endostatin, as well as regions of intense tumstatin and endostatin accumulation (fig. S6D). These results are consistent with a model in which α(II)PH expression augments production of antiangiogenic collagen fragments. In culture, α(II)PH-expressing H1299 cells and control H1299 cells had comparable growth rates and apoptotic indices (Fig. 4B, inset). Thus, the differences in tumor size in xenografted mice were not because α(II)PH expression reduced the intrinsic growth rate of, or induced apoptosis in, H1299 cells.

Loss of p53 function is a common event in tumor progression (*11*), and our data suggest that p53-negative tumors may lack an important mechanism for limiting tumor vascularization. In support of this idea, xenografted tumors derived from $p53^{-/-}$ HCT116 cells were significantly more vascularized and had large areas lacking tumstatin staining compared with tumors derived from wild-type HCT116 cells (fig. S7).

We next asked whether adenovirus-mediated α(II)PH expression could reduce growth of tumors derived from B16 cells, a highly aggressive mouse melanoma cell line (*12*). Treatment of B16-derived melanomas with α(II)PH-expressing adenovirus significantly reduced tumor volume (Fig. 4C). Thus, α(II)PH can inhibit tumor growth when intracellularly expressed or exogenously delivered.

Although it is well established that C-terminal collagen fragments have an antiangiogenic activity (*13*), their physiological role and connection to cellular growth pathways have not been elucidated. Our results reveal a genetic and biochemical linkage between the p53 tumor suppressor pathway and the production of antiangiogenic collagen fragments. We propose that α(II)PH induction by p53 results in increased synthesis and secretion of full-length collagens, which are then proteolytically processed in the extracellular matrix to produce antiangiogenic peptides. Although this extracellular proteolytic processing occurs, at least to some extent, constitutively in the absence of p53 (Fig. 2D), p53 expression greatly enhances the processing of full-length collagen 18 to endostatin. On the basis of these observations, we propose that p53 activates a transcriptional program that increases synthesis and processing of collagen-derived antiangiogenic peptides. Several alternative mechanisms by which p53 could negatively affect angiogenesis in tumors have also been suggested (*14*–*16*).

In this study, we focused on collagen 18 because its antiangiogenic properties have been extensively characterized. Consistent with our observations, a recent report has demonstrated that a modest increase (~1.6-fold) in the synthesis of endostatin can lead to marked reduction in tumor growth rates in mice (*17*). Our data indicate that p53-dependent up-regulation of α(II)PH also results in increased synthesis of the collagen 4 C-terminal fragment, tumstatin; by analogy, increased synthesis of other collagen 4 C-terminal fragments, such as canstatin and arresten, seems likely. The shedding of multiple collagen-derived antiangiogenic fragments at the tumor-host interface may be part of a general p53-dependent mechanism of inhibiting tumor vascularization and growth.

References and Notes

1. B. Vogelstein, D. Lane, A. J. Levine, *Nature* **408**, 307 (2000).
2. Materials and methods are available as supporting material on *Science* Online.
3. W. S. el-Deiry *et al.*, *Cell* **75**, 817 (1993).
4. K. I. Kivirikko, R. Myllyla, *Methods Enzymol.* **82**, 245 (1982).
5. In H1299 cells, collagen 18 appeared as a doublet, consistent with previous findings that the collagen 18 gene encodes two major splice variants in human cells (*18*).
6. The observed size difference between endogenous and ectopically expressed endostatin is primarily due to the ~4-kD epitope tag present at the C terminus of the ectopically expressed collagen 18. In addition, sequence variation, as well as slight species differences in the site of proteolytic cleavage, may also contribute to small differences in electrophoretic mobility.
7. Y. Maeshima *et al.*, *Science* **295**, 140 (2002).
8. M. S. O'Reilly *et al.*, *Cell* **88**, 277 (1997).
9. Expression of vascular endothelial growth factor (VEGF) was not affected by EDHB treatment (fig. S8) and therefore cannot explain the inability of CM from EDHB-treated, p53-expressing H1299 cells to induce apoptosis in HUVECs.
10. P. Carmeliet, R. K. Jain, *Nature* **407**, 249 (2000).
11. P. Hainaut *et al.*, *Nucleic Acids Res.* **25**, 151 (1997).
12. I. J. Fidler, *Cancer Res.* **35**, 218 (1975).
13. A. G. Marneros, B. R. Olsen, *Matrix Biol.* **20**, 337 (2001).
14. R. Ravi *et al.*, *Genes Dev.* **14**, 34 (2000).
15. L. Zhang *et al.*, *Cancer Res.* **60**, 3655 (2000).
16. K. M. Dameron, O. V. Volpert, M. A. Tainsky, N. Bouck, *Science* **265**, 1582 (1994).
17. M. Sund *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 2934 (2005).
18. J. Saarela, R. Ylikarppa, M. Rehn, S. Purmonen, T. Pihlajaniemi, *Matrix Biol.* **16**, 319 (1998).

19. We thank S. Benchimol for providing the plasmid pECH53; B. Vogelstein for the HCT116 cell lines; F. Graham for the adenovirus vectors Ad-LacZ, Ad-p53, and Ad-p53mut; B. Olsen for the collagen 18 cDNAs; and R. Kalluri for the antibody to tumstatin. We also thank members of the Green lab, both past and present, for useful discussions; C. Welch and S. Griggs for technical assistance; and S. Evans for editorial assistance. J.G.T. was supported by postdoctoral fellowships from the National Cancer Institute of Canada and the Medical Foundation Charles A. King Trust. M.R.G. is an investigator of the Howard Hughes Medical Institute.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5789/968/DC1
Materials and Methods
Figs. S1 to S9
References

17 February 2006; accepted 27 June 2006
10.1126/science.1126391

Mutations That Increase the Life Span of *C. elegans* Inhibit Tumor Growth

Julie M. Pinkston, Delia Garigan, Malene Hansen, Cynthia Kenyon*

Mutations in *gld-1* cause lethal germline tumors in the nematode *Caenorhabditis elegans*. We find that a wide variety of mutations that extend *C. elegans*' life span confer resistance to these tumors. The long life spans of *daf-2*/insulin-receptor mutants were not shortened at all by *gld-1* mutations; we attribute this finding to decreased cell division and increased DAF-16/p53–dependent apoptosis within the tumors. Mutations that increase life span by restricting food intake or inhibiting respiration did not affect apoptosis but reduced tumor cell division. Unexpectedly, none of these longevity mutations affected mitosis in normal germlines; this finding suggests that cellular changes that lead to longevity preferentially antagonize tumor cell growth.

In nature, there is a strong correlation between physiological aging and tumor susceptibility. Mice, which have short (2-year) mean life spans, frequently acquire tumors after ~1 year, whereas dogs do so after ~10 years and humans only after many decades. Understanding how youthful animals resist tumors may provide new insights into tumor biology, particularly if genes that regulate aging influence tumor susceptibility. Many apoptotic, signaling, and other genes that affect tumors in mammals have orthologs in *C. elegans*. Moreover, mutations affecting insulin/IGF-1 (insulin-like growth factor–1) signaling, mitochondrial activity, and food intake each extend life span in both worms and mammals (*1*–*4*). Thus, *C. elegans* may be a valuable organism in which to investigate links between aging and tumors.

In *C. elegans gld-1* tumor-suppressor mutants, germ cells in the early stages of oogenesis reenter the mitotic cell cycle and overproliferate (*5*). The cells eventually break out of the gonad and fill the body, killing the animal early in life. Like transformed vertebrate cells, these cells proliferate in a growth

Department of Biochemistry and Biophysics, University of California, San Francisco, CA 94158, USA.

*To whom correspondence should be addressed. E-mail: ckenyon@biochem.ucsf.edu

factor–independent manner (*5*), and they do not undergo programmed cell death (*6*). As they do not exhibit an angiogenesis-inducing or clear metastatic phenotype, these cells most likely approximate vertebrate cells in the early stages of tumorigenesis. In this study, we asked whether the lethal effects of this overproliferation could be delayed by longevity mutations that inhibit insulin/IGF-1 signaling [the receptor mutations *daf-2(e1370)* and *daf-2(mu150)*] (*7*, *8*), a mutation that extends life span via caloric restriction [the feeding mutant *eat-2(ad1116)*] (*9*), and mitochondrial mutations that disrupt respiratory chain components [*isp-1(qm150)*] (*10*) or ubiquinone production [*clk-1(qm30)*] (*11*). These mutations affect distinct pathways (*3*, *4*, *9*), although they may converge on common downstream processes. In principle, longevity mutations might not affect the tumor, in which case they would not extend the short *gld-1* mutant life span. Alternatively, as certain p53 mutations that inhibit tumors also accelerate aging and shorten life span (*12*), mutations that lengthen wild-type life span might promote tumor growth and accelerate death. In contrast, all of the longevity mutations tested extended the life spans of *gld-1* mutants (Fig. 1). In fact, the long life spans of the two *daf-2* mutants were not shortened at all by *gld-1* mutations. Thus, we observed a strong correlation between life-span extension and tumor resistance.

How do these longevity mutations affect the tumor? We found that none of the mutations restored oocyte production, but they all reduced germ cell number (Fig. 2). Thus, these mutations were likely to inhibit germ cell proliferation and/or increase germ cell death.

In the wild type, many germ cells undergo apoptosis as they enter oogenesis, but in *gld-1* mutants, this apoptosis is blocked (*6*). Using the dye SYTO12 to label apoptotic cells (*6*), we found that *daf-2* mutations stimulated apoptosis in the *gld-1* germlines (Fig. 3, A and B). *daf-2* mutations also stimulated apoptosis in wild-type germ cells undergoing oogenesis (Fig. 3D). Therefore, *daf-2* mutations appear to trigger a general increase in germline cell death.

How might *daf-2* mutations affect apoptosis? Timed RNA interference (RNAi) experiments showed that *daf-2* acts during adulthood to influence cell death (fig. S1). We found that the transcription factor DAF-16/FOXO, which is required for the longevity of *daf-2* mutants (*8*), was required for *daf-2* mutations to increase germline apoptosis in both the wild type and *gld-1* mutants (Fig. 3D). The *C. elegans* p53 gene *cep-1* (*13*) was required as well (Fig. 3E). p53/*cep-1* does not appear to be a DAF-16 transcriptional target (*14*, *15*). Together, these findings suggest that *daf-2* inhibition during adulthood increases the expression of DAF-16 target genes whose products function, directly or indirectly, along with p53/CEP-1 to stimulate germline apoptosis.

In *C. elegans*, p53 stimulates germline apoptosis in response to genotoxic stress but plays only a minor role in germline apoptosis under normal conditions (*13*). Thus, we hypothesized that *daf-2* mutations might affect apoptosis by shifting cells into a physiological state resembling that induced by genotoxic stress. This idea is attractive because inhibiting *daf-2* activity is known to activate multiple stress resistance processes (*1*, *3*). Consistent with this, we found that DAF-16 was required for the increase in germline apo-

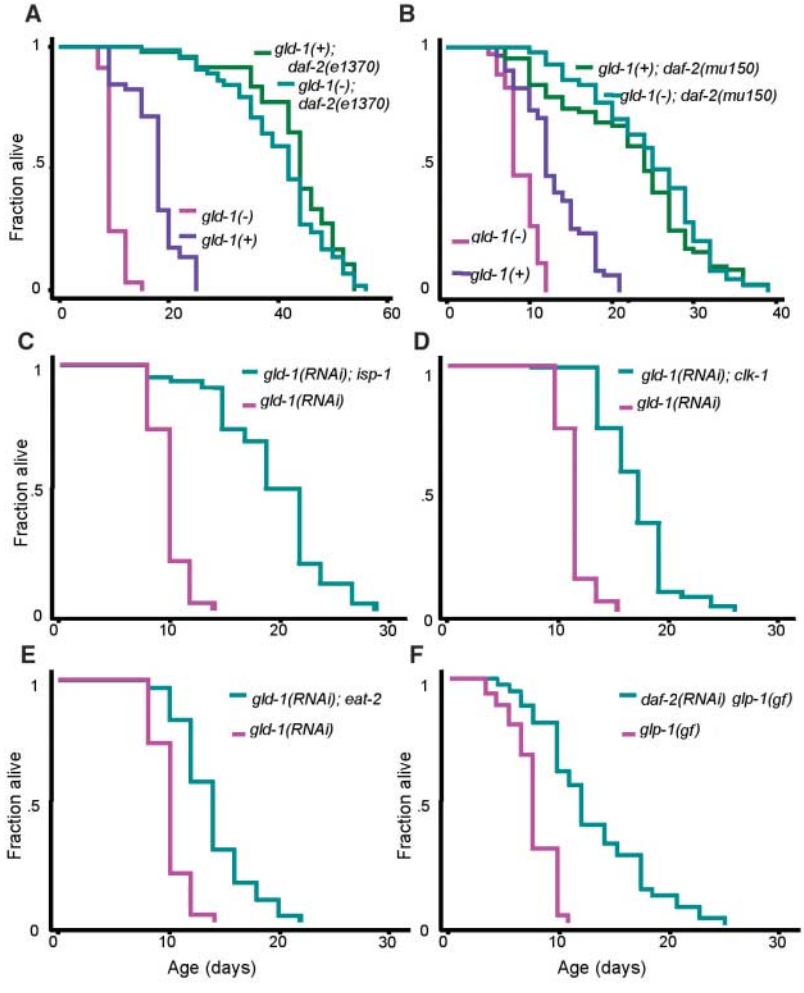

Fig. 1. Mutations that increase longevity delay the death caused by germline-tumor mutations. For each chart, experimental and control animals were grown in parallel. In this and other figures, *gld-1(-)* refers to the null allele *gld-1(q485)*, and all RNAi-treated animals are labeled accordingly. (**A** and **B**) *daf-2(e1370)* (A) and *daf-2(mu150)* mutations (B) suppressed the lethality caused by *gld-1(-)*. Similar results were obtained with *gld-1* RNAi (table S5). In control experiments, a nontumorous *gld-1* mutant (*op236*) (*18*) behaved like the wild type (table S5). (**C** to **E**) *isp-1(qm150)* (C), *clk-1(qm30)* (D), and *eat-2(ad1116)* mutations (E) delayed the death caused by *gld-1* RNAi, although the animals did not live as long as *isp-1*, *clk-1*, or *eat-2* mutants exposed to control RNAi. The magnitude of the life-span extension caused by these mutations was roughly the same in *gld-1(-)* and *gld-1(+)* backgrounds (table S5). (**F**) *daf-2(RNAi)* delayed the death caused by another germline tumor mutant, *glp-1(ar202)*. All experiments were repeated at least twice with similar results (table S5). We considered the possibility that germline tumors shorten life span by down-regulating the life span–extending protein DAF-16, because removing the germ cells in the wild type extends life span in a *daf-16*–dependent manner. However, changes in DAF-16 activity cannot be responsible, because *gld-1* RNAi further shortens the life spans of *daf-16(null)* mutants (table S5). As they near death, the animals become rigid and so packed with germ cells that none of their organs are visible by Nomarski microscopy, a finding consistent with tumors' killing the animal.

ptosis caused by gamma irradiation in wild-type animals (Fig. 3G). In addition, the checkpoint genes *hus-1*, *mrt-2*, and *clk-2*, which act upstream of p53 in the DNA damage–induced apoptotic pathway (*16*), were required for *daf-2* mutations to increase apoptosis in otherwise wild-type animals (Fig. 3G).

The cell death that occurs in wild-type and *gld-1; daf-2(-)* double-mutant germ cells is restricted to cells entering oogenesis. For this reason, we also examined a germline-tumor mutant in which cells do not progress to meiosis. The *glp-1(ar202gf)* mutation renders the GLP-1/Notch receptor, which maintains the germ cells in the stem-cell state, constitutively active (*17*). We found that *daf-2* mutations were unable to stimulate apoptosis in this germline tumor (table S1). Thus, *daf-2* mutations may trigger cell death specifically in cell populations that are already "primed" for apoptosis. Consistent with this interpretation, p53 levels are known to be elevated in *gld-1* mutants (*18*).

The life spans of *gld-1(RNAi); daf-2(-)* animals were shortened when cell death was prevented with either the *ced-3(n1286)* caspase mutation (*19*) or the *cep-1(gk138)* p53 mutation (*20*). [Cell death mutations do not reduce wild-type or *gld-1(+); daf-2(-)* life spans (*7*) (table S2).] Nonetheless, *daf-2* mutations still more than doubled the life spans of both of these apoptosis-defective tumor strains (Fig. 3C). This result suggested that *daf-2* mutations might influence cell proliferation as well as apoptosis.

We assayed cell proliferation using an M phase–specific (phospho–histone H3) antibody (*21*) and found that *daf-2* mutations decreased the number of actively dividing germ cells in *gld-1* mutants (Fig. 4, A and B). We observed a similar decrease when apoptosis was blocked by a *ced-3* mutation (table S3), indicating that the decrease in labeling was not due to increased apoptosis. *daf-2* mutations also inhibited cell division in the *glp1/Notch* germline tumors; consistent with this finding, these animals had a smaller overall germ cell number and increased life spans (Fig. 1F and fig. S2).

daf-16 was required for *daf-2(mu150)* mutations to inhibit *gld-1* germline mitosis. However, a small portion of the effect caused by the stronger allele, *daf-2(e1370)*, was *daf-16* independent (Fig. 4C). Likewise, *daf-2(e1370)* mutations slightly extended the life spans of *gld-1(RNAi) daf-16(null)* mutants (~20%, $P < 0.0001$) (fig. S3).

Unexpectedly, *daf-2(-)* mutations did not affect the number of M-phase germline cells in a wild-type background (although we may not have detected a subtle change with this antibody) (Fig. 4C). Thus, *daf-2(-)*'s antiproliferative effect appeared to be specific to tumor cells. This was particularly intriguing for the *glp-1/Notch* mutant, because its tumors are thought to consist of an overabundance of normal germline stem cells. Perhaps the insulin/IGF-1 pathway, which controls food usage and growth in many species, becomes limiting for cell proliferation in the face of such a large metabolic load.

In *Drosophila*, insulin/IGF-1–pathway mutations were recently reported to prevent normal germ cell growth and maturation (*22*). The discrepancy between this finding and ours may reflect the strength of the mutations examined, because stronger *daf-2* alleles in *C. elegans* inhibit reproduction severely (*23*). In contrast, the *daf-2(mu150)* mutants reproduced normally, in terms of both reproductive timing and brood size, and *daf-2(e1370)* mutants grown at 20°C had only a slight reduction in fecundity (table S4), as reported previously (*7*, *8*).

In humans, PTEN tumor-suppressor mutations stimulate oncogenesis by elevating insulin/IGF-1 signaling (*24*). This inhibits the DAF-16 ortholog FOXO3a, which in turn prevents apoptosis and promotes cell proliferation (*25*). Here, we decreased insulin/IGF-1 signaling (and elevated DAF-16 activity) throughout the animal and obtained a strong tumor-suppressive effect. This may have implications for mammalian tumors, including those that do not carry mutations in the insulin/IGF-1 signaling pathway. Mutations that inhibit growth hormone signaling extend life span and increase tumor resistance in rodents (*26*). Our findings suggest that reduced insulin/IGF-1 signaling, which results from growth hormone deficiency, may be responsible for their cancer resistance, and that this resistance is mediated by FOXO (and possibly p53) activity.

Are these tumor-suppressive mechanisms activated by other longevity pathways? To address this question, we analyzed mutations

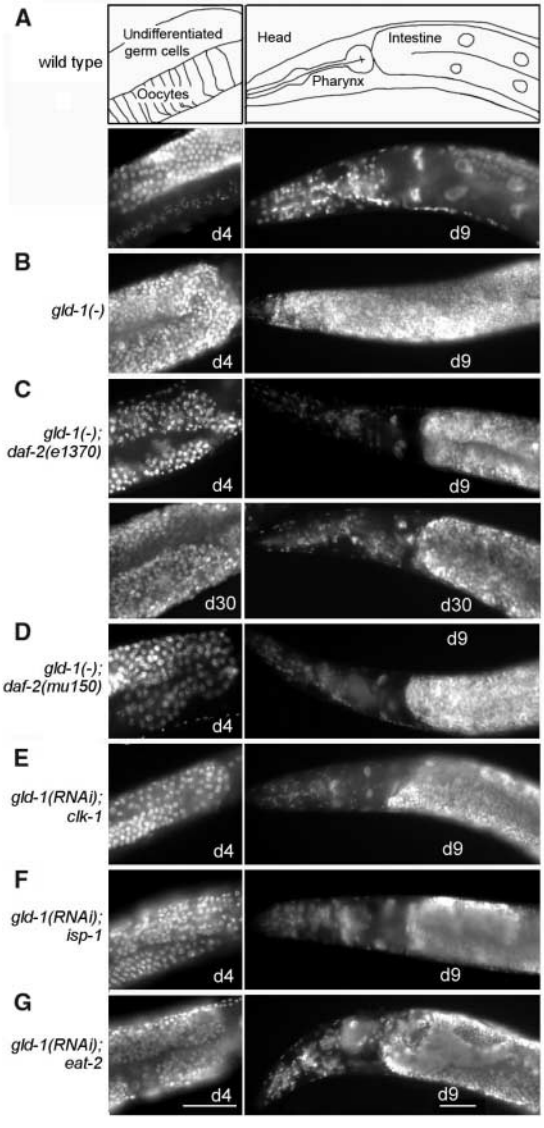

Fig. 2. Mutations that increase longevity reduce germ cell number in *gld-1(-)* mutants. Adult animals were stained with the DNA-intercalating dye DAPI (4′,6′-diamidino-2-phenylindole). Left panels, midpoints of the gonad arms; right panels, heads. In all panels, anterior is to the left; d, day of adulthood. (**A**) Wild type. (**B**) *gld-1(q485)* mutants lack oocytes and have many undifferentiated germ cells in their gonads (left), which later break out of the gonad and fill the head and body (right). (**C**) *gld-1(q485); daf-2(e1370)* and (**D**) *gld-1(q485); daf-2(mu150)* double mutants lack oocytes; however, they have far fewer undifferentiated germ cells (left) and maintain the integrity of their gonads (right), even into old age [(C), lower panel]. The number of germ cells in *gld-1(op236)*, a nontumorous mutant of *gld-1*, is unaffected by *daf-2* RNAi (table S6). (**E** to **G**) *isp-1(qm150)* (E), *clk-1(qm30)* (F), and *eat-2(ad1116)* mutants (G) also have fewer undifferentiated germ cells in the presence of *gld-1* RNAi (left), and the spread of germ cells into the body is delayed (right). Scale bars, 20 μm. For germ cell estimates and statistical analysis, see table S6. We considered the possibility that *daf-2* mutations increased the (low) frequency of transdifferentiation of *gld-1(-)* germ cells into somatic cells. We found, using Nomarski optics, DAPI staining, and neuronal green fluorescent protein markers, that this was not the case (fig. S5).

that cause caloric restriction [*eat-2(ad1116)*] (*9*) or impair mitochondrial activity [*isp-1(qm150)* and *clk-1(qm30)*] (*10*, *11*). None of these mutations affected germline apoptosis in *gld-1* tumor mutants (Fig. 3F and fig. S4). Thus, this effect appears to be specific to the insulin/IGF-1 pathway. However, all of the mutations reduced the number of M-phase germ cells (Fig. 4D). Remarkably, as with *daf-2* mutations, none of these mutations reduced the number of M-phase germ cells in *gld-1(+)* animals (Fig. 4D). Thus, all of the longevity mutations we examined preferentially affected the proliferation of tumor cells. This implies a molecular link between processes that protect or repair cells and processes that disadvantage tumor cell growth.

Caloric restriction inhibits tumorigenesis in mammals, and our findings suggest that inhibiting the mammalian orthologs of *isp-1* and *clk-1* may do so as well. *isp-1* mutations are thought to increase life span by inhibiting respiration (*3*, *4*, *10*). Perhaps tumor cells, which

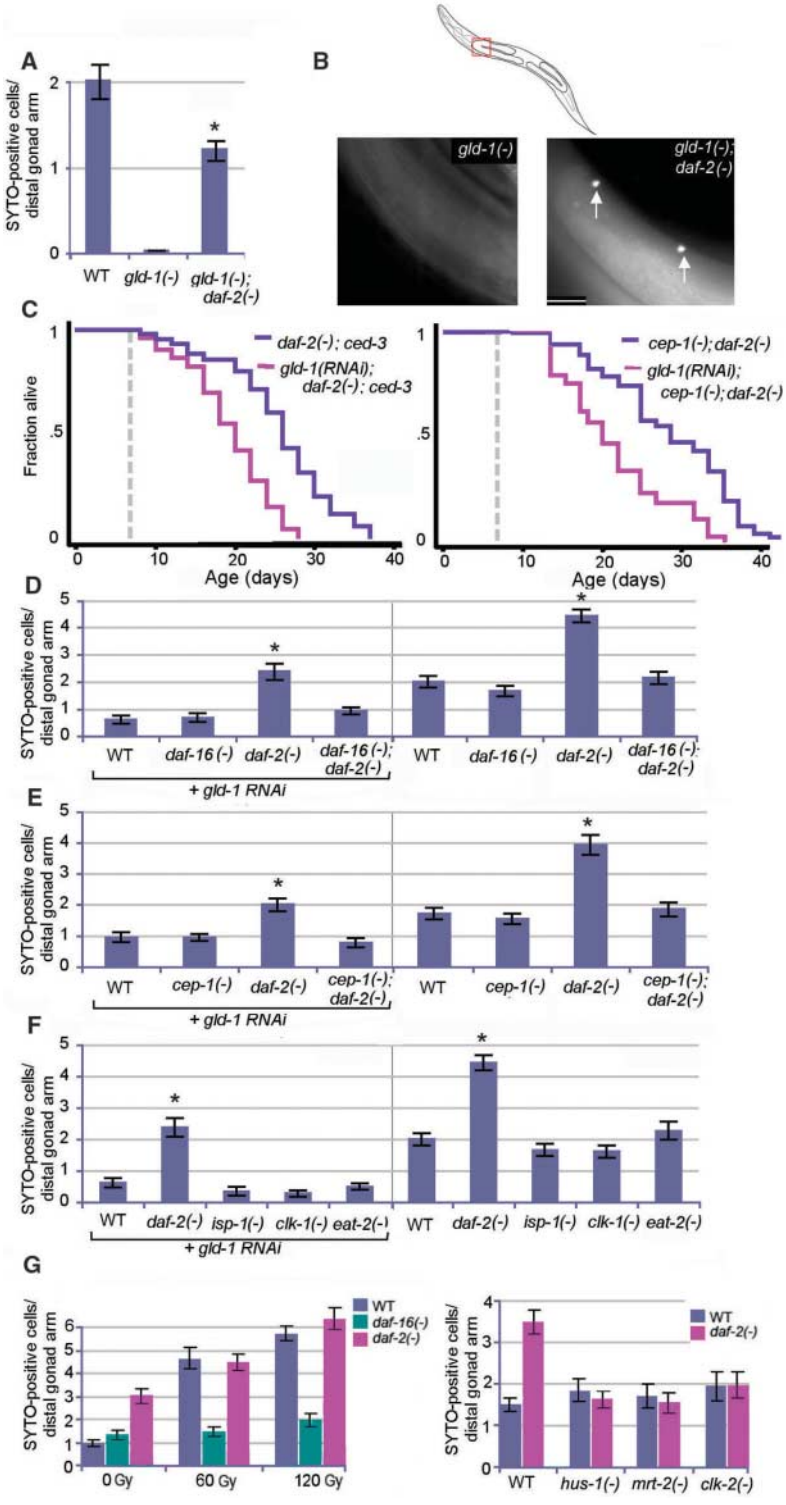

Fig. 3. p53- and DAF-16–dependent germline apoptosis is triggered by insulin/IGF-1 but not by mitochondrial or feeding mutations. Germ cell corpses were identified by SYTO12 labeling of day 2 (48-hour) adults. Each bar represents the mean (±SE) of at least three combined experiments (for details, see table S1); **P* < 0.0001. (**A**) *daf-2(e1370)* mutations partially restored germ cell death in *gld-1(q485)* mutants. This was blocked by a *ced-3(-)* mutation (table S1). (**B**) Representative SYTO12-labeled *gld-1(q485)* mutant (left) and *gld-1(q485); daf-2(e1370)* double mutant (right). Arrows, stained germ cell corpses; scale bar, 20 μm. (**C**) Life spans of *gld-1(RNAi); daf-2(e1370)* animals were reduced ~20% by either *ced-3(n1286)* (left), *cep-1(gk138)* (right), or *ced-4(n1162)* (table S2) mutations. The number of germ cells in a *gld-1(-); daf-2(-)* animal was increased by either a *ced-3(-)* or *cep-1(-)* mutation (table S6). Dashed line, mean life span of animals treated with *gld-1* RNAi. (**D** to **F**) Left panels, *gld-1(RNAi)* animals; right panels, *gld-1(+)* animals; **P* < 0.0001. (D) *daf-2* mutations increased germ cell apoptosis in wild-type as well as *gld-1(-)* animals; this increase was *daf-16* dependent. Residual germ cell apoptosis in *gld-1(RNAi)* animals was likely due to incomplete RNAi knockdown. The requirement of *daf-16* for this apoptosis is consistent with life-span data (fig. S6). (E) The *daf-2*–dependent increase in germ cell apoptosis was reversed by *cep-1(gk138)*. (F) *isp-1(qm150)*, *clk-1(qm30)*, and *eat-2(ad1116)* mutations did not affect germ cell apoptosis in *gld-1(-)* or wild-type animals. (**G**) *daf-16* was necessary for DNA damage–induced apoptosis (left). Germ cell corpses were scored 18 hours after irradiation. The checkpoint genes *hus-1*, *mrt-1*, and *clk-2* were required for *daf-2* RNAi to increase apoptosis in the wild type (right). For details and statistical data, see table S1.

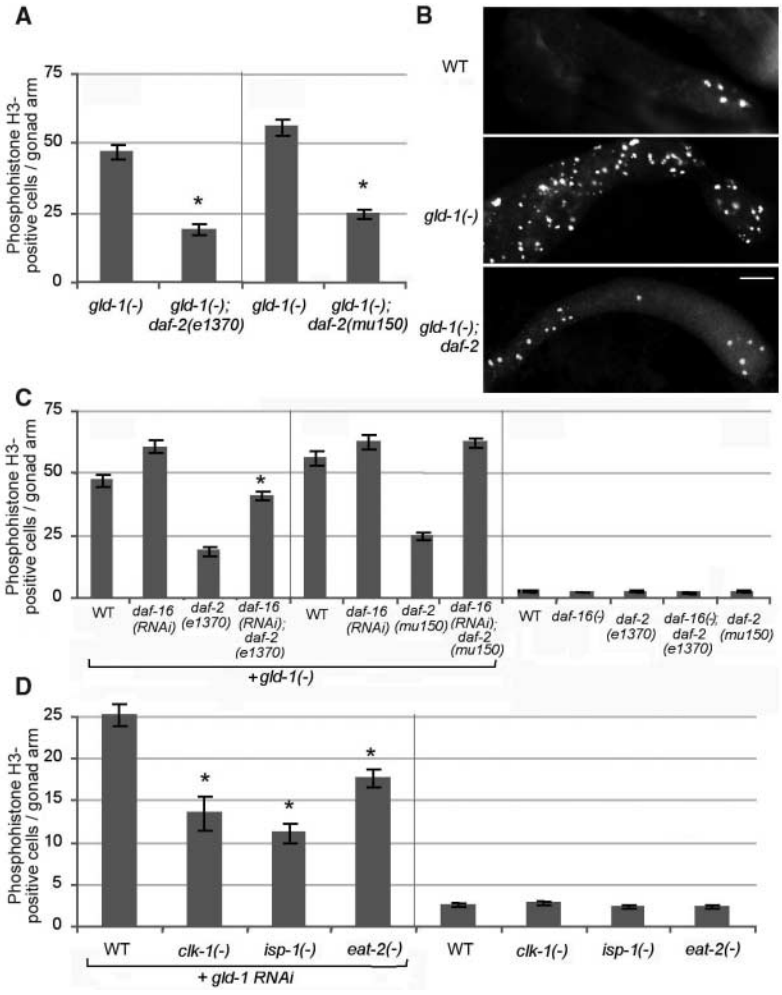

Fig. 4. Longevity mutations reduce the number of M-phase germ cells in *gld-1(-)* but not wild-type animals. Dividing germ cells were scored by antibody labeling to phospho–histone H3 at 36 hours of adulthood. Each bar represents the mean (±SE) of at least three combined experiments; *$P < 0.0001$. (**A**) *daf-2(mu150)* and *daf-2(e1370)* mutations reduced labeled cells in *gld-1(-)* mutants by about 50%. Similar results were obtained with *gld-1(RNAi)* (table S3). (**B**) Representative gonads of wild-type (top), *gld-1(q485)* (middle), and *gld-1(q485); daf-2(e1370)* (bottom) animals labeled with antibody to phospho–histone H3. Scale bar, 20 μm. (**C**) The reduction of mitotic germ cells by *daf-2(e1370)*, but not *daf-2(mu150)*, mutations was incompletely reversed by *daf-16* RNAi. Similar results were obtained in *gld-1(RNAi) daf-16(mu86); daf-2(e1370)* animals (table S3). In contrast, a *cep-1(-)* mutation had no effect on the number of mitotic cells (table S3). In a wild-type background, *daf-2* mutations had no effect on germ cell divisions (right). (**D**) *clk-1(qm30)*, *isp-1(qm150)*, and *eat-2(ad1116)* mutations reduced the number of M-phase cells in *gld-1(RNAi)* animals by about 35%, 50%, and 30%, respectively, but had no effect in a wild-type background. The scale in (D) differs from that in (A) and (C) because *gld-1* RNAi has a less severe effect on proliferation than a *gld-1(q485)* mutation. For details and statistical data, see table S3.

have low rates of respiration, are particularly sensitive to further reductions, or perhaps reducing respiration rates in surrounding cells inhibits tumor growth. The mechanism by which *clk-1* mutations increase life span is not known. *C. elegans clk-1* mutants, which receive essential quinones from the environment (*27*), do not have reduced metabolic output (*28*). The longevity of *clk-1*$^{+/-}$ mice has been associated with increased stress resistance (*2*). It will be interesting to investigate the effects of *clk-1* mutations on mammalian tumors.

Our results show that many *C. elegans* longevity mutations are tumor protective. The generality of our findings, along with the cancer resistance of long-lived calorically restricted and endocrine-mutant rodents, argues that mutations such as the p53 alleles that inhibit tumors but accelerate aging (*12*) may be the exception rather than the rule. It may be that during evolution, longevity and delayed tumorigenesis arose together—for example, from endocrine pathway and mitochondrial mutations that influenced both processes.

References and Notes

1. M. Tatar, A. Bartke, A. Antebi, *Science* **299**, 1346 (2003).
2. X. Liu *et al.*, *Genes Dev.* **19**, 2424 (2005).
3. C. Kenyon, *Cell* **120**, 449 (2005).
4. S. Hekimi, L. Guarente, *Science* **299**, 1351 (2003).
5. R. Francis, E. Maine, T. Schedl, *Genetics* **139**, 607 (1995).
6. T. L. Gumienny, E. Lambie, E. Hartwieg, H. R. Horvitz, M. O. Hengartner, *Development* **126**, 1011 (1999).
7. D. Garigan *et al.*, *Genetics* **161**, 1101 (2002).
8. C. Kenyon, J. Chang, E. Gensch, A. Rudner, R. Tabtiang, *Nature* **366**, 461 (1993).
9. B. Lakowski, S. Hekimi, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 13091 (1998).
10. J. Feng, F. Bussiere, S. Hekimi, *Dev. Cell* **1**, 633 (2001).
11. S. Felkai *et al.*, *EMBO J.* **18**, 1783 (1999).
12. S. D. Tyner *et al.*, *Nature* **415**, 45 (2002).
13. B. Schumacher, K. Hofmann, S. Boulton, A. Gartner, *Curr. Biol.* **11**, 1722 (2001).
14. C. T. Murphy *et al.*, *Nature* **424**, 277 (2003).
15. J. McElwee, K. Bubb, J. H. Thomas, *Aging Cell* **2**, 111 (2003).
16. A. Gartner, S. Milstein, S. Ahmed, J. Hodgkin, M. O. Hengartner, *Mol. Cell* **5**, 435 (2000).
17. A. S. Pepper, D. J. Killian, E. J. Hubbard, *Genetics* **163**, 115 (2003).
18. B. Schumacher *et al.*, *Cell* **120**, 357 (2005).
19. J. Yuan, S. Shaham, S. Ledoux, H. M. Ellis, H. R. Horvitz, *Cell* **75**, 641 (1993).
20. X. Deng *et al.*, *Nat. Genet.* **36**, 906 (2004).
21. K. Ajiro, K. Yoda, K. Utsumi, Y. Nishikawa, *J. Biol. Chem.* **271**, 13197 (1996).
22. L. LaFever, D. Drummond-Barbosa, *Science* **309**, 1071 (2005).
23. D. Gems *et al.*, *Genetics* **150**, 129 (1998).
24. K. Inoki, M. N. Corradetti, K. L. Guan, *Nat. Genet.* **37**, 19 (2005).
25. A. Essafi *et al.*, *Oncogene* **24**, 2317 (2005).
26. M. M. Ramsey *et al.*, *Endocrinology* **143**, 4139 (2002).
27. T. Jonassen, P. L. Larsen, C. F. Clarke, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 421 (2001).
28. B. P. Braeckman *et al.*, *Mech. Ageing Dev.* **123**, 1447 (2002).

29. We thank D. Toczyski, as well as S. Henis-Korenblit and other Kenyon lab members, for helpful discussions and the *Caenorhabditis* Genetics Center for strains. Supported by NIH and the Ellison Foundation for Medical Research (C.K.), NIH training grant AG000278 (J.M.P.), and the Danish Medical and Natural Science Research Councils (M.H.). C.K. is the director of the UCSF Hillblom Center for the Biology of Aging, an ACS Research Professor, and a founder of Elixir Pharmaceuticals.

Supporting Online Material

www.sciencemag.org/cgi/content/full/313/5789/971/DC1
Materials and Methods
Figs. S1 to S6
Tables S1 to S6
References

27 October 2005; accepted 16 June 2006
10.1126/science.1121908