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Article

Tissue-specific knockdown of genes of the *Argonaute* family modulates lifespan and radioresistance in *Drosophila melanogaster*

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Abstract: Small RNAs are essential for the coordination of many cellular processes, including the regulation of gene expression patterns, the prevention of genomic instability, and the suppression of mutagenic transposon activity. These processes determine aging, longevity, and sensitivity of cells and an organism to stress factors (particularly, ionizing radiation). The biogenesis and activity of small RNAs are provided by proteins of the Argonaute family. These proteins participate in the processing of small RNA precursors and the formation of an RNA-induced silencing complex. However, the role of Argonaute proteins in the regulation of lifespan and radioresistance remains poorly explored. We studied the effect of knockdown of *Argonaute* genes (*AGO1*, *AGO2*, *AGO3*, *piwi*) in various tissues on the *Drosophila melanogaster* lifespan and survival after the γ-irradiation at a dose of 700 Gy. In most cases, these parameters were reduced or did not change significantly in flies with tissue-specific RNA interference. Surprisingly, *piwi* knockdown in both the fat body and the nervous system caused a lifespan increase. But changes in radioresistance depended on the tissue in which the gene was knocked out. In addition, analysis of changes in retrotransposon levels and expression of stress response genes allowed us to determine associated molecular mechanisms.

Keywords: lifespan, aging, radioresistance, ionizing radiation, *Agronaute*, *Piwi*, *Drosophila melanogaster*

1. Introduction

Lifespan is determined by the processes that occur at the molecular, cellular, tissue, and organism levels, as well as the influence of damaging environmental factors and other external conditions. Among the molecular mechanisms of lifespan regulation, epigenetic mechanisms have a special place. On the one hand, it provides the implementation of hereditary information embedded in cells of an organism. On the other hand, it is necessary for fine-tuning of gene expression in accordance with the entering of outside stimuli. The well-coordinated work of these two processes allows maintaining the vitality of an organism and ensures its longevity. However, a disturbance of epigenetic regulation can lead to cumulative negative consequences associated with the loss of functionality of cells and an organism, a decrease in its adaptive capabilities [1,2]. This is exactly what happens during the aging of an organism, therefore epigenetic alterations are one of the basic hallmarks of aging [3]. During aging, there is a change in the structure of chromatin (for

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example, a loss of nucleosomes and a decrease in the amount of heterochromatin), DNA methylation status, modification of histone marks, changes in the patterns of noncoding RNA activity, epigenetic drift [4]. In addition to the fact that such changes lead to a disturbance of gene expression, they also cause a number of other fatal consequences. For example, the loss of heterochromatin, DNA hypomethylation and changes in histone labels lead to the activation of the expression of silent mobile genetic elements (or transposons), which increases the accumulation of DNA damages and mutations, and causes genome instability [2,4,5]. Epigenetic dysregulation contributes to the pathogenesis of age-related pathologies such as cancer, atherosclerosis, type 2 diabetes, mental and neurodegenerative diseases, and a decrease in the immune response [6,7].

Among epigenetic mechanisms, small RNAs are required for the coordination of many cellular processes, including post-transcriptional regulation of gene expression, regulation of heterochromatin formation, prevention of genome instability, and suppression of mutagenic activity of transposons [8-11]. Small RNAs include three classes and differ in the mechanism of their biogenesis and the type of protein with which they are associated. These are endogenous short interfering RNAs (endo-siRNAs) that are targeted on mRNA and transposons, microRNAs (miRNAs) that regulate mRNA expression, and P-element induced wimpy testis (PIWI) -interacting RNAs (piRNAs) that are essential for the suppression of transposons' activity. In addition, there are exogenous short interfering RNAs (exo-siRNAs) that are derived from viral double-stranded RNAs (dsRNAs) or artificial dsRNAs, and are aimed to restrict viral and external activity [8-10,12]. They are important for coordinating the organism development, forming various organs and tissues, controlling metabolism, and maintaining the genome integrity [8-10]. Moreover, there are evidences for the important role of small RNAs in regulating lifespan and providing resistance to a range of environmental stressors [2,11-15,16 1].

The biogenesis and activity of small RNAs are provided by proteins of the Argonaute family. These proteins are involved in the processing of small RNA precursors and the formation of the RNA-induced silence complex (RISC). At the same time, an Argonaute protein loaded with a mature small RNA forms active RISC, which targets a corresponding molecule (mRNA or transposon), carries out its catalytic degradation, and inhibits translation [9,17]. Previous studies have demonstrated the role of some genes of the *Argonaute* family in the lifespan regulation. For example, it was found that in *Caenorhabditis elegans* the *alg-1* and *alg-2* genes conversely regulate lifespan: *alg-1* promotes longevity, while *alg-2* limits lifespan. This is mediated by their different roles in the regulation of DAF-2/insulin/IGF-1 and DAF-16/FOXO signaling pathways [18]. In *Drosophila melanogaster*, a mutation in the *AGO2* gene leads to a significant reduction in lifespan, which is associated with an increase in transposon expression in the brain and age-dependent memory impairment [19]. In addition, the activity of genes of small RNA biogenesis, including the *Argonaute* family, mediates beneficial effects of pro-longevity interventions such as intermittent fasting [20]. However, there is almost no data on the effects of partial downregulation or tissue-specific shutdown of *Argonaute*.

It should be noted that the range of studied functions of small RNAs and proteins of their biogenesis is currently expanding. In particular, it is known that some miRNAs (as well as lncRNAs) are involved in the response to DNA damage and DNA repair due to participating in the network of signaling pathways [21-23]. At the same time, disruption of the activity of small RNA biogenesis genes and proteins encoded by them (such as AGO2 and PIWIL2) reduces the survival of human cells after exposure by genotoxic agents (such as UV light, ionizing radiation and others) as a result of distorted regulation of cell cycle, apoptosis, and DNA repair [24-26]. Similar data were obtained in an *in vivo* model of *Caenorhabditis elegans* with a mutation in the *alg-2* gene [27]. In addition, the role of complexes of DSB-induced small RNAs (diRNAs) and AGO2 (diRISCs) in the repair of double-stranded DNA breaks (mainly by the way of homologous recombination) has been described [28,29]. Thus, small RNAs and Argonaute proteins are important for the response to DNA damage and, apparently, play a significant role in the response of cells and an organism to genotoxic agents.

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Thus, it is obvious that the Argonaute proteins (as well as the small RNAs associated with them) are involved in the regulation of lifespan and the organism's resistance to radiation. However, these functions remain poorly understood and require investigation. The fruit fly *Drosophila melanogaster* is an appropriate model for this task. Its genome contains 5 genes encoding proteins of the Argonaute family. Among them, the Argonaute subfamily includes AGO1 (provides maturation and functioning of miRNAs) and AGO2 (performs biogenesis and specifically binds to siRNAs). The PIWI subfamily includes piwi, AGO3, and Aubergine, which are required for piRNA processing and functioning [30,31]. Thus, Argonaute proteins in *Drosophila* are relatively specific for types of small RNAs, which makes it possible to analyse the contribution of biogenesis and functioning of each of them to the studied processes. In addition, fruit flies have known advantages due to their short life cycle, ease of maintenance, accessibility of genetic manipulation, and evolutionary conservatism of many signaling pathways [32].

In this work, we studied the effect of knockdown of *Argonaute* genes (*AGO1, AGO2, AGO3, piwi*) in various tissues on the *Drosophila melanogaster* lifespan and survival after the γ-irradiation at a dose of 700 Gy. In addition, changes in the levels of retrotransposons and expression of stress response genes (including DNA damage response genes) were analyzed to determine the involved molecular mechanisms.

2. Materials and Methods

2.1. Drosophila Melanogaster Strains and Induction of Argonaute Genes' Knockdown

The wild-type Canton-S strain was used to assess age-related changes in gene expression.

In experiments to study the influence of tissue-specific knockdown of genes of the *Argonaute* family (*AGO1, AGO2, AGO3, piwi*) on the lifespan and the effects of γ-irradiation, the tested flies were obtained on the basis of the GAL4/UAS system [33-35]. We used strains carrying double-stranded RNA (dsRNA) for RNA interference of these genes under the control of the *UAS* promoter (*RNAi-AGO1, RNAi-AGO2, RNAi-AGO3, RNAi-piwi*, respectively) and strains expressing the conditional (mifepristone-inducible) driver GAL4-GeneSwitch in specific tissues (*GS-elav* - in the nervous system, *GS-S106* - in the fat body, *GS-TIGS-2* - in the digestive system, *GS-Mhc* - in the muscles) (see Table S1).

The use of GAL4-GeneSwitch allows to control the expression level of a studied gene, the stage of development (imago) and the age of flies, at which the expression is induced, as well as the localization of suppression of the studied genes (ubiquitous or tissue-specific). First, the choice of tissue-specific expression was associated with the topicality of studying the role of *Argonaute* genes in various tissues in the regulation of lifespan and aging. Second, ubiquitous RNA interference in itself reduces the lifespan of *Drosophila*, while tissue-specific (including the drivers that were used in our study) does not have a negative effect on the lifespan [36]. In addition, the use of conditional GAL4 excludes the influence on the lifespan of an unequal genetic background in experimental and control animals.

To obtain experimental flies of each of the genotypes, virgin females of a line with UAS-construction and males of a line with GAL4-GeneSwitch were crossed. In males and virgin females obtained by crossing, an *Argonaute* gene knockdown was induced by mifepristone (RU486, Merk, USA) at a concentration of 3.2 mg/ml in ethanol, which was dripped onto a nutrient medium at 30 μl [37]. Control variants were obtained by the same crosses, but were kept in the medium without mifepristone. The decrease in the activity of Argonaute genes was verified by using RT-PCR analysis (Figure S1).

2.2. Lifespan Assay

Flies were kept at 25 C, 12:12 day-night regime in climate chamber Binder KBF720-ICH (Binder, Germany) on nutrient medium (gram per 1 liter): agar agar - 5.2, dry yeast - 32.1, glucose - 136.9, yellow cornmeal - 92 [38]. To prevent

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simple fungus and bacteria growth a 10 % solution of methyl 4-hydroxybenzoate (Sigma-Aldrich, USA) and a 50% solution of propionic acid (Sigma-Aldrich, USA) were added.

To induce the silence of target genes, females expressing dsRNA under control of UAS sequences were crossed with GAL4 driver males. The F1 males and virgin females were used. Experimental flies were sorted by sex using CO_2 anesthesia and were kept separately, 30 animals per Drosophila vial (Genesee Scientific, USA) with 5 ml of nutrient medium (see above) and 30 μl mifepristone solution which was applied to the surface of the nutrient medium [37]. Control F1 flies were maintained on medium without mifepristone (with 30 μl ethanol).

Flies were transferred to fresh medium without anesthesia twice a week. The number of dead flies were counted daily. Further lifespan parameters (particularly, the mean and median lifespan, the age of 90 % mortality, the mortality rate doubling time (MRDT)) mean rate of were calculated. Experiments were made in 1-2 independent biological replicates (two replicates were used for flies with RNA interference of *Argonaute* genes in the nervous system and the adipose body to confirm the positive lifespan effects).

Statistical processing was carried out by using nonparametric criteria. The comparison of the shape of survival curves was done using Kolmogorov-Smirnov test [39]. The Mantel-Cox test [40] and Gehan-Breslow-Wilcoxon test [41] was used to estimate the statistical differences in the median lifespan. A Wang-Allison test was used to estimate differences in the age of 90 % mortality [42]. The statistical analyses of the data were carried out using STATISTICA software, version 6.1 (StatSoft, USA) and R, version 2.15.1 (The R Foundation).

2.3. Irradiation Conditions

The radiosensitivity to acute gamma irradiation of male imago from the control groups was measured in the preliminarily tests (Figure S2), according to which radiation dose of 700 Gy was selected.

Experimental and control flies were obtained and cultivated in the same manner as for lifespan assay. At the age of 14 days experimental *Drosophila* was irradiated at a dose of 700 Gy for 15 h 45 min using a Cs-137 gamma source "Issledovatel'" (USSR), the dose rate in the central part was 0.74 Gy/min. Further, experimental and control flies were placed under standard conditions in medium with mifepristone and without it. Next, their survival was assessed. Statistical analysis was similar to lifespan assay.

2.4. Real-time RT-PCR

The gene expression analyses were carried out using whole *Drosophila* bodies or their parts (heads, toraxes or abdomens). In the case of whole flies, 10 males or 10 females were prepared per variant of experiment. In other cases, 30 males or 30 females were partitioned into heads, toraxes or abdomens, places to separate tubes, and used for further procedures.

RNA was isolated by Aurum Total RNA mini kit (Bio-Rad, USA). To determine total RNA concentration was used Quant-iT RNA Assay Kit (Invitrogen, USA). Reverse transcription was performed using the iScript cDNA Synthesis Kit (Bio-Rad, USA). The mix for RT-PCR was prepared by iTaq Universal SYBR Green Supermix (Bio-Rad, USA) with primers listed in Table S2. The reaction was carried out on the CFX96 Real-Time PCR Detection System (Bio-Rad, USA) using the following parameters: one cycle of 95 °C for 30 s; 40 cycles of 95 °C for 10 s and 60 °C for 30 s. Expression levels of target genes were calculated relative to the expression of reference genes (*β-Tubulin, RpL32, EF1α*) using the CFX Manager 3.1 software (Bio-Rad, USA). Experiments were made in two independent biological replicates, with three technical replicates in each.

RNA and cDNA samples were prepared using the equipment of the Molecular Biology Core Facility (IB FRC Komi SC UB RAS, Syktyvkar, Russia).

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3. Results

3.1. Effects of Down-Regulation of Argonaute Genes on the Drosophila Lifespan

Tissue-specific knockdown of genes of the *Argonaute* family in the most cases either did not have a statistically significant effect, or led to a decrease in the median lifespan (by 3.0-89.3 %, $p < 0.05$) and the parameter of maximum lifespan (the age of 90 % mortality) (by 3.1-62.9 %, $p < 0.05$) in *Drosophila* males and females (Figures 1-2, Table S3). However, in some replicates of the experiment, the studied longevity parameters were increased in flies with RNA interference of the *AGO1* and *AGO3* genes. Moreover, the median lifespan was reproducibly higher in males and females with *piwi* knockdown in the nervous system and in the fat body compared with flies without induction of RNA interference (by 2.1-12.5 %, $p < 0.05$) (Figures 2E, 2F, Table S3).

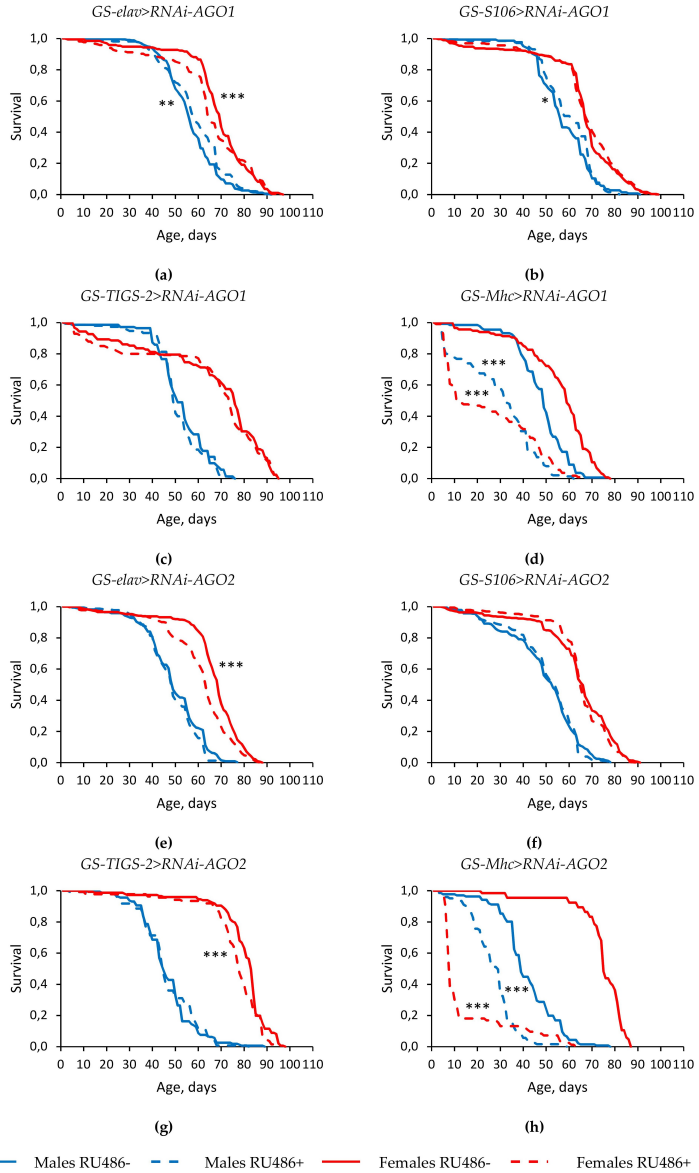

Figure 1. Influence of *AGO1* (**a-d**) and *AGO2* (**e-h**) knockdown in the nervous system (**a, e**) (two replicates combined), fat body (**b, f**) (two replicates combined), guts (**c, g**), muscles (**d, h**) on the survival of *Drosophila melanogaster*. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$ (Kolmogorov-Smirnov test).

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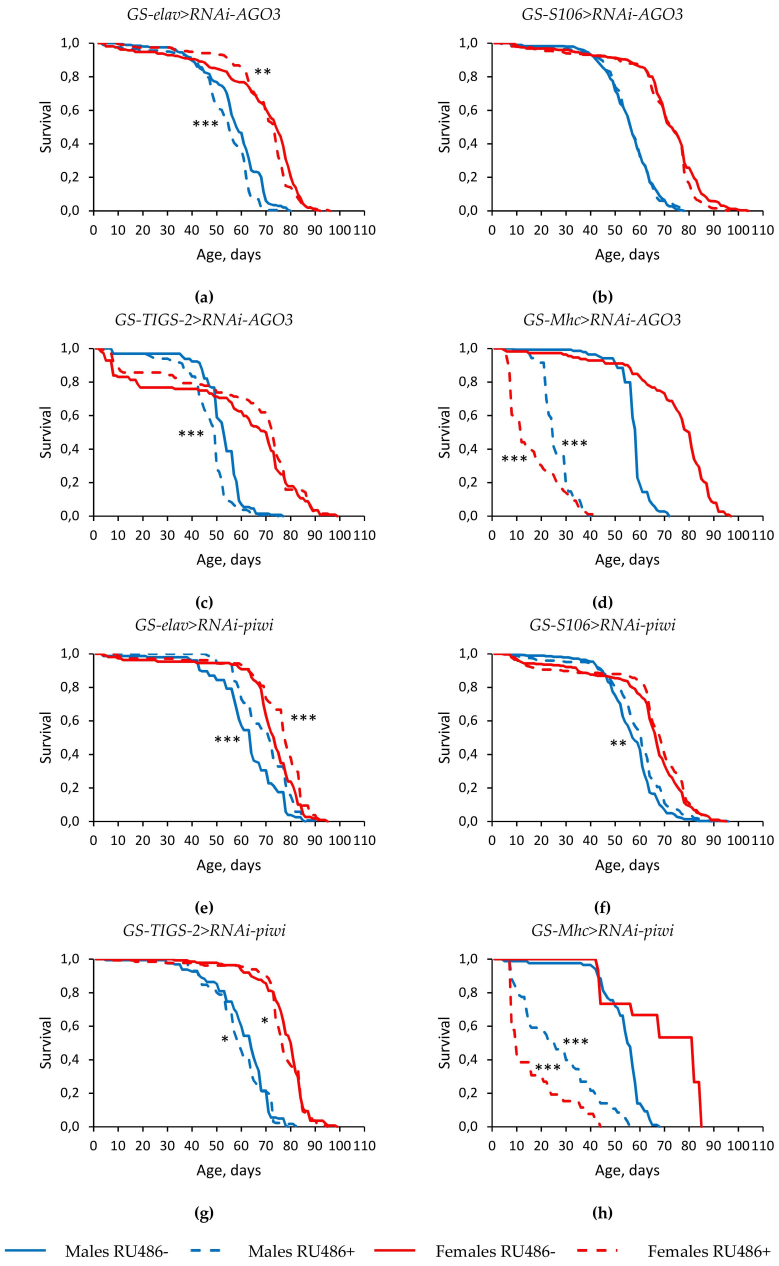

Figure 2. Influence of *AGO3* (**a-d**) and *piwi* (**e-h**) knockdown in the nervous system (**a, e**) (two replicates combined), fat body (**b, f**) (two replicates combined), guts (**c, g**), muscles (**d, h**) on the survival of *Drosophila melanogaster*. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$ (Kolmogorov-Smirnov test).

Since the positive effect of knockdown of some of the studied *Argonaute* genes on longevity was manifested in the case of their RNA interference in the nervous system and the fat body, we carried out further research only with these variants.

3.2. Radioresistance of Drosophila with Knockdown of Argonaute Genes

The exposure with γ-irradiation at a dose of 700 Gy extremely reduced the survival of *Drosophila* in both sexes, regardless of the tissue-specific expression of the *Argonaute* family genes. The median survival was decreased by 17.4-

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76.7 % ($p < 0.001$), and the age of 90 % mortality was lower by 18.8-73.8 % ($p < 0.001$) in irradiated flies compared to non-irradiated ones (Figure 3, Table S4).

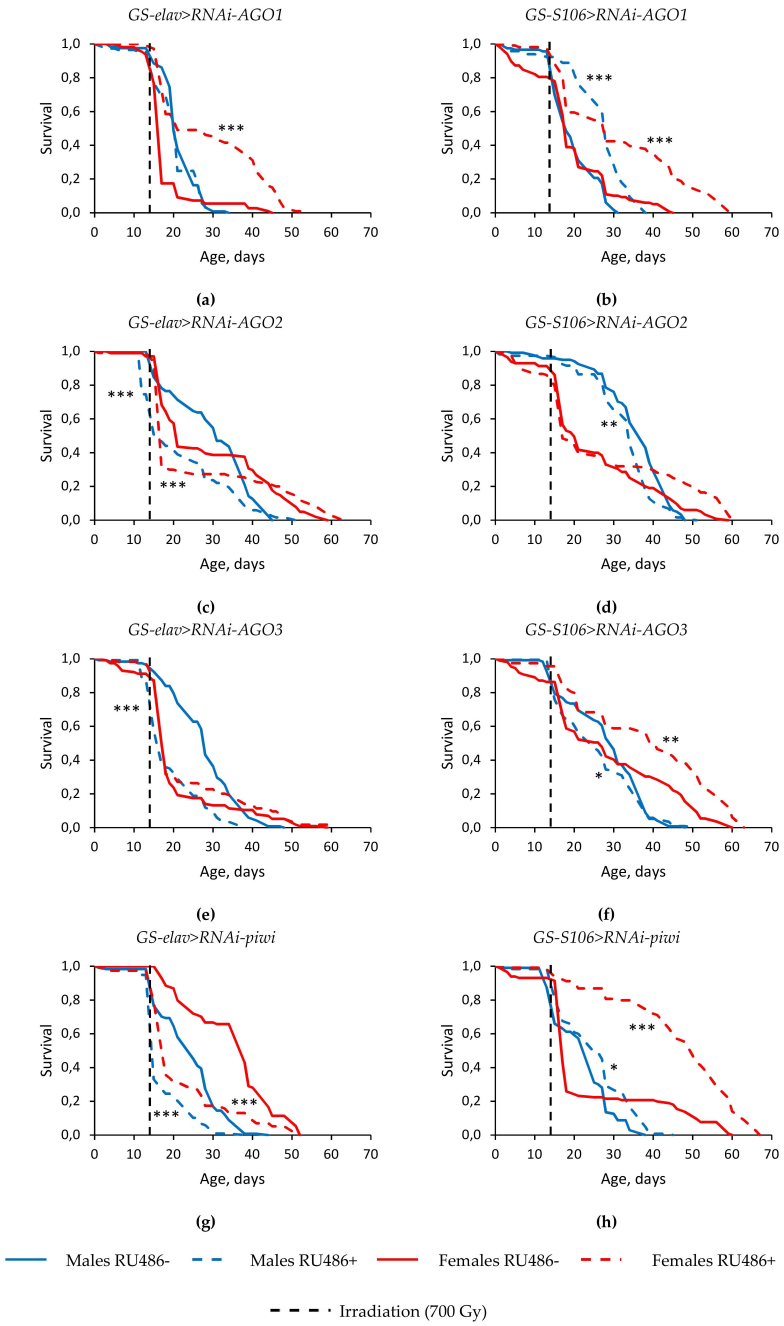

Figure 3. Influence of *AGO1* (**a, b**), *AGO2* (**c, d**), *AGO3* (**e, f**), *piwi* (**g, h**) knockdown in the nervous system (**a, c, e, g**) and fat body (**b, d, f, h**) on the survival of *Drosophila* flies after γ-irradiation at the dose of 700 Gy. * - $p < 0.05$, ** - $p < 0.01$, *** - $p < 0.001$ (Kolmogorov-Smirnov test).

In the most experimental variants, tissue-specific knockdown of genes of the *Argonaute* family negatively affected the radioresistance of *Drosophila* of both sexes, decreased the median survival (by 10.5-55.3 %, $p < 0.001$) and the maximum survival rate (by 21.1-23.5 %, $p < 0.001$) in conditions of γ-irradiation (Figure 3, Table S4). However, flies of both sexes with RNA interference of the *AGO1* and *piwi* genes in the fat body (Figures 3B, 3H), females (but not males) with

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RNA interference of *AGO2* and *AGO3* in the fat body (Figures 3B, 3H), and females with *AGO1* neuronal knockdown showed a high resistance to the radiation exposure (Figure 3A). In these variants of the experiment, the median survival rate was increased by 23.5-200 % ($p < 0.001$), the age of 90 % mortality was higher by 17.6-123.8 % ($p < 0.001$) compared with irradiated flies without induction of RNA interference.

It should be noted that an increase in lifespan did not coincide in all cases with an increase in radioresistance. In particular, flies with reduced *piwi* activity in the nervous system under irradiation conditions had a reduced survival rate compared to variants without induction of RNA interference (Figures 2E, 3G).

3.3. Age-Related Changes in the Expression of Argonaute Genes, Retrotransposons, and Stress Response Genes

In flies of the wild-type *Canton-S* strain, a slight increase in the expression of genes of the *Argonaute* family (by 1.6-2.4 times, $p < 0.05$) and a pronounced activation of retrotransposons (by 1.7-12.0 times, $p < 0.05$) at the age of 10 weeks was observed (Figures 4A-4D). It should be noted that this tendency was repeated separately in the abdomens of *Drosophila* and, in part, in the heads (but not in the thoraxes) (Figures S3, S4).

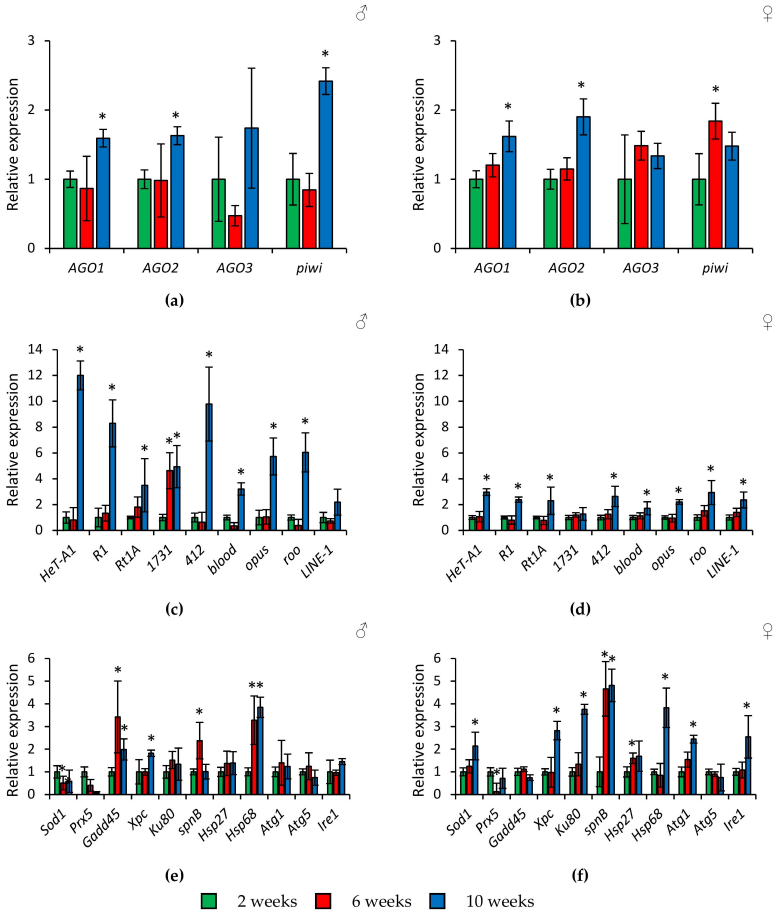

Figure 4. Age-related changes in the expression of *Argonaute* genes (**a, b**), transposable elements (**c, d**) and stress response genes (**e, f**) in wild-type *Canton-S* males (**a, c, e**) and females (**b, d, f**). * - $p < 0.05$ (Mann-Whitney U-test).

In addition, at the ages of 6 and 10 weeks, flies had increased transcription of some stress response genes both in whole bodies and in individual parts of the body (Figures 4E, 4F, S5). In particular, activation (by 1.8-15.2 times, $p <$

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0.05) is shown for genes of response and repair of DNA damages (*Gadd45, Xpc, Ku80, spn-B*) and proteostasis genes (*Hsp27, Hsp68, Atg1, Ire1*). At the same time, the activity of the *Prx5* gene was decreased.

3.4. Changes in Expression Levels of Retrotransposons Associated with Argonaute Genes' Knockdown and γ-Irradiation

In *Drosophila* without the induction of RNA interference of genes of the *Argonaute* family, γ-irradiation at a dose of 700 Gy caused the activation of retrotransposons (by 1.3-9.3 times, $p < 0.05$), or did not lead to statistically significant changes (Figures 5, 6). Exceptions are flies with the genotypes *GS-elav>RNAi-AGO2* and *GS-elav>RNAi-AGO2*, in which radiation exposure suppressed the retrotransposons' expression in some cases (Figures 5E-5H).

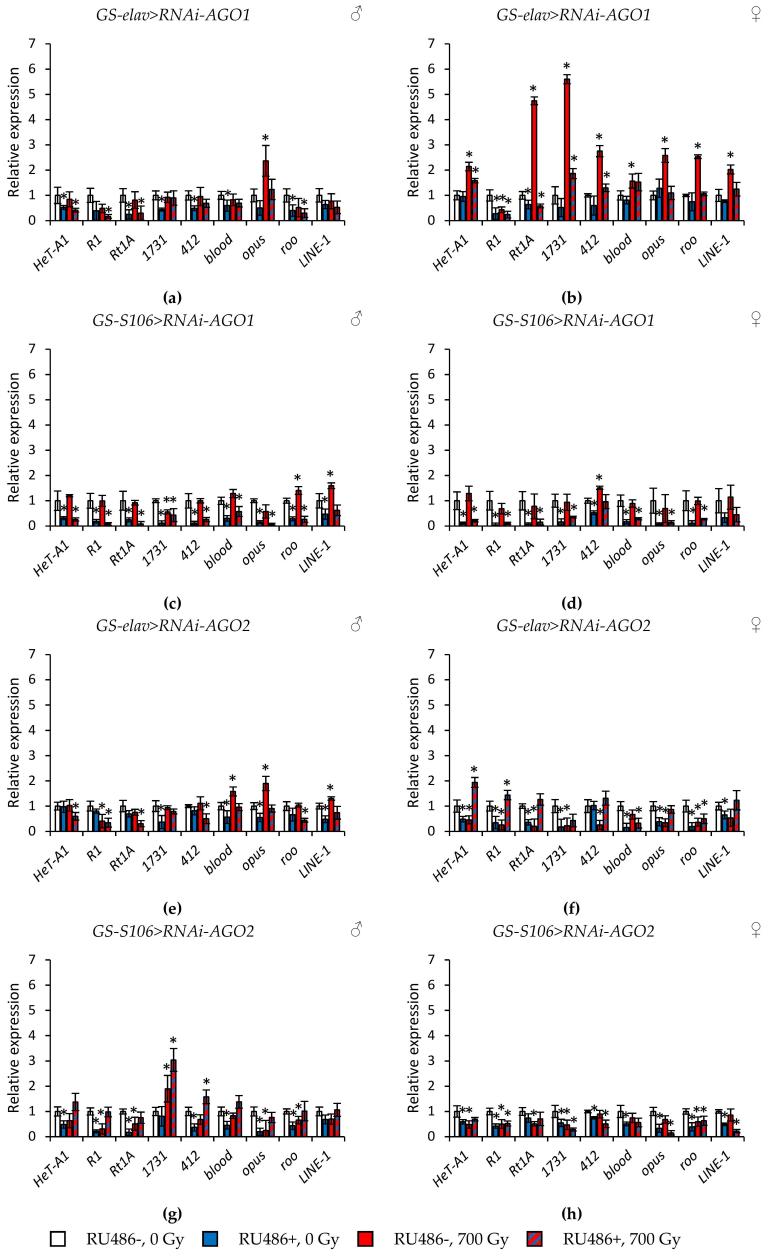

Figure 5. Changes in expression levels of transposable elements in irradiated and unirradiated males (**a, c, e, g**) and females (**b, d, f, h**) with *AGO1* (**a-d**) and *AGO2* (**e-h**) knockdown. * - $p < 0.05$ (Mann-Whitney U-test).

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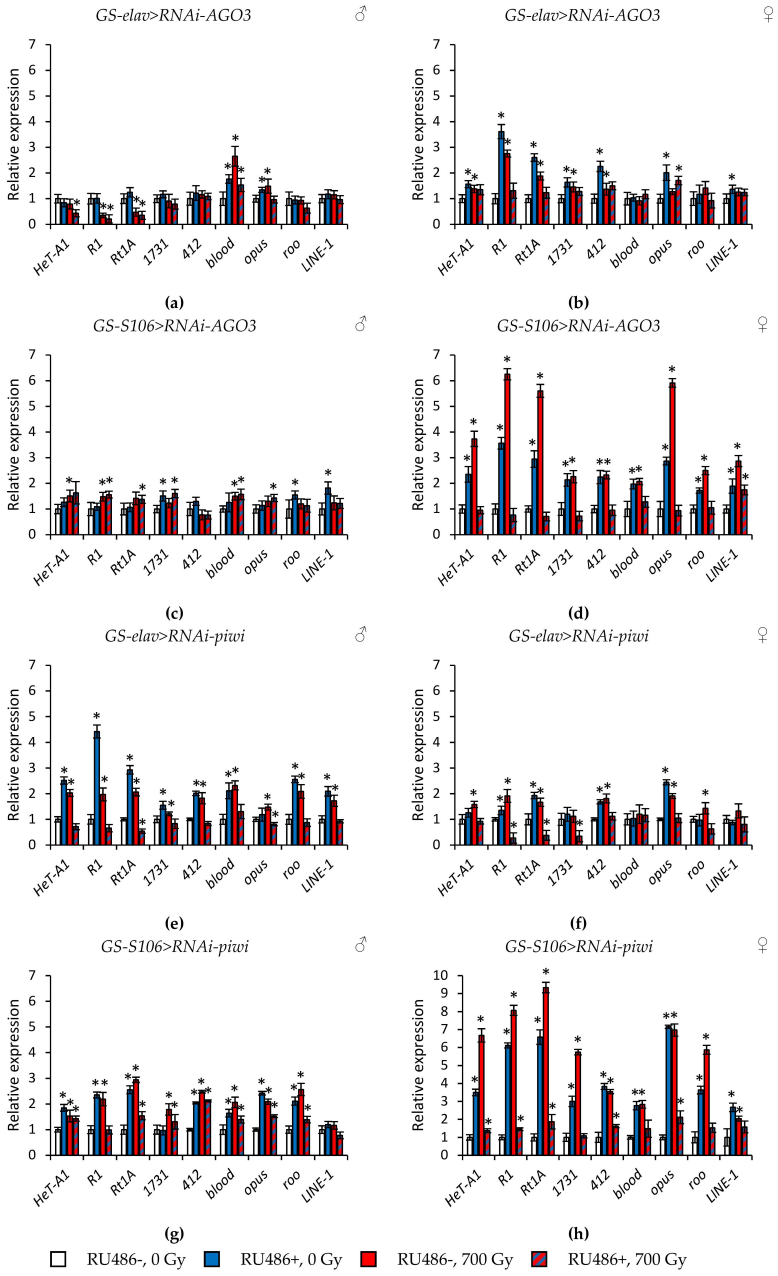

Figure 6. Changes in expression levels of transposable elements in irradiated and unirradiated males (**a, c, e, g**) and females (**b, d, f, h**) with *AGO3* (**a-d**) and *piwi* (**e-h**) knockdown. * - $p < 0.05$ (Mann-Whitney U-test).

At the same time, knockdown of genes of the subfamilies *Argonaute* and *PIWI* had different effects on the activity of retrotransposons. In flies with knockdown of genes of the *Argonaute* subfamily (*AGO1* and *AGO2*) in the nervous system and the fat body both under γ-irradiation and without irradiation, the activity of retrotransposons decreased by 1.4-22.1 times ($p < 0.05$) compared with the variants without induction of RNA interference (Figure 5). RNA interference of genes of the *Piwi* subfamily (*AGO3* and *piwi*) significantly increased the activity of retrotransposons (by 1.3-9.3 times, $p < 0.05$) in unirradiated flies. However, γ-irradiation, on the contrary, reduced the activity of mobile elements in males and females with knockdown of *PIWI* genes by 1.2-8.2 times ($p < 0.05$) compared with irradiated flies without induction of RNA interference (Figure 6).

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3.5 *Changes in Expression Levels of Stress Response Genes Associated with Argonaute Genes' Knockdown and γ-Irradiation*

Tissue-specific knockdown of the *AGO1* gene caused the greatest activation of stress response genes. In particular, the expression of genes of antioxidant defense (*Sod1, Prx5*), genes of DNA damage response and repair (*Gadd45, spn-B*), genes of heat shock proteins (*Hsp27, Hsp68*) was increased by 1.7-14.3 times ($p < 0.05$) (Figures 7A-7D). The most pronounced induction of their activity was observed in males with *AGO1* RNA interference in the fat body. In addition to these genes, *Ku80, Atg1*, and *Ire1* were also activated in this variant of the experiment (Figure 7C). Similar but less pronounced changes were observed in flies with *piwi* knockdown in the nervous system and the fat body (Figures 8E-8H). At the same time, the decreased activity of *AGO2* and *AGO3* mainly decreased the activity of stress response genes (Figures 7E-7H, 8A-8D).

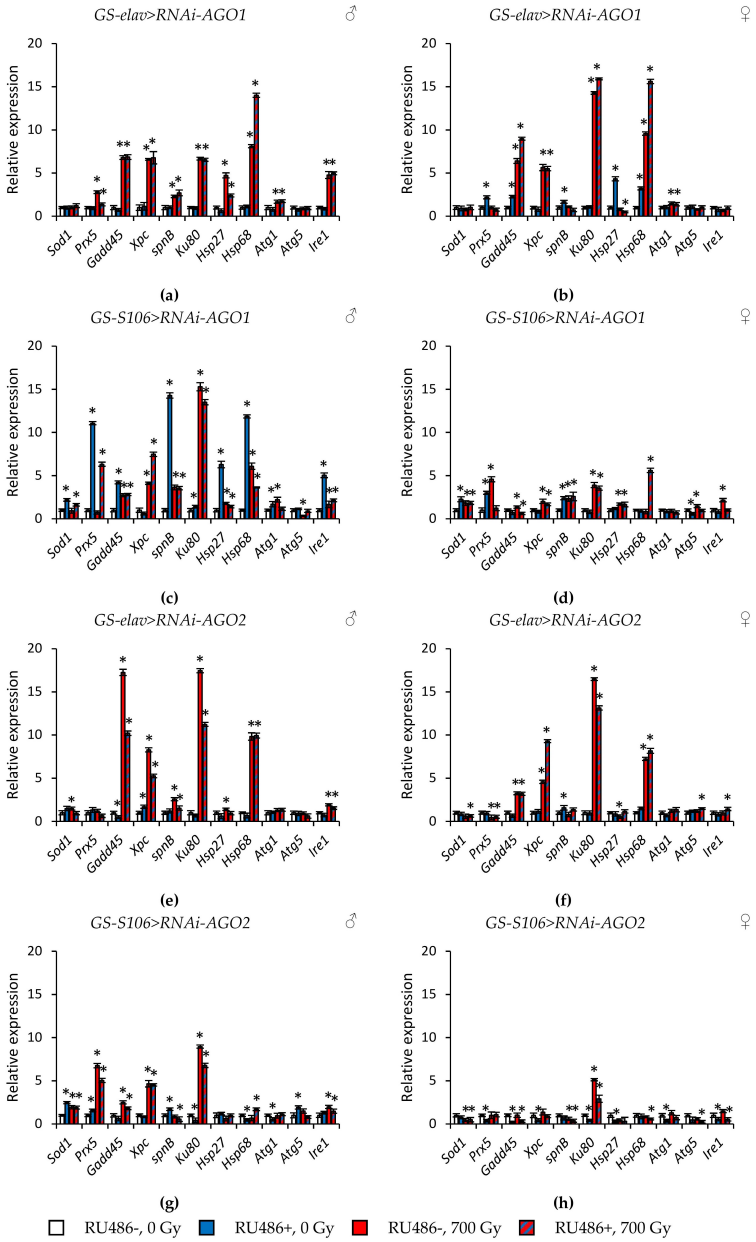

Figure 7. Changes in expression levels of stress response genes in irradiated and unirradiated males (**a, c, e, g**) and females (**b, d, f, h**) with *AGO1* (**a-d**) and *AGO2* (**e-h**) knockdown. * - $p < 0.05$ (Mann-Whitney U-test).

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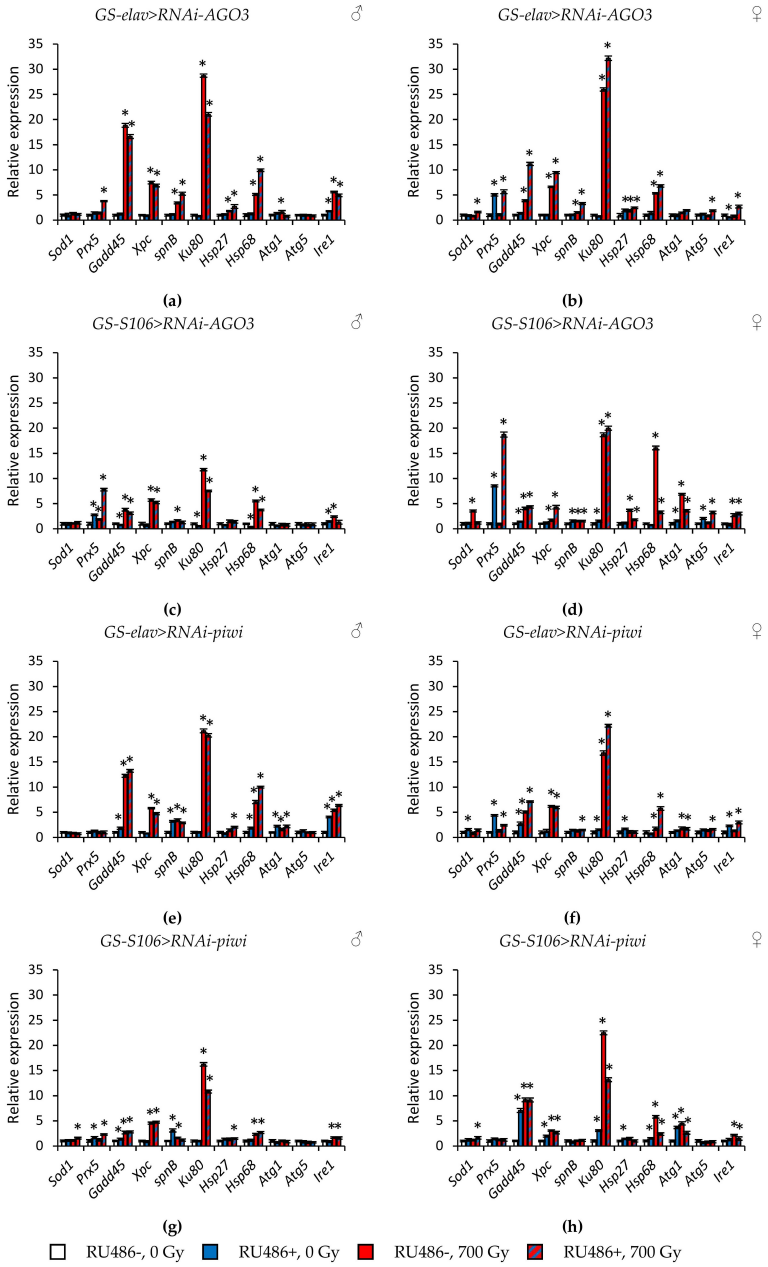

Figure 8. Changes in expression levels of stress response genes in irradiated and unirradiated males (**a, c, e, g**) and females (**b, d, f, h**) with *AGO3* (**a-d**) and *piwi* (**e-h**) knockdown. * - $p < 0.05$ (Mann-Whitney U-test).

γ-Irradiation led to a significant activation (by 1.4-32.2, $p < 0.05$) of genes responsible for the response to genotoxic stress (*Gadd45, Xpc, Ku80*) and proteotoxic stress (*Hsp68*). This effect was observed both in variants with RNA interference of *Argonaute* genes and without induction of RNA interference (Figures 7, 8). Other studied stress response genes were also activated in some variants of the experiment, but by a lesser extent.

4. Discussion

Aging is accompanied by age-related differential changes in the expression of small RNAs, which is closely associated with impaired biogenesis and regulation. Dysregulation of small RNA biogenesis proteins and corresponding

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changes in the functioning of miRNAs, siRNAs, and piRNAs lead to a global disruption of gene expression and chromatin structure with subsequent negative consequences at the molecular, cellular, tissue, and organismal levels. For example, they include loss of genome integrity and genetic instability, impaired stress response, metabolism, immunity, regenerative abilities, increased inflammatory responses, and others. Such changes significantly deplete the organism's life support systems, cause age-related disorders and aging [4,43].

During aging, depending on the tissue and physiological state of the organism, both a critical decrease in the expression of small RNA biogenesis proteins and their excessive activation can occur. Predominantly, aging human cell cultures, as well as cells obtained from old donors are characterized by reduced activity of small RNA biogenesis genes, such as *Drosha, Dicer, Exportin 5,* and *AGO2*. Such changes are accompanied by shifts in the expression patterns of miRNAs [44-47]. Similar data were obtained in studies of age-related changes in various tissues of rodents [46-48] and in nematodes [47]. Nevertheless, some data indicate the nonlinear pattern of the dynamics of the activity of genes encoding enzymes of small RNA biogenesis. Thus, in the hearts of rats, *AGO1* and *AGO2* firstly increase the expression, but in the end of life they decrease it [49]. In addition, it should be noted that not only the levels of small RNAs depend on the activity of proteins of its biogenesis, but there is a feedback. For example, a miRNA-directed mechanism of age-related changes in the expression of an *Argonaute* gene has been described using the *Caenorhabditis elegans* model. In particular, *miR-71*, which is activated during aging, suppresses *alg-1* and limits the lifespan of nematodes [14].

In this work, the analysis of gene expression showed that there is an age-related increase in the expression of genes of the *Argonaute* family in whole *Drosophila* bodies, in heads and abdomens (but not in thoraxes). At the same time, increased activity was also observed in retrotransposons and some stress response genes. In other words, despite the fact that aging flies activate mechanisms aimed at the production of piRNAs, miRNAs, and siRNAs, which suppress the activity of mobile genetic elements and target mRNAs, we did not observe the corresponding effect. We assume at least two explanations for the obtained data. First, an increase in the transcriptional activity of the *Argonaute* genes does not indispensably indicate an increase in the level of its proteins and their functional activity. Deregulation of their activity may occur at post-transcriptional levels. For example, it was found that there is a decrease in *AGO2* mRNA methylation in human cells during aging, probably leading to deregulation of miRNA expression [44]. Second, an increase in the activity of the *Argonaute* genes may be a manifestation of a compensatory response to the increasing age-related activity of retrotransposons, disruption of the heterochromatin structure, and cellular stress. This may also be the reason for the activation of stress response genes in old fruit flies. Indeed, the chronic activation of stress-sensitive pathways during aging has been previously described. In a number of experimental models, the induction of stress response genes was found both in individual organs and throughout the body [50-53]. In the early stages of aging or in the case of a short period of time after an acute damaging impact, this tendency can provide faster recovery and better survival of an organism. However, their chronic activation and dysregulation during aging causes the destruction of homeostasis and depletion of energy. There is a general decrease in the efficiency of cellular and organismal responses to stressful influences, a decrease in the work of repair systems, an increase in the number of senescent and malfunctioning cells and other destructive processes [52,54-56].

An increase in the activity of retrotransposons (which we observed in the experiment) is both a consequence of age-related deregulation of the mechanisms of maintaining the structure of repressive heterochromatin and cellular defense, and the cause of genotoxic stress with the subsequent development of degenerative processes [5,57]. Earlier it was found that the activity of mobile genetic elements increases in various organs of aging animals. For example, such changes have been shown in the brain [19] and the adipose body of fruit flies [58]. These changes are accompanied by age-related depletion of the functions of these organs.

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In the present research, we studied the effect of knockdown of the *Argonaute* genes in various tissues on the *Drosophila melanogaster* lifespan. Based on the data described above, we assumed two possible consequences of the *Argonaute* knockdown. First, a decrease in the activity of the *Argonaute* genes will increase age-related changes in the tissues of flies as a result of deregulation of the activity of small RNAs, and will lead to a decrease in lifespan. Secondly, suppression of the *Argonaute* genes will partly help smooth out the imbalance in the work of proteins encoded by them and age-related hyperactivation, at least at the level of conserving energy resources.

We found that decreased activity of genes in the *Argonaute* family causes changes in the lifespan depending on a gene and a tissue in which a gene was knocked down. In most cases, tissue-specific RNA interference of genes of the *Argonaute* family either did not have a statistically significant effect, or led to a shortened lifespan, which is consistent with the first hypothesis. It is worth noting that there are few studies where reduced activity of the *Argonaute* genes also led to a lifespan reduction in model animals. For example, in *Drosophila melanogaster*, mutations in the *AGO2* gene led to a progressive deterioration in the functions of the nervous system and a lifespan decrease [19]. At the same time, *piwi* mutations lead to opposite effects on the lifespan and health of fruit flies, depending on the allele. *Drosophila* with a heterozygous $piwi^{2}$ mutation had a short lifespan, increased sensitivity to starvation, and reduced immunity. In the fat body of flies, the $piwi^{2}$ mutation caused a decrease in the level of piRNAs, activation of mobile elements, an increase in DNA damages, and a loss of lipid stores [59]. However, the $piwi^{c362}$ mutation led to an increase in lifespan [60]. In our study, RNA interference of the *piwi* gene in the nervous system and the fat body, as well as knockdown of the *AGO1* and *AGO3* genes in individual cases also led to an increase in the lifespan. These data are consistent with the second hypothesis and indicate a critical role in the emerging epigenetic imbalance in the biogenesis mechanisms of miRNAs and piRNAs, but not siRNAs. Activated Argonaute proteins can enhance gene repression (as a response to an increase in the proportion of heterochromatin and activation of transposons) and, at the same time, suppress the activity of genes important for survival. Indeed, it has been found that proteins of the Argonaute family in nematodes [18] and piwi in fruit flies [61] affect the DAF-16/FOXO and DAF-2/IGF-1/insulin signaling pathways, which regulate longevity and aging. In our study, knockdown of *AGO1* and *piwi* genes in the nervous system and the fat body caused activation of stress response genes, especially antioxidant defense genes (*Sod1, Prx5*), genes of DNA damage response and repair (*Gadd45, spn-B*), and genes encoding heat shock proteins (*Hsp27, Hsp68*). Several of these genes have been identified previously as pro-longevity genes [62-66]. At the same time, suppression of *AGO2* and *AGO3* expression mainly reduced the activity of stress response genes.

At the same time, unexpected data were obtained on the effect of RNA interference of *Argonaute* genes on the activity of retrotransposons. Since the genes of the *PIWI* subfamily are an important part of the mechanism for controlling the activity of transposons, a disruption of their regulation leads to a surge in the activity of these genetic elements. This effect we observed in flies with tissue-specific knockdown of *AGO3* and *piwi*. Surprisingly, knockdown of genes of the *Argonaute* subfamily (*AGO1* and *AGO2*), on the contrary, reduced the activity of retrotransposons. The mechanism of siRNAs is also aimed at suppressing the activity of mobile genetic elements, mutations in *AGO2* were previously found to increase their expression in the *Drosophila* brain [19]. Despite the fact that it is not clear how the knockdown of *AGO1* and *AGO2* reduces the activity of retrotransposons in our experiment, it is obvious that the change in the activity of these genetic elements did not play a key role in the observed effects.

Currently, there are few data on the contribution of the activity of *Argonaute* genes to age-related changes and lifespan regulation, therefore we can only assume the mechanisms of the lifespan effects of tissue-specific *Argonautes*' knochdown. It is known that AGO2 takes over part of the functions of AGO1 in aging fruit flies. Deep sequencing of small RNAs revealed a global increase in miRNAs loaded into AGO2, but not AGO1, with age. This process is mediated by an increase in the level of 2'-O-methylation of miRNAs. Despite the fact that this mechanism is assumed to be associated with age-related events, its violation has even greater negative consequences. Thus, the *AGO2* mutation or the

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disruption of miRNA 2′-O-methylation leads to accelerated neurodegeneration and a reduction in the lifespan of flies [67]. Thus, the age-related activation of *AGO2* can be justified in connection with the increasing load on it; therefore, its knockdown caused rather a negative effect on the lifespan. At the same time, *AGO1* hyperactivation can enhance the growing imbalance with aging, so we observed the positive effects of its knockdown in some cases.

The piRNAs-PIWI mechanism was initially identified in germline cells. However, the understanding of the functions of piRNAs and proteins of the PIWI subfamily in somatic tissues and their role in the regulation of lifespan is now expanding [5,68]. For example, the regulation of transposon activity and the functioning of PIWI proteins is important for the maintenance of somatic stem cells and the prevention of aging-related tissue degeneration. Thus, it was shown that piwi is crucial for the suppression of age-related expression of transposons in stem cells of the *Drosophila* intestine and maintenance of epithelial homeostasis [69]. The piwi activity in the fat body is essential for the regulation of metabolism and the normal lifespan of flies [59]. A number of studies in rodents have established the role of PIWI proteins and piRNAs in the regeneration of axons of sensory neurons [70] and in the implementation of neuronal functions, for example, memory [71]. It is known that they not only regulate the formation of heterochromatin and break down mobile genetic elements, but can also affect the activity of genes encoding proteins [70,72]. In addition, the activity of genes encoding PIWI proteins affects the fertility of model animals (their defects lead to infertility), determining age-related changes in reproductive abilities and affecting longevity [61,73].

We observed that neuronal knockdown of the *piwi* gene in males and females, as well as *AGO3* in females, increases the lifespan. Recent studies on *Drosophila melanogaster* have shown that the brain is characterized by genomic heterogeneity, and the mobility of retrotransposons is important for the activity of some parts of the brain. For example, transpositions in $\alpha\beta$ neurons of mushroom bodies are important for the implementation of some functions, for example, for memory. In these neurons, the activity of piRNA biogenesis proteins is reduced [74]. Thus, the elimination of age-related hyperactivation of the *piwi* and *AGO3* genes specifically in the nerve cells could lead to the preservation of the fly's brain activity and an increase in the lifespan. In addition, *piwi* knockdown in the fat body of males also increased lifespan. But further research is required to identify possible mechanisms for this effect.

Currently, data on the role of small RNA biogenesis proteins in the response of cells and an organism to the action of stress factors, as well as their interaction with proteins and signaling pathways of stress response, are expanding. Previously, small RNAs called double-strand break-induced RNAs (diRNAs) have been identified. In human cells, they are loaded onto AGO2 (forming diRISC) and are important for triggering the repair of DNA double-strand breaks (mainly homologous recombination) by recruiting repair factors (particularly, Rad51) to target sites [28,29]. There are studies indicating the relationship of the AGO2 protein with isoforms of the transcription factor p53, one of the central regulators of the genotoxic stress response and the anticancer mechanisms. Studies on human cancer cell cultures have shown that p53 interacts (including indirectly through miRNAs) with AGO2 after DNA damage, affecting the biogenesis and activity of specific miRNAs. In turn, they can regulate the activity of p53 targets (such as GADD45A) and determine cellular processes, in particular, cell cycle arrest and apoptosis [75,76]. In addition, there is evidence of Argonaute- and miRNA-dependent mechanisms of regulation of the activity of other DNA damage response proteins, for example, ATM [23] and CDK [24]. It should be noted that proteins of the PIWI subfamily may also be involved in the repair of DNA damage caused by genotoxic agents, in particular, through the regulation of histone acetylation and chromatin relaxation [26].

For the Argonaute proteins' functioning, they must interact with certain heat shock proteins. In particular, Hsp90 activity is required for the efficient targeting of AGO2 to processing bodies and stress granules, and also affects the production and functional activity of miRNAs and siRNAs [77]. Similarly, the association of the PIWI proteins with chaperones, for example, with the heat shock protein DNAJA1 in planarians, has been shown. Homologues of these

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proteins also interact in human gastric cancer cells [78]. In addition, the *Drosophila* organizing protein homologue Hsp70/90 (Hop) interacts with piwi and mediates the maintenance of genome stability in germline cells [79].

As indicated above, in our study, long-lived flies with knockdown of the *AGO1* and *piwi* genes in the nervous system and the fat body were characterized by activation of stress response genes. This effect also indicates the contribution of small RNA biogenesis genes to the stress response.

As a rule, changes in stress resistance and, in particular, radioresistance correspond to changes in lifespan. Thus, organisms that are more resistant to the action of negative environmental factors have higher viability and longevity [80]. We compared the survival rate of fruit flies under normal conditions and after acute exposure with γ-radiation. Nevertheless, the obtained data did not always correspond to the described pattern. For example, fruit flies with *AGO1* knockdown in the fat body and the nervous system and *piwi* knockdown in the fat body showed both increased lifespan and radioresistance. However, *piwi* RNA interference in the nervous system, which had a pro-longevity effect, significantly reduced the survival under irradiation conditions. RNA interference of *AGO2* and *AGO3* in the fat body of females did not significantly affect the lifespan of females under normal conditions, but increased the survival rate after γ-irradiation.

It should be noted that the relationship between radioresistance and the tissue in which an *Argonaute* gene was knocked out is more likely than the relationship with a particular gene. In general, *Drosophila* with neuronal knockdown of the *Argonaute* genes were sensitive to the action of γ-radiation, which indicates the important role of the proteins encoded by them in the stability of the nervous system functioning under stressful conditions. At the same time, females (and to a lesser extent males) with knockdown of the *Argonaute* genes in the fat body, on the contrary, showed a higher resistance to radiation. Previous data do not explain the observed effects. In contrast, *piwi* mutants exhibit piRNA depletion in the fat body, enhanced transposon mobilization, increased levels of DNA damage, decreased lipid stores, and increased stress sensitivity [59]. Mutations of *AGO2* and *PIWIL2* in human and rodent cells reduced their survival under exposure with UV light and ionizing radiation, and led to impaired responses to DNA damage [24-26]. Similar results were obtained for germ cells in irradiated *Caenorhabditis elegans* with loss of the *alg-2* gene. In this case, an increased cell apoptosis associated with MAPK hyperactivation was observed [27]. In addition, studies on non-small cell lung cancer cells indicate no effect of *AGO2* gene knockdown on their radiosensitivity [81].

The data obtained for the activity of transposons and the expression of stress response genes also do not allow drawing conclusions about the mechanisms of the observed effects of tissue-specific *Argonaute* genes' knockdown on the radioresistance of *Drosophila*. γ-Irradiation caused the activation of genes provided the response to genotoxic stress, in particular, its coordination, nucleotide excision repair, and repair of double-strand breaks by non-homologous end joining (*Gadd45, Xpc, Ku80*), as well as the response to proteotoxic stress (*Hsp68*). These genes belong to the basic signaling pathways of reaction to acute irradiation, and their activation ensures survival in adverse conditions [80,82]. However, we observed such changes both in variants with RNA interference of the *Argonaute* genes and without activation of RNA interference.

Irradiation increased the activity of retrotransposons in experimental variants without activation of RNA interference of the *Argonaute* genes. Indeed, it is known that damaging environmental factors (including ionizing radiation) can disrupt epigenetic control and, as a consequence, cause the activation of mobile genetic elements. An increase in their activity is characterized by early manifestation and persistence, which makes it possible to use transposons as biomarkers of exposure with environmental stressors [83-85]. Surprisingly, irradiated *Drosophila* with *Argonaute* genes' knockdown had lower levels of retrotransposon expression than irradiated animals without knockdown. One of the reasons may be a general blockage of the transcriptional apparatus, which is often observed during extensive disruption of the integrity and stability of the genome [86]. However, the exact mechanisms also require further study.

Additionally, it should be noted that we observed a greater sensitivity to irradiation in males than in females. This may be due to the specificity of the epigenome in different sexes. For example, males have more heterochromatic DNA than females due to the presence of a Y chromosome with a large number of repeats [87]. Consequently, they are more sensitive to changes in the functioning of systems that regulate gene expression and repression of mobile genetic elements.

5. Conclusions

We found that tissue-specific decrease in the activity of genes of the *Argonaute* family causes changes in lifespan and resistance to γ-irradiation at a dose of 700 Gy, depending on the gene and tissue in which a gene knockdown was triggered. In the most cases, these parameters were reduced or did not change significantly in flies with tissue-specific RNA interference. Surprisingly, *piwi* knockdown in both the fat body and the nervous system, as well as *AGO1* and *AGO3* RNA interference in some cases caused a lifespan increase. Such positive changes were associated with increased expression of some stress response genes, but, apparently, did not depend on the activity of transposons. At the same time, changes in radioresistance depended on the tissue in which the gene was knocked out. Thus, neuronal RNA interference of the *Argonaute* genes predominantly reduced the survival of irradiated flies, while RNA interference in the fat body increased the radioresistance of females.

The mechanism of epigenetic control using small RNAs is highly evolutionary conserved and persists through animal phylogeny [13]. Accordingly, *in vivo* studies in animal models (such as fruit flies or nematodes) suggest the function of small RNA orthologues as well as proteins of their biogenesis in other animals, including humans. At the same time, epigenetic mechanisms are highly susceptible to external stimuli and affect a wide range of cellular processes, and small RNA biogenesis genes and proteins can be targets for potential geroprotectors and drugs in age-related diseases [1,2]. Indeed, it was found that the dysregulation of their activity is associated with the development of a number of age-related diseases, including cancer, inflammatory, neurodegenerative, cardiovascular, metabolic and immune disorders [88-96]. We have found that suppression of some genes of the *Argonaute* family can prolong the life of fruit flies or enhance their radioresistance. This indicates the potential for their use as targets for geroprotective or radioprotective interventions (for example, using selective pharmacological drugs). However, a detailed study of the molecular mechanisms associated with the observed effects and possible negative consequences affecting quality of life is required.

Supplementary Materials: The following are available online at www.mdpi.com. Table S1: *Drosophila melanogaster* strains. Table S2: Primers for real-time PCR. Table S3: Lifespan parameters of flies with tissue-specific knockdown of the *Argonaute* genes. Table S4: Survival of flies with tissue-specific knockdown of the *Argonaute* genes in the condition of γ-irradiation. Figure S1: Knockdown of *AGO1, AGO2, AGO3,* and *piwi* in investigated flies. Figure S2: Effects of acute gamma irradiation on the survival of *Drosophila* male imago from the controls for RNAi of *AGO1, AGO2, AGO3, piwi* in the fat body and nervous system. Figure S3: Age-related changes in the expression of *Argonaute* genes in heads, toraxes, abdomens of wild-type *Canton-S* males and females. Figure S4: Age-related changes in the expression of transposable elements in heads, toraxes, abdomens of wild-type *Canton-S* males and females. Figure S5: Age-related changes in the expression of stress response genes in heads, toraxes, abdominals of wild-type *Canton-S* males and females.

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