GENETICS

Geroprotective Effects of Activation of *D-GADD45* DNA Reparation Gene in *Drosophila Melanogaster* Nervous System

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The expression of *D-GADD45* gene involved in DNA reparation in *Drosophila melanogaster* decreases with age. Overexpression of *D-GADD45* in the drosophila nervous system prolongs the median and maximum life span without deterioration of the quality of life parameters (fertility and neuromuscular activity). The life span prolongation effect is due to more effective DNA reparation, as spontaneous level of DNA aberrations in the nerve tissue of larvae with *D-GADD45* overexpression is reduced significantly.

Key Words: *life span; DNA reparation; D-GADD45; overexpression*

The life span of an organism depends on its capacity to effectively react to stress exposure. The DNA aberration recognition and reparation genes play the key role in the stress response. Long-living individuals are often highly resistant to genotoxic stress factors [4]. Mutations in DNA reparation genes reduce the life span [1]. In humans, hereditary syndromes of accelerated aging (Werner syndrome, *etc.*) are caused by mutations in DNA reparation genes [8]. However, only prolongation of organism's life span under conditions of overexpression of DNA reparation genes can be regarded as a direct proof of the geroprotective characteristics of these proteins. The evolutionally conservative GADD45 family proteins play the key role in stress response and reparation of DNA in animals and humans. GADD45 orthologue has been detected in drosophila [10]. We hypothesized that *GADD45* overexpression leads to more effective reparation of DNA aberrations and causes prolongation of life span without reducing fertility and neuromuscular activity (NMA). In order to verify this hypothesis, we superstimulated *D-GADD45* in drosophila nervous system (NS), because the neurohumoral regulation of homeostasis determined the life span and cell and organism aging processes [2].

MATERIALS AND METHODS

***Drosophila melanogaster* strains.** Wild type *Canton-S* strain. The *UAS-D-GADD45* strain containing an extra copy of *D-GADD45* gene controlled by *UAS* promotor, induced by *GAL4* driver (kind gift from Dr. Uri Abdu, Ben Gurion University, Israel), constitutively induced in the NS (Bloomington Stock Center, USA). *Strain ELAV-GeneSwitch* containing mifeptristone-inducible driver *GAL4* in the NS (gift from Dr. Haig Keshishian, Yale University, USA).

UAS-D-GADD45 females were crossed with *GAL4-1407* males for constitutive overexpression of *D-GADD45* in the NS. In order to attain conditional overexpression of *D-GADD45* in the NS, *UAS-D-GADD45* females were crossed with *ELAV-GeneSwitch* males, after which mifepristone RU486 (Mifepristone, Sigma) was added to the fodder.

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RT-qPCR. Imago heads were used for the analysis. Standard RNA isolation (TRIzol Reagent, Invitrogen) and reverse transcription (SuperScriptIII, Invitrogen) procedures were used. PCR was carried out in an ANK-32 amplifier (Institute of Analytical Engineering) using SYBR Green I stain (Applied Biosystems) and *D-GADD45* and β-*Tubulin* primers (SINTOL). The expression of *D-GADD45* gene was calculated by the $2^{-\Delta\Delta Ct}$ method [6].

The drosophilas were kept at 25°C and 12 h light: darkness regimen on agar-year medium. Dead flies were counted daily. The differences between the samples were statistically evaluated by nonparametric Gehan–Breslow–Wilcoxon test. The differences in the maximum life span were evaluated by Wang–Allison method.

The eggs laid by the flies over 24 h were counted weekly, the pupae were counted on day 10 after laying. The significance of differences was evaluated by χ^2 test.

Spontaneous NMA and negative geotaxis were evaluated by the Drosophila Population Monitor programmed complex (TriKinetics). The significance of differences was evaluated by χ^2 test.

Nervous ganglia of age 3 larvae were used for evaluation of level of DNA aberrations by DNA-comet assay [7,9] and the incidence of apoptosis by DNA diffusion in gel [11]. The differences between the samples were evaluated by Student *t* and Fisher φ test for sampling fractions.

RESULTS

The expression of *D-GADD45* increased by 2.5-2.9 times after 28 days of life in wild type *Canton-S* imagoes, while on day 56 this expression decreased by more than 10 times. Hence, a decompensatory reduction of *D-GADD45* gene expression with aging was seen in drosophila.

Constitutive overexpression of *D-GADD45* (10-fold in males and 3-fold in females) prolonged median life span: by 73-77% ($p<0.001$) in males and by 22-46% ($p<0.001$) in females in comparison with the parental strains *UAS-D-GADD45* and *GAL4-1407* and by 6-17% ($p<0.001$) in males and up to 7% ($p<0.05$) in females in comparison with *Canton-S/GAL4-1407* strain. In addition, the age of 90% mortality was delayed by 5-59% ($p<0.001$) in flies with constitutive overexpression of *D-GADD45*, their minimum life span being many-fold prolonged (Table 1). These data indicated slower aging of individuals with *D-GADD45* overexpression in the NS.

Life span prolongation in comparison with representatives of the parental strains was more pronounced than in comparison with the *Canton-S/GAL4-1407* flies due to the heterosis contribution. Therefore, we studied the life span using a mifepristone-inducible (conditional) system for *UAS-D-GADD45* promotor stimulation. Mifepristone has no effect on drosophila life span [3]. The life span median in the drosophila

TABLE 1. Effect of Constitutive Overexpression of *D-GADD45* Gene in the NS on Life Span

Variant of experiment	*M*	$\bar{X}\pm\Delta m$	90%	min	max	*n*
♂ *UAS-D-GADD45*	43***	45.0±1.2	71***	3	84	258
♀ *UAS-D-GADD45*	57***	56.6±1.2	76***	3	84	211
♂ *GAL4-1407*	44***	45.6±0.8	56***	4	73	136
♀ *GAL4-1407*	48***	45.0±1.0	56***	6	64	138
♂ *Canton-S/GAL4-1407* (1-)	72***	65.3±1.4	87***	6	91	238
♀ *Canton-S/GAL4-1407* (2-)	77***	69.1±1.5	84***	6	90	200
♂ *Canton-S/GAL4-1407* (1-)	75***	74.6±0.6	84*	7	90	273
♀ *Canton-S/GAL4-1407* (2-)	72	68.3±1.2	84	6	90	199
♂ *UAS-D-GADD45/GAL4-1407* (1+)	76	74.2±1.4	89	34	98	186
♀ *UAS-D-GADD45/GAL4-1407* (2+)	84	81.8±1.1	96	6	102	176
♂ *UAS-D-GADD45/GAL4-1407* (3+)	82	77.8±1.2	91	20	98	175
♀ *UAS-D-GADD45/GAL4-1407* (1+)	70	67.0±1.1	84	15	98	173
♂ *UAS-D-GADD45/GAL4-1407* (2+)	80	77.0±0.8	89	6	96	271
♀ *UAS-D-GADD45/GAL4-1407* (3+)	70	69.1±1.0	84	21	97	211

Note. Here and in Table 2: *M*: median life span; $\bar{X}\pm\Delta m$: mean life span and error of the mean; 90%: age of 90% mortality; min: minimum life span; max: maximum life span; *n*: number of flies; "-": flies without overexpression; "+": with *D-GADD45* overexpression; ♂ — males; ♀ — females; *1*, *2*, *3*: repeats. ***$p<0.001$, *$p<0.05$ (*M* by Gehane–Breslow–Wilcoxon test; 90% by Wang–Allison test).

TABLE 2. Effect of Conditional Overexpression of *D-GADD45* Gene in the NS on Life Span

Experiment variant	*M*	$\bar{X}\pm\Delta m$	90%	min	max	*n*
♂ *UAS-D-GADD45*	40***	39.1±0.7	52***	10	54	162
♀ *UAS-D-GADD45*	55***	56.1±0.9	69***	4	85	173
♂ *ELAV-GeneSwitch*	37***	32.5±1.2	57***	1	70	221
♀ *ELAV-GeneSwitch*	35***	33.8±1.1	57***	4	63	223
♂ *Canton-S/ELAV-GeneSwitch*	57*	55.5±1.2	74***	9	81	172
♀ *Canton-S/ELAV-GeneSwitch*	55***	55.6±0.9	72***	10	80	176
♂ *UAS-D-GADD45/ELAV-GeneSwitch* (1-)	48***	45.4±1.8	74*	4	80	110
♀ *UAS-D-GADD45/ELAV-GeneSwitch* (2-)	43***	39.5±2.1	64*	2	80	105
♂ *UAS-D-GADD45/ELAV-GeneSwitch* (1-)	67*	59.9±2.0	83	4	95	145
♀ *UAS-D-GADD45/ELAV-GeneSwitch* (2-)	68	63.7±1.7	83	2	92	127
♂*UAS-D-GADD45/ELAV-GeneSwitch* (1+)	68	61.3±1.5	78	7	85	124
♀ *UAS-D-GADD45/ELAV-GeneSwitch* (2+)	60	54.4±1.8	74	4	85	112
♂ *UAS-D-GADD45/ELAV-GeneSwitch* (1+)	71	65.0±2.1	86	9	92	125
♀ *UAS-D-GADD45/ELAV-GeneSwitch* (2+)	70	65.4±1.8	88	4	85	139

with conditional overexpression (4-fold in males and 2-fold in females) of *D-GADD45* in the NS increased by 27-102% ($p<0.001$) in comparison with the parental *UAS-D-GADD45* and *ELAV-GeneSwitch* parental strains. Mifepristone induction of overexpression was associated with prolongation of the median life span by 40-42% ($p<0.001$) in males and by 3-6% ($p<0.05$) in females. The age of 90% mortality was also delayed in comparison with flies without overexpression: by 5-54% ($p<0.05$; Table 2). Hence, overexpression of *D-GADD45* in the NS prolonged life span of the drosophila irrespective of the heterosis impact and genetic background. The life span was prolonged by just overstimulation of the *D-GADD45* gene in the NS at the imago stage. The life span prolongation in association with *D-GADD45* overexpression was more manifest in the males than in the females, which agrees with higher level of transgene expression.

Prolongation of organism's life span associated with mutations of certain genes is often paralleled by reduction of reproduction and deterioration of motor activity [12]. Fertility of females with constitutive and conditional overexpression of *D-GADD45* in the NS evaluated by the number of eggs per female did not decrease throughout the entire life span or increased by 1.7-2.6 times ($p<0.001$). The number of pupas changed similarly. Physical activity of males and females with constitutive and conditional overexpression of *D-GADD45* was retained in comparison with flies without overexpression ($p<0.001$). Hence, overexpression of *D-GADD45* in the NS was not associated with deterioration of drosophila quality of life.

The GADD45 proteins are essential for the maintenance of genome stability in response to DNA aberrations and are involved in the nucleotide excision reparation support [5]. DNA-comet assay showed a 21-27% ($p<0.001$) decrease in the incidence of single-strand DNA breaks in the larval neuroblasts under conditions of constitutive and conditional overexpression of *D-GADD45*. On the other hand, the GADD45 proteins are involved in apoptosis regulation [10]. Hypersensitivity to apoptosis induction could lead to selection of neuroblasts with the highest resistance to DNA aberrations. However, the incidence of apoptosis in the larval neuroblasts under conditions of *D-GADD45* overexpression did not differ from the apoptosis level in control genotypes.

Hence, overexpression of *D-GADD45* gene in the NS prolonged the median and maximum life span of the drosophila without deterioration of its quality of life due to more effective recognition and repair of spontaneous DNA aberrations.

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