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Drosophila foxo acts in males to cause sexual-dimorphism in tissue-specific *p53* life span effects

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ABSTRACT

Sex-specific selective pressures are hypothesized to lead to sexually antagonistic gene functions that contribute to phenotypes such as aging and cancer. However, relatively little is known about the identity of such genes and possible mechanisms. Here we report that nervous system-specific over-expression of wild-type *p53* in *Drosophila* caused decreased life span in males and increased life span in females. In contrast, tissue-general over-expression produced the opposite pattern: increased life span in males and decreased life span in females. In a *foxo* null background, *p53* life span effects in males were reversed, becoming similar to the effects in females. In contrast, a *Sir2* null background tended to reduce the magnitude of *p53* effects. The data demonstrate that wild-type *p53* over-expression can regulate life span independent of *foxo*, and suggest that *foxo* acts in males to produce sexually antagonistic life span effects of *p53*.

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1. Introduction

The mammalian p53 transcription factor is a multi-functional tumor suppressor that regulates apoptosis, cell senescence, oxidative stress responses, and mitochondrial metabolism (Green and Kroemer, 2009), and it is preferentially required in females for neural tube closure, embryonic viability and adult fecundity (Chen et al., 2008; Hu et al., 2008; Kang et al., 2009). Several lines of evidence implicate *p53* in aging. Truncated forms of p53 protein in mice can produce a premature aging-like phenotype, apparently by causing a state of p53 hyperactivation (Maier et al., 2004; Moore et al., 2007), whereas increasing the dose of wild-type p53 along with the p53 activator p19ARF could delay aging (Matheu et al., 2007). These data suggest that increased activity of wild-type p53 can promote longevity, whereas mis-regulated and activated p53 forms can promote aging. Taken together, the results from mammals suggest that *p53* exhibits antagonistic pleiotropy, in that it favors development, fecundity and cancer resistance in young animals, but may promote aging in old animals (Hu et al., 2008; Kang et al., 2009; Rodier et al., 2007; Ungewitter and Scrable, 2009).

Inhibition of the insulin/IGF1-like signaling (IIS) pathway increases life span in *Caenorhabditis elegans*, *Drosophila* and mice, and in *C. elegans* this has been shown to be dependent upon the forkhead-family transcription factor Daf16 (Kenyon, 2005; Murphy et al., 2007; Samuelson et al., 2007). The most closely related transcription factor in *Drosophila* is Foxo, which can increase life span when over-expressed in fly fat-body tissue (Giannakou et al., 2004; Hwangbo et al., 2004). It has been suggested that one way p53 might affect life span in mammals and other species is by interacting with the IIS pathway (Scrable et al., 2009). For example, in *C. elegans*, inactivation of the *p53* homolog *cep-1* causes increased life span, and this was dependent upon function of Daf16 (Arum and Johnson, 2007). The phenotype of a mutation, which will affect the animal throughout development and adulthood, can sometimes differ from the effects of transgenic and RNAi manipulations, which may be targeted to specific tissues and life-cycle stages; indeed, both IIS (Broughton and Partridge, 2009) and *p53* (this study; Waskar et al., in press) manipulations can have contrasting effects on life span depending upon tissue, developmental stage and gender. Mutations in the p53 DNA binding domain can result in dominant mutant forms of the protein, that sometimes antagonize normal p53 function (Brodsky et al., 2000). Expression of a dominant mutant form of p53 in adult *Drosophila* has been shown to increase life span in females (Bauer et al., 2005; Shen et al., 2009), and this was found to correlate with decreased IIS (Bauer et al., 2007); however, it has not been determined if *Drosophila* life span regulation by *p53* requires Foxo.

One potential problem in interpreting the effects of dominant-mutant *p53* transgenes is that the phenotypes might be neomorphic, i.e., not necessarily representative of the function(s) of wild-type *p53*. For example, dominant mutant forms of p53 con-

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taining a disruption of the DNA binding domain are expected to antagonize the transcriptional activity of p53, but may also promote one or more of the transactivation-independent effects of p53, in a tissue-specific manner (Green and Kroemer, 2009). We have recently shown that, in *Drosophila*, wild-type *p53* can cause increased life span when over-expressed during development, and that in adults, wild-type *p53* has sex-specific effects on life span: Over-expression of wild-type *p53* in a tissue-general pattern in adult flies caused decreased life span in females, and increased life span in males (Waskar et al., in press); and consistent with these observations, null mutation of *Drosophila p53* caused increased life span preferentially in females.

Developmental stage-specific and sex-specific selective pressures are hypothesized to produce antagonistically-pleiotropic gene functions, that in turn affect phenotypes such as reproductive fitness, behavior and life span (Hughes and Reynolds, 2005; Leips et al., 2006; Long and Rice, 2007; Tower, 2006). Here we determine that the sexually antagonistic life span effects of *p53* are tissue-specific, and that these effects are modulated in a sex-specific way by *foxo* and *Sir2*.

2. Materials and methods

2.1. Drosophila culture and strains

Drosophila melanogaster culture, life span assays, Geneswitch driver strains, and the multi-copy UAS-eGFP strain ("UAS-ultra-GFP") are as previously described (Shen et al., 2009). Flies were cultured at 25 °C until eclosion, and then adults were maintained at 29 °C, or at 25 °C as indicated for specific experiments; additional details are provided in Supplementary materials. UAS-*p53* transgenic lines and chromosomal deficiency lines that uncover the *foxo* and *Sir2* loci were obtained from Bloomington *Drosophila* Stock Center. "p53WT1" is *P{GUS-p53}2.1*, "p53WT2" is *P{UAS-p53.Ex}2*, and "p53WT3" is *P{UAS-p53.Ex}3*. The *foxo[21rec7A]* and *foxo[w24]* null mutation lines were provided by M. Tatar (Min et al., 2008), and the *Sir2[4.5]* and *Sir2[5.26]* null mutation lines were provided by S.L. Helfand (Rogina and Helfand, 2004). Certain genotypes were generated by chromosomal recombination and/or crosses to double-balancer strains. Details of strain construction and characterization are provided in Supplementary materials; all strains and genotypes are listed in Supplementary Table S1.

2.2. Quantitative real-time RT-PCR

Total RNA was isolated from 15 male or female flies, using TRIzol reagent (Invitrogen). Flies were 12 days of age, and had been cultured for 10 days at 29 °C on RU486 food, or on control (ethanol-only) food. Quantitative real-time RT-PCR was performed using the Bio-RAD MyiQ™ Real-time PCR detection system and SYBR green dye, according to the manufacturer's instructions, and *Rp49* gene expression was used as control. Values are plotted as means ± SD of triplicate biological replicates. Additional details of methods and primer sequences are presented in Supplemental materials.

2.3. Western blot assay

Total protein was isolated by homogenizing 15 male or female flies in Laemmli sample buffer (Bio-Rad). Flies were 12 days of age, and had been cultured for 10 days at 29 °C on RU486 food, or on control (ethanol-only) food. Antibodies were specific for the phosphorylated form of Akt (#4054, Cell Signaling Technology; 1:1000 dilution), or for total Akt (#4691, Cell Signaling Technology; 1:1000 dilution). Anti-beta-actin antibody (#4967, Cell Signaling Technology; 1:5000 dilution) was used as a loading control. Additional details are provided in Supplemental materials.

2.4. Statistical analyses

For life span assays, mean, standard deviation, median, percent change in mean, percent change in median, and log rank (Breslow) p value were calculated using R 2.6.2 (RDevelopmentCoreTeam, 2006). Unpaired, two-sided t-tests were used to determine the significance of differences in mRNA levels between the RU486-treated and control groups; statistically significant differences ($p < 0.05$) are indicated along with the fold change in mean above the bar graphs.

3. Results

3.1. Conditional over-expression of transgenes using the Geneswitch system

To over-express *p53* in adult flies, the Geneswitch system was utilized, where transgene expression is induced by feeding the flies the drug RU486 (Mifepristone) (Nicholson et al., 2008). Characterization of the system using UAS-LacZ and UAS-GFP reporter strains demonstrated that the Act-GS-255B driver yields target gene expression throughout all the tissues of male and female flies, and that the Elav-GS driver produces expression that is strictly limited to the central nervous system (Ford et al., 2007; Shen et al., 2009) (Supplemental Fig. S1). To control for any potential effects on life span of the drug, the driver lines were crossed to the *w[1118]* injection strain and to Oregon-R wild-type strain to produce progeny containing only the drivers and no target transgene ("Control" flies). In the first set of control experiments with the Elav-GS driver, the drug-treated flies exhibited a small increase in life span in males (+4%) and no significant change in females (Fig. 1A and B). For both the Elav-GS driver and the Act-GS-255B driver the change in life span observed in the absence of a target transgene was on average approximately +3%, which we interpret as the background variability of the life span assay (results summarized in Fig. 4; details of statistical analyses for all life span data are presented in Table 1 and Supplemental Table S2).

3.2. Sexually-antagonistic effects of p53 on adult life Span

When *p53* was over-expressed specifically in adult nervous tissue using the Elav-GS driver, and either one of two independent p53-WT transgenes, this produced decreased life span in males (−9% and −13%, respectively), and increased life span in females (+11% and +12%, respectively)(Fig. 1C and D; Supplemental Fig. S2C and D; data summarized in Fig. 4 and Table 1). This result is in striking contrast to our previous observation that tissue-general over-expression of p53-WT3 using the Act-GS-255B driver produces the opposite effect: increased life span in males and decreased life span in females (Waskar et al., in press). That result was confirmed here using three independent p53-WT transgenes, and life span was increased in males by +10 to +18%, and decreased in females by −4 to −6% (Fig. 1I and J; Supplemental Fig. S3C–F; data summarized in Fig. 4 and Table 1). Because tissue-general over-expression includes nervous-system expression, the opposite effects produced by nervous system-specific expression versus tissue-general expression suggests that the effect of *p53* in peripheral tissues dominates over the effect in nervous system, and/or that signaling between tissues is involved in producing the effects on adult life span. Quantitative real-time RT-PCR (qPCR) assays con-

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