and over expression of DmChk2, but not Dmp53, leads to mid-oogenesis cell death

To study the effect of overexpression of DmChk2 and Dmp53 *in vivo* we used the UAS-Gal4 binary system (35) in which ubiquitous or tissue-specific expression can be induced. We cloned the full length *DmChk2* and *Dmp53* into the pUASp vector allowing expression in both somatic and germline tissues (36). To test the functionality of these constructs we determined whether expression of DmChk2 could enhance Dmp53-mediated apoptosis in the eye (17–18). We found that overexpression of Dmp53 in the eye using GMR-Gal4 resulted in a reduced eye size with partial fusion of the ommatidia and some remaining bristles due to apoptosis (Fig. S1 C). Expression of DmChk2 in the *Drosophila* eye had no effect on eye morphology (Fig. S1 B). Co-expression of DmChk2 and Dmp53 resulted in a considerably more severe phenotype with almost complete loss of the eye compared to flies expressing Dmp53 alone (Fig S1 D). Thus, similar to previous findings (17–18), we found that Dmp53-induced apoptosis was enhanced by DmChk2 expression, showing that DmChk2 regulates Dmp53 mediated apoptosis. Thus, our DmChk2 and Dmp53 constructs and transgenic flies are functional and suitable for further investigation of the role of DmChk2 and Dmp53 during oogenesis.

To test the role of DmChk2 and Dmp53 in apoptosis during oogenesis we expressed these proteins in the *Drosophila* germline. We found that females over-expressing Dmp53 by *nanos-Gal4VP16*, which drives expression during ovarian stem cell development and also during oogenesis (37), are agametic with rudimentary ovaries probably due to loss of ovarian stem cells (Fig. 1B). We showed that over-expression of DmChk2 with the same

driver had no effect on stem cell development but led to massive cell death of stage 8/9 egg chambers in mid-oogenesis (56%, n=456) (Fig. 1C), visualized by nurse cell DNA condensation. These results show ovarian germline stem cells are sensitive to the level of Dmp53 but not of DmChk2.

Since expression of Dmp53 using the *nanos-Gal4VP16* driver affected stem cell development, we could not use this driver for studying the role of Dmp53 during mid-oogenesis. To test the role of DmChk2 and Dmp53 specifically during mid-oogenesis we decided to use the *P{matalpha4-GAL-VP16}V2H* flies which drives expression during mid to late oogenesis and is loaded into eggs. We found that over-expression of DmChk2 in germ cells using the above driver caused cell death of stage 8/9 egg chambers (52%, n=378) (Fig. 2B), as again was evident by nurse cell DNA condensation. Most importantly, we found that over-expression of Dmp53 in the germ line using this driver had no effect on survival of the germline (Fig. 2A). To check the expression of Dmp53 we stained the ovaries with p53 monoclonal specific antibody (18) and found that p53 expressed in the ovaries and is localized to the cell nucleus (data not shown). To verify these results we repeated these experiments with all of our transgenic lines (DmChk2 and Dmp53) and found similar results. These results suggest that over-expression of DmChk2, but not Dmp53, is sufficient for induction of cell death during mid-oogenesis.

Mid-oogenesis DmChk2-mediated cell death is not suppressed by mutation in *Dmp53*

The up-regulation of *Dmp53* under apoptotic conditions and the finding that *Dmchk2* regulates irradiation-induced, *Dmp53*-mediated apoptosis in *Drosophila* (17) suggested that DmChk2 may also regulate p53 during mid-oogenesis cell death. Thus, we studied the ability of *Dmp53* mutants to suppress Chk2-induced cell death and found that mutations in *Dmp53* did not block DmChk2-dependent cell death (Fig. 2C). Ovaries from flies over-expressing DmChk2 in the germline showed 52% degenerating stage 8/9 egg chambers (n=129), and the level of cell death of egg chambers was similar (54%, n=86) in *Dmp53* mutant background. These results indicate that cell death caused by DmChk2 during mid-oogenesis is *Dmp53* independent.

Over expression of *Dmp53* but not of *DmChk2* in the somatic follicle cells leads to egg chamber cell death

It was shown that induction of egg chamber apoptosis in *Drosophila* could be achieved not only in the germline but also in the follicle cell layer (38). Thus, we decided to check whether expression of either Dmp53 or DmChk2 in the follicle cell layer could also induce death of the egg chamber. We found that whereas over-expression of DmChk2 using several follicle cell specific Gal4 drivers had no effect on either follicle or germline cell survival (Fig. 3B); expression of Dmp53 led to the degeneration of mid-oogenesis egg chambers (Fig. 3C). These results suggest that DmChk2 induced cell death is germline-specific but Dmp53 could induce cell death in the somatic follicle cells.

Stem cell loss by *Dmp53* and mid-oogenesis cell death mediated by *DmChk2* are not suppressed by over-expression of caspase inhibitors

To determine whether cell death occurring during mid-oogenesis in flies over-expressing DmChk2 in the germline was dependent only on caspase activity, we co-expressed the caspase inhibitor, DIAP1, in the germline along with DmChk2. Expression of pUASp – DIAP1 (Fig. 4A) did not suppress mid-oogenesis DmChk2 mediated cell death (63%, n=150). Since DIAP1 and another caspase inhibitor, p35, may inhibit different caspases (39), we decided to check whether expression of other caspase inhibitors, p35 or p49 (40–41), could suppress DmChk2 mid-oogenesis cell death. We found that overexpression of p35 (Fig. 4B, 51%, n=87) or p49 (Fig. 4C, 55%, n=98) did not affect cell death caused by

DmChk2. These results suggest that DmChk2 expression leads to mid-oogenesis cell death that is not dependent on caspase activity.

Next we analyzed whether ovarian stem cell loss is dependent on caspase activity by expressing either DIAP1, p35 or p49 (Fig. 5) together with Dmp53. We found that similar to DmChk2, these caspase inhibitors did not suppress Dmp53 ovarian stem cell loss (Fig. 5). Thus, our results show that the cell death effects of over-expression of Dmp53 and DmChk2 in the germline do not require caspase activity.

Expression of p53 lead to loss of PGC's during larval development

We showed that expression of Dmp53 led to loss of ovarian stem cells which was not suppressed by expression of caspase inhibitors. To determine how these stem cells were lost, we studied the effects of Dmp53 on PGC's development. First we analyzed whether expression of Dmp53 affects PGC's survival. We found that larval gonads from flies expressing Dmp53 had significantly lower numbers of PGC's (number of PGC's= 36 ± 20 ; Fig. 6B) compared to wild type (number of PGC's= 97 ± 10 , Fig. 6A), as revealed by using Vasa antibody as a marker for germ cells. Thus, our results showed that expression of Dmp53 led to loss of PGC's.

Next, we studied whether over-expression of Dmp53 in the germline affected PGC proliferation by staining the ovaries for a mitotic marker, phosphorylated histone-3 (PH3). In ovaries from Dmp53 over-expression flies (Fig 6D) we detected a significantly lower number of PGC stained for anti- PH3 as compared to wild type (Fig. 6C).

We also analyzed whether loss of PGC's is accompanied by increased apoptosis. To accomplish this, we stained ovaries from flies over-expressing Dmp53 with terminal deoxyribonucleotidyl transferase (TdT)-mediated biotin-16-dUTP nick end labeling (TUNEL). We were unable to detect positive TUNEL staining in these ovaries (data not shown). Since we were unable to detect TUNEL staining in ovaries expressing Dmp53, we checked whether loss of PGC's by Dmp53 led to other forms of cell death, such as autophagic cell death. We used LysoTracker (LT) Red, which labels lysosomes and autolysosomes, and GFP-LC3 as a reporter for autophagy (42). We found that larval ovaries from flies expressing Dmp53 in the germline showed a significant increase in LT positive dots in the PGC area (Fig. 6F, number of ovaries= 28) as compared to wild type larval ovaries (Fig. 6E, number of ovaries=30). However, when we tested for activation of autophagy by p53 in the ovaries using the GFP-LC3 marker, we found that larval ovaries from flies expressing p53 with GFP-LC3 (Fig. 6H) had diffuse GFP-LC3 staining as in ovaries expressing GFP-LC3 alone (Fig. 6G), suggesting that expression of p53 did not lead to activation of autophagy.

Mid-oogenesis DmChk2-mediated cell death is characterized by DNA fragmentation

Next we examined DNA fragmentation in *DmChk2* over-expressing egg chambers using TUNEL. As previously shown, we found that 84% of degenerating mid-stage egg chambers from nutrient deprived females (Fig. 7A) stained positively for TUNEL (n=108 egg chambers with highly condensed nurse cell chromatin). Similarly, 82% of degenerating egg chambers were TUNEL-positive in *DmChk2* (Fig. 7E) over-expression females (n=94). Our results suggest that over-expression of *DmChk2* leads to both chromatin condensation and DNA fragmentation.

Mid-oogenesis DmChk2-mediated cell death shows delayed autophagy induction

It has previously been shown that autophagy is induced during mid-oogenesis cell death following nutrient deprivation (43–44). Thus, we asked whether over-expression of

DmChk2 in the germline also regulated autophagy in mid-stage egg chambers using LT. As previously shown (43–44), degenerating egg chambers of wild-type nutrient-deprived flies displayed increasing amounts of LT staining as the nurse cell nuclei became condensed and began to fragment (Fig. 8A–C). In flies over-expressing DmChk2, LT staining was seen much later during degeneration, when the nurse cell nuclei had fragmented into tiny pieces (Fig. 8D–F). To quantify this delay in lysosome increase, we staged degenerating egg chambers carefully with respect to the degree of nuclear condensation and fragmentation. While 100% (n=11) of control *NGT;nosGAL4* egg chambers at stage 8–9 showing fragmented nurse cell nuclei were LT-positive, only 20% (n=62) of DmChk2-expressing egg chambers were LT-positive at that stage. At later stages of degeneration, when nurse cell nuclei were largely degraded, 100% (n=14) of control egg chambers and 96% (n=28) of DmChk2-expressing egg chambers were LT-positive. Because autophagy mutants have been shown to partially disrupt normal mid-oogenesis cell death (43–44), we tested whether DmChk2-mediated cell death required the autophagy machinery. We found that a mutation in *Atg7* did not suppress DmChk2-mediated cell death (data not shown). Taken together, these findings indicate that DmChk2 does not induce autophagic cell death.

Discussion

In this study we focused on analyzing the role of DmChk2 and Dmp53, apoptosis-inducing factors during oogenesis in *Drosophila*. We demonstrated, for the first time in *Drosophila*, that expression of DmChk2 alone induced a tissue specific massive cell death; expression of DmChk2 in the germline had a specific cell death effect on mid-oogenesis egg chambers but not on ovarian stem cells. In support of these results, it was shown that the level of DmChk2 transcripts increase in response to nutrient deprivation that leads to mid-oogenesis cell death (45), suggesting an important role of DmChk2 in the induction of cell death in response to starvation. In contrast to DmChk2, expression of Dmp53, a Chk2-dependent effector protein, in the germline, affected stem cell survival but not mid-oogenesis egg chambers. Thus, to that end it seems that the only tissue that is refractory to DmChk2 but not to Dmp53 levels are mid-oogenesis egg chambers. Moreover, DmChk2 mid-oogenesis induced cell death is not dependent on *Dmp53*. Thus, our study reveals in *Drosophila* a novel role of DmChk2 in inducing cell death in a *Dmp53*-independent manner.

Surprisingly, egg chamber cell death induced by expressing DmChk2 was not prevented by co-expression of caspase inhibitors. Two other major forms of cell death are autophagic cell death and necrosis (46). We found that DmChk2-induced cell death was associated with a delay in autophagy induction and occurred normally in an *Atg7* mutant background, indicating that Chk2-induced cell death is not occurring by autophagic cell death. Further, the cell death observed was not morphologically necrotic, and looked indistinguishable from apoptosis. Taken together, Chk2-induced cell death appears to occur by a caspase-independent apoptosis-like death.

We found that over-expression of Dmp53 led to loss of ovarian stem cells. It was previously shown that during gonad formation, Dmp53 is required for programmed cell death of PGC's (14). In *Dmp53* mutant, an excess of PGC's were found ectopic of the gonads. The role of *Dmp53* in this process was found to be due to germ cell death but not of PGC's migration. Interestingly enough, in contrast to our results, it was reported that *p53* expression in wild-type embryos using the same Gal4 line used by us (*nanos-Gal4VP16*) did not affect survival of PGCs in the gonads (14). The difference between our results and the previous findings is likely due to the use of different pUAS vectors. In this study, we express Dmp53 using the pUASp vector, which allow expression in both somatic and germline tissues (36), whereas Yamada et al. expressed Dmp53 in the pUAST vector which drives expression only in somatic cells.

We demonstrated that loss of ovarian stem cell by Dmp53 was not suppressed by expression of caspase inhibitors. Moreover, we were unable to detect DNA fragmentation by TUNEL in PGC's from larval ovaries expressing Dmp53. A similar type of cell death was seen with overexpression of lipid phosphate phosphatases Wun or Wun2 in somatic tissues (47–51). Wun/Wun2- induced germ cell death is not affected by the expression of the inhibitor-of-apoptosis proteins. Moreover, cells dying in response to Wun/Wun2-mediated signals were negative for TUNEL staining and did not label for another marker of apoptosis, cleaved caspase 3. Thus, Dmp53 and Wun/Wun2 induces cell death of the PGC's in a caspase independent manner. To check what other cell death mechanism led to the loss of PGC's by Dmp53, we stained larval ovaries with the lysosomal marker, LT and autophagy marker, GFP-LC3. Using LT and GFP-LC3 staining we detect that activation of a lysosomal process is accompanying the loss of PGC's by Dmp53. Thus, our results revealed that lysosomal cell death may contribute to loss of PGC's by Dmp53.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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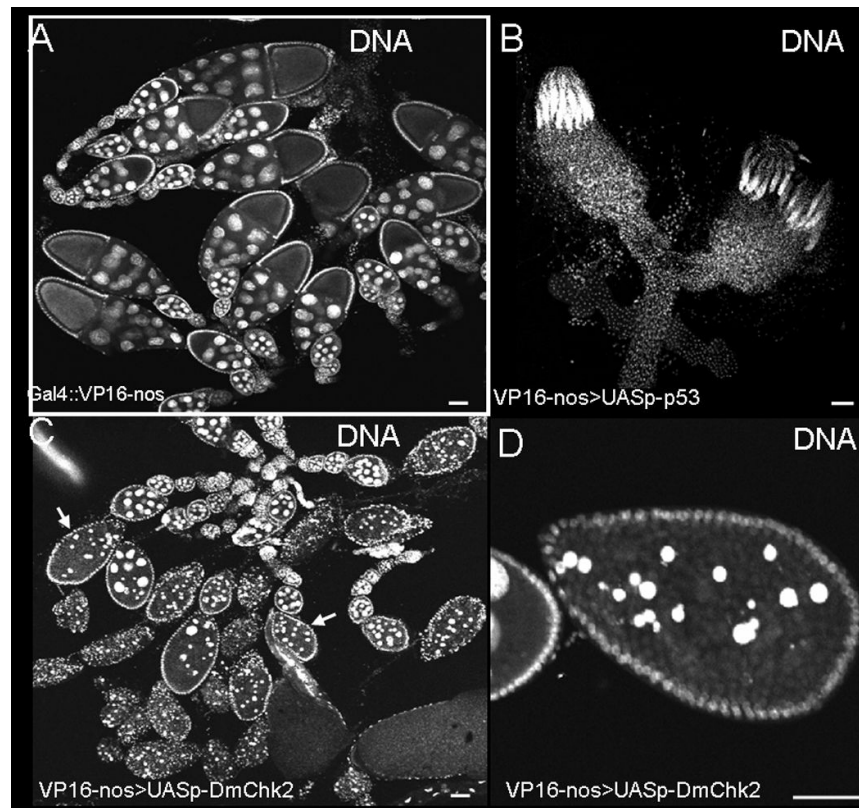


Figure 1. Effects of Dmp53 and DmChk2 over-expression using *nanos*-Gal4VP16 in germline
 Egg chambers were stained with Hoechst. A) *nanos*-Gal4VP16 *Drosophila* ovaries. B) Over-expression of Dmp53 in the germline using the above Gal4 driver resulted in small ovaries probably due to loss of germline stem cells. C) Over-expression of DmChk2 in the germline leads to massive egg chamber death; degenerating egg chambers are indicated by arrows. D) Closer examination of a degenerating egg chamber over-expressing *DmChk2*. Scale bar is 50µm.

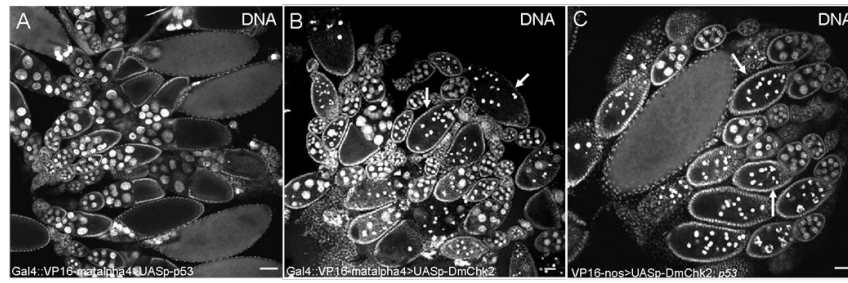


Figure 2. Effects of Dmp53 and DmChk2 over-expression using P{matalpha4-GAL-VP16} V2H in the germline

A) DNA staining of egg chambers from P{matalpha4-GAL-VP16}V2H:UASp-p53 fly. B) DNA staining of egg chambers from P{matalpha4-GAL-VP16}V2H : UASp-HA-Chk2 fly. Degenerating egg chambers of stages 8–9 are indicated by arrows. C) DNA staining of egg chambers from P{matalpha4-GAL-VP16}V2H/ UASp-HA-Chk2; *p53* flies. Degenerating egg chambers of stages 8–9 are indicated by arrows. Scale bar is 50 μ m.

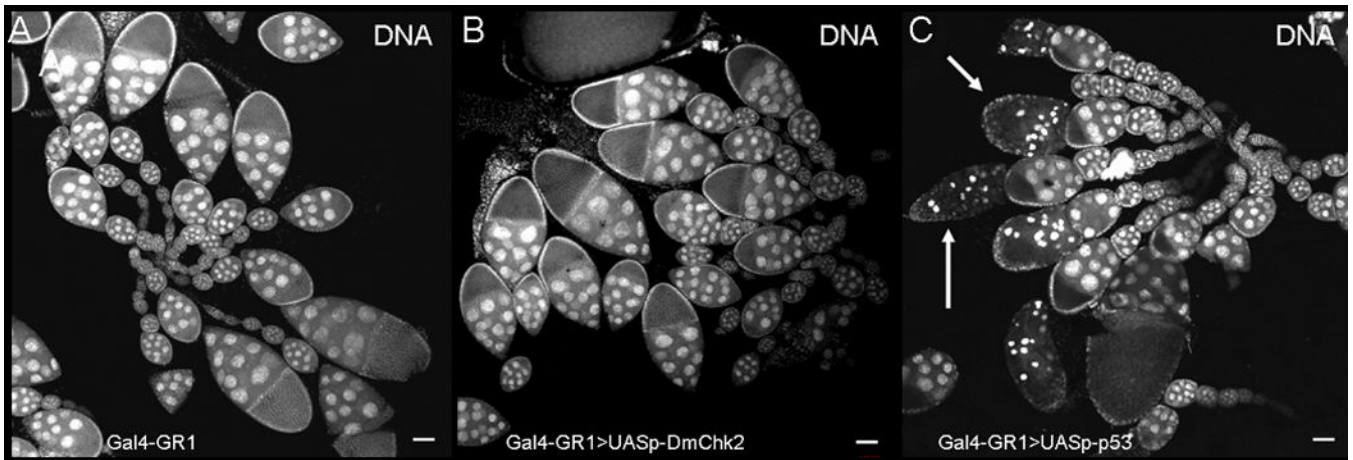


Figure 3. Over-expression of Dmp53 but not of DmChk2 in the follicle somatic cells using GR1-Gal4 driver induced germline cell death

Egg chambers were stained with Hoechst. A) Egg chambers from GR1-GAL4 flies. B) Expressing DmChk2 in the follicle cell does not affect egg chamber survival. C) Expressing Dmp53 in the follicle cells lead to egg chamber death (marked with arrows).

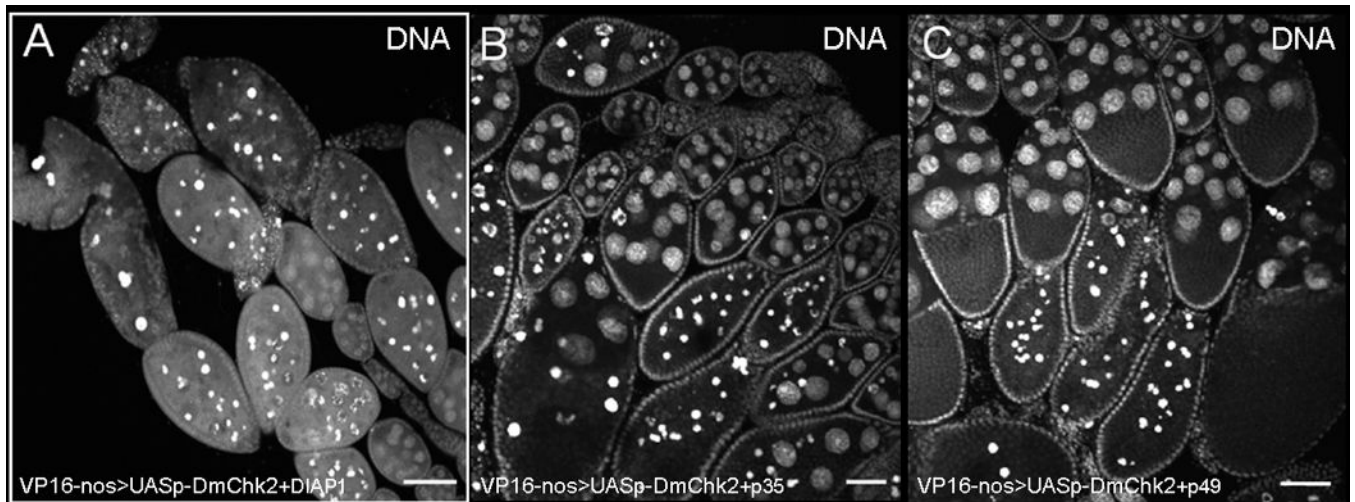


Figure 4. DmChk2-mid oogenesis cell death is caspase-independent

Egg chambers were stained with Hoechst. A) Egg chambers from flies over-expressing DmChk2 and DIAP1 undergo cell death similar to egg chambers expressing DmChk2 alone. Over-expressing p35 (B) or p49 (C) does not suppress DmChk2-mid oogenesis cell death. Scale bar is 50 μ m.

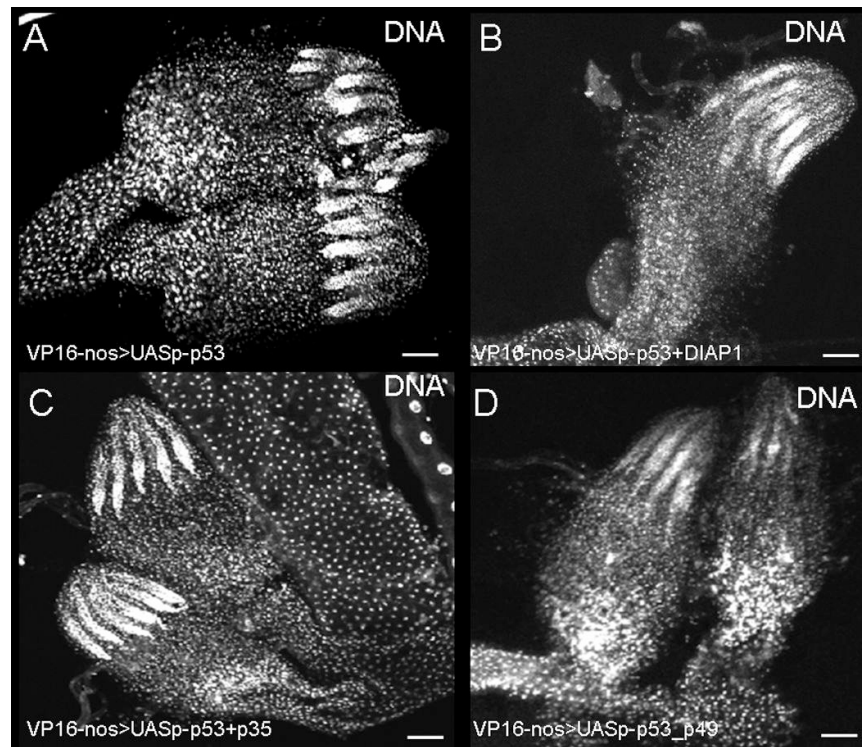


Figure 5. Expression of caspase inhibitors do not suppress p53-mediated stem cell loss
 Ovaries were stained with Hoechst. A) ovaries from *nanos-Gal4VP16; pUASp53*.
 Expression of p53 led to loss of stem cells. Over-expressing DIAP1 (B) or p35 (C) or p49
 (D) does not suppress Dmp53-stem cell loss.

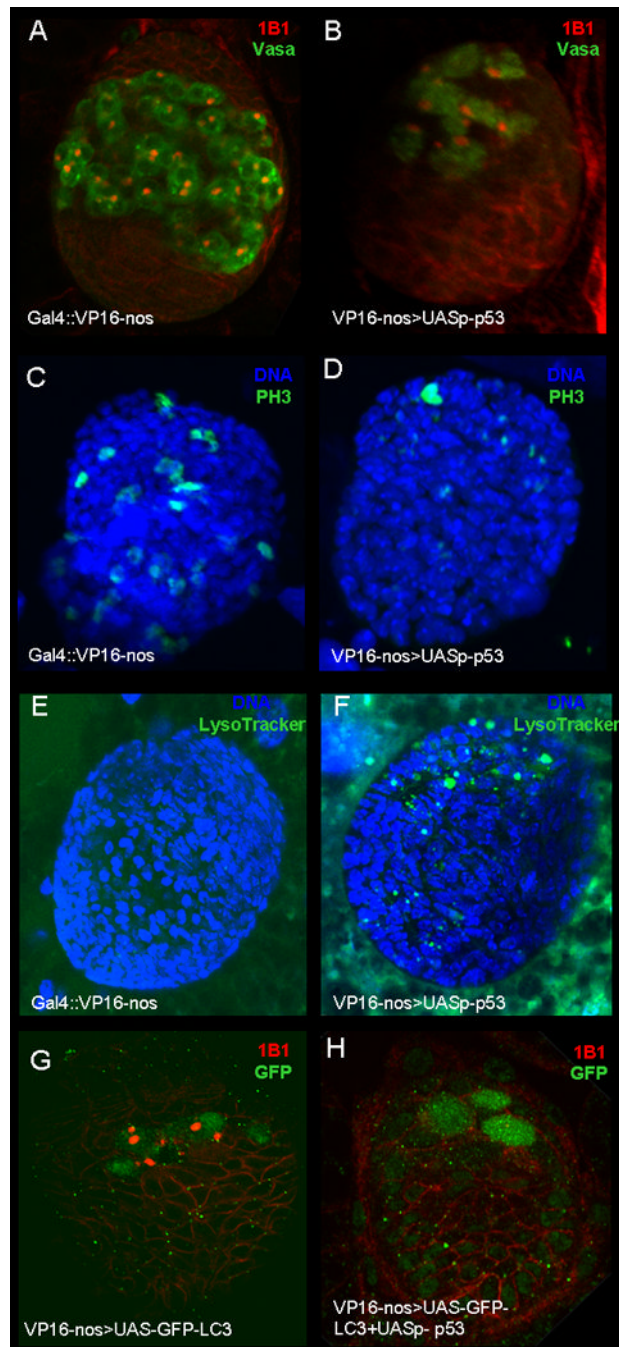


Figure 6. Expression of Dmp53 in the germline lead to loss of primordial germ cells (PGC's) Gonads from third instar larvae were stained with germ cell marker, vasa (A–B; Green), anti-*Adducin-like* antibodies (A–B and G–H; Red), anti-phospho-histone 3 (C–D, Green), LysoTracker (E–F, Green), anti-GFP (G–H) and DAPI (C–F, Blue). A) *nanos-Gal4VP16* ovary. B) Ovary from *nanos-Gal4VP16; UASp- p53* fly, over-expressing of Dmp53 lead to loss pf PGC's. C) *nanos-Gal4VP16*. D) Ovary from *nanos-Gal4VP16; UASp- p53* fly, over-expressing of Dmp53 lead to decrease in numbers of proliferating PGC's. E) Ovary from *nanos-Gal4VP16*. F) Ovary from *nanos-Gal4VP16; UASp- p53* fly, LysoTracker positive puncta (arrows) have begun to accumulate in the ovary. G) Ovary from *nanos-Gal4VP16*;

GFP-LC3 stained with anti-GFP. H) Ovary from *nanos-Gal4VP16/UASp-p53*; GFP-LC3 stained with anti-GFP.

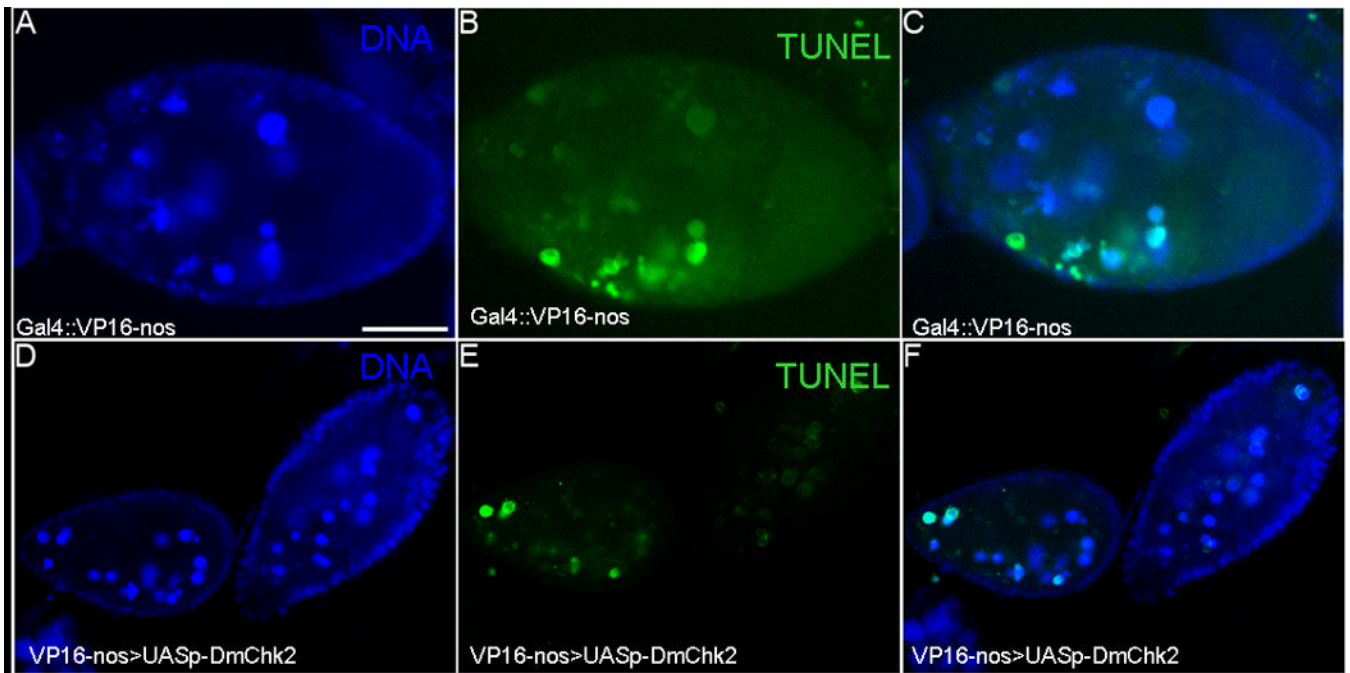


Figure 7. DmChk2-mediated cell death is characterized by DNA fragmentation

All egg chambers were stained with DAPI (Blue) and TUNEL (Green). A) A control degenerating egg chamber from a nutrient deprived fly has condensed and fragmented nurse cell nuclei. B) TUNEL-positive puncta are detected in the same egg chamber. C) Merge of A–B. D) A degenerating egg chamber from a female over-expressing *DmChk2* has condensed and fragmented nurse cell nuclei. E) The degenerating egg chamber over-expressing *DmChk2* also has TUNEL-positive puncta. F) Merge of D–E. Scale bar is 50 μ m. All images were taken at the same magnification.

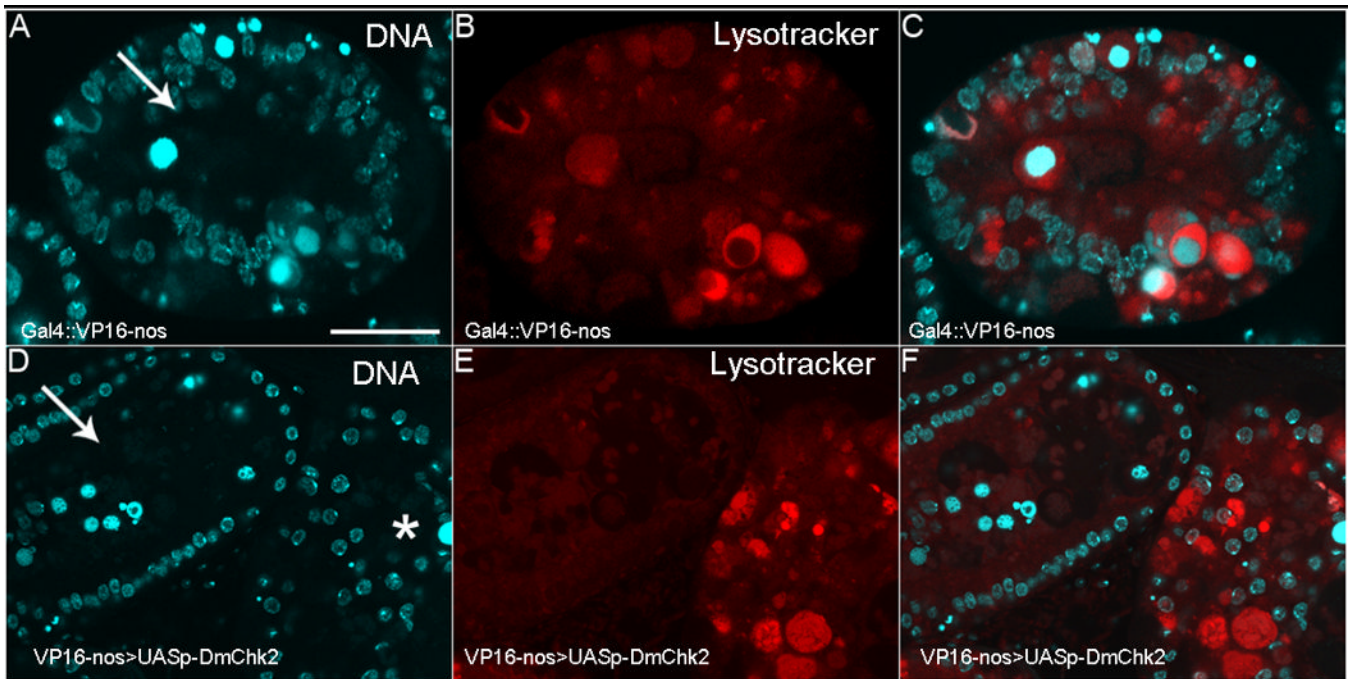


Figure 8. Lysosome activity is delayed in flies over-expressing *DmChk2*

All egg chambers were stained with DAPI (Blue) and LysoTracker (Red). A) Control *NGT;nosGAL4* egg chamber from a nutrient deprived fly with condensed nurse cell nuclei that have fragmented. B) LysoTracker positive puncta have begun to accumulate in this egg chamber. C) Merge of A–B. D) Egg chambers over-expressing *DmChk2* at different stages of degeneration (arrow, middle and *, late) E) LysoTracker staining can only be seen in the later degenerating egg chamber (*). F) Merge of D–E. Scale bar is 20 μ m. All egg chambers were taken at the same magnification.