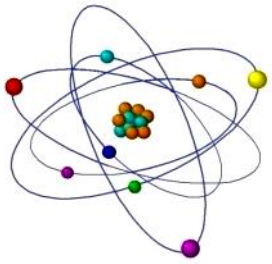
EVALUATION OF DIFFERENTLY EXPRESSED GENES IN IRRADIATED AND INTACT MICE TO STUDY RADIOACTIVE AGING PHENOMENON



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ABSTRACT: *The question about processes which lie behind aging of organisms still does not have an unambiguous answer. The main reason of this uncertainty are numerous manifestation forms and effects participating in this process at various structural and functional levels of the organization of living organisms. In order to specify the processes involved in the aging effect, we used two methodological stages: 1. we analyzed RNA sequences from publicly available databases to find differently expressed genes in old and young mice and mice after radiation exposure; 2. compared obtained set of genes with genes recognized as age related. If we take into the account that we can possibly dose the acceleration of aging process by varying radiation exposure, then the identification of specific genes can significantly narrow the genetic spectrum which is most important for the aging of organisms. Given the high degree of similarity of the investigated mice genes with the human genome, this methodological approach can lead us towards unification of integrative mechanisms of aging, and the development of methods for biomedical monitoring of this process.*

Key words: aging, longevity, genes, bioinformatics, RNA-sequence, irradiation

INTRODUCTION

According to the World Health Organization (WHO), age-related diseases are currently leading among the main causes of death: coronary heart disease (CHD), stroke, cancer, and diabetes. Aging is the main risk factor for these and many other socially significant chronic neurodegenerative disorders such as dementia, Alzheimer's and Parkinson's diseases [1]. Such a variety of ailments increasingly dictates the need to study more general mechanisms underlying the aging process, without focusing only on individual specific cases. Accumulation of mutations and antagonistic pleiotropy, oxidative effects of free radicals, self-destruction of cells (apoptosis), telomeres shortening, neuroendocrine theory and other popular hypotheses are currently discussed in scientific publications regarding aging theories [2].

Thus a large number of different versions create a necessity to study adequate methods to trigger the aging process. Ionizing radiation, which is capable of dosing the influence on the regulatory mechanisms of aging, favorably differs from other methodologies. An assessment of the aging associated biological processes reveals the acceleration of normal aging by ionizing radiation [3].

Analyzing this phenomenon, we should mention that radiation has pluripotent effects, including such fundamental processes as DNA repair and blocking of proliferative activity. At the same time, the postradiational fate of cellular populations, including aging and apoptosis, mainly depends on the accuracy of implementation of reparation processes [4]. Obviously, that there is regularity associated with different levels of high doses of radiation, because of an increasing probability of DNA double-strand breaks. In this case, active induction of cellular aging processes is observed [5]. Thus, radiation aging can be considered as more general form of premature decrease of vital activity of organisms,

here caused by radiation exposure [6]. Aging is defined as the age-related deterioration of biological functions necessary for life [7]. In other words, ionizing radiation affects aging through molecular and cellular mechanisms on the various structural and functional levels of biological objects.

Experiments on irradiated (5Gy) mice of different age groups confirmed that the cognitive abilities for spatial learning were markedly reduced in groups of 3-month-old mice, while 1-year-old mice did not show a very noticeable deterioration in cognitive abilities [8]. These observations lead us to the question about genetic nature of radiosensitivity of older mice to compare with younger mice.

Genetic variability of longevity may be observed due to the fact that genes that influence lifespan represent various molecular functions but may be involved in similar biological processes and health disorders, which could contribute to genetic heterogeneity of longevity and the lack of replication in genetic association studies [9]. It is very important that the inheritance of longevity is still poorly understood. There are several controversial opinions about it [10,11]. So the question of the genetic inheritance of longevity is still open for study.

The process of gene expression is responsible for using the information from gene to synthesize a functional product, which can result in proteins production. In the case when the level of gene expression in different conditions of organism is statistically different, then the study of these differences gives us the possibilities to understand the biological difference between these conditions [12]. To provide us with right direction for further investigations, ascertain the genetic nature of changes in the body caused by radiation, and identify genes that are radiosensitive and specific to the aging organism, we used Publicly available database and analyzed differently expressed mouse genes (Mus Musculus C57Bl/6J SPF) of irradiated organism and young (7-12 weeks) and old (12-19 months) non-irradiated mice.

MATERIALS AND METHODS

In this work the RNA - seq study identified differently expressed genes between irradiated and not irradiated conditions in mice. The main steps of the analysis was performed on Galaxy web platform - free, open source software, which provides necessary tools for step-by-step analysis of different expressed genes [13].

We used RNA sequence from Publicly available data from European Nucleotide Archive (ENA) which provides a comprehensive record of the world's nucleotide sequencing information, covering raw sequencing data, sequence assembly information and functional annotation. Data was obtained from project: PRJEB38394, from ENA archive. Referenced investigation was created by Service Laboratory of Functional Genomics and Bioinformatics Institute of Molecular Genetics of the ASCR, RNA sequences were obtained by NextSeq 500 sequencing, the name of study is "Comparison of CD8+ T-cell sub-types from peripheral lymph nodes and spleen from Balb/c or C57Bl/6J SPF mice mice by transcription profiling".

For our purposes RNA sequencing data from C57Bl/6J SPF mice (10-12 weeks) who were irradiated with 4 Gy and sacrificed 16 days after irradiation and young (7-12 weeks) and old (12-19 months) non-irradiated mice were studied. According to published data each sample consisted of a pool of lymphocytes originating from 2-5 mice. For each population, data were obtained from 3 independent biological triplicates [14].

On Galaxy server we analyzed 6 irradiated with 4Gy samples - ERR4164688, ERR4164689, ERR4164690, ERR4164691, ERR4164692, ERR4164693, and 4 not irradiated samples ERR4164694, ERR4164695, ERR4164696, ERR4164697.

We started from quality control of the reads using FastQC and Cutadapt Galaxy tools. Reads mapping to a reference genome was made by STAR tool. Referenced genome file was taken from ZENODO

database [15]. The number of reads from the mapped sequences per annotated genes were counted using featureCounts tool. For each step, Quality reports of each step were aggregated using MultiQC. DESeq2 tool was used on the read counts to normalize them and extract the differentially expressed genes. DeSeq2 results for different expressed (DE) genes were visualized by variance plot, heatmap and Volcano Plot (Fig.1).

Analysis revealed 322 differently expressed genes of irradiated and not - irradiated young mice, and 658 differently expressed genes of irradiated and not – irradiated old mice. We considered only those genes where expression differences (2FoldChange) exceeded 1, and the level of statistical significance was p.value > 0.05.

RESULTS AND DISCUSSION

We can see (Fig.1, Fig.2) that irradiation quite strongly affects the expression of many genes in both young and old mice. But as the aim of the study was to identify the genes that are supposedly responsible for the process of radioactive aging, we compared differently expressed genes under the influence of radiation in young and old mice separately Fig.3. All further calculations were made by seaborn and pandas packages, in python.

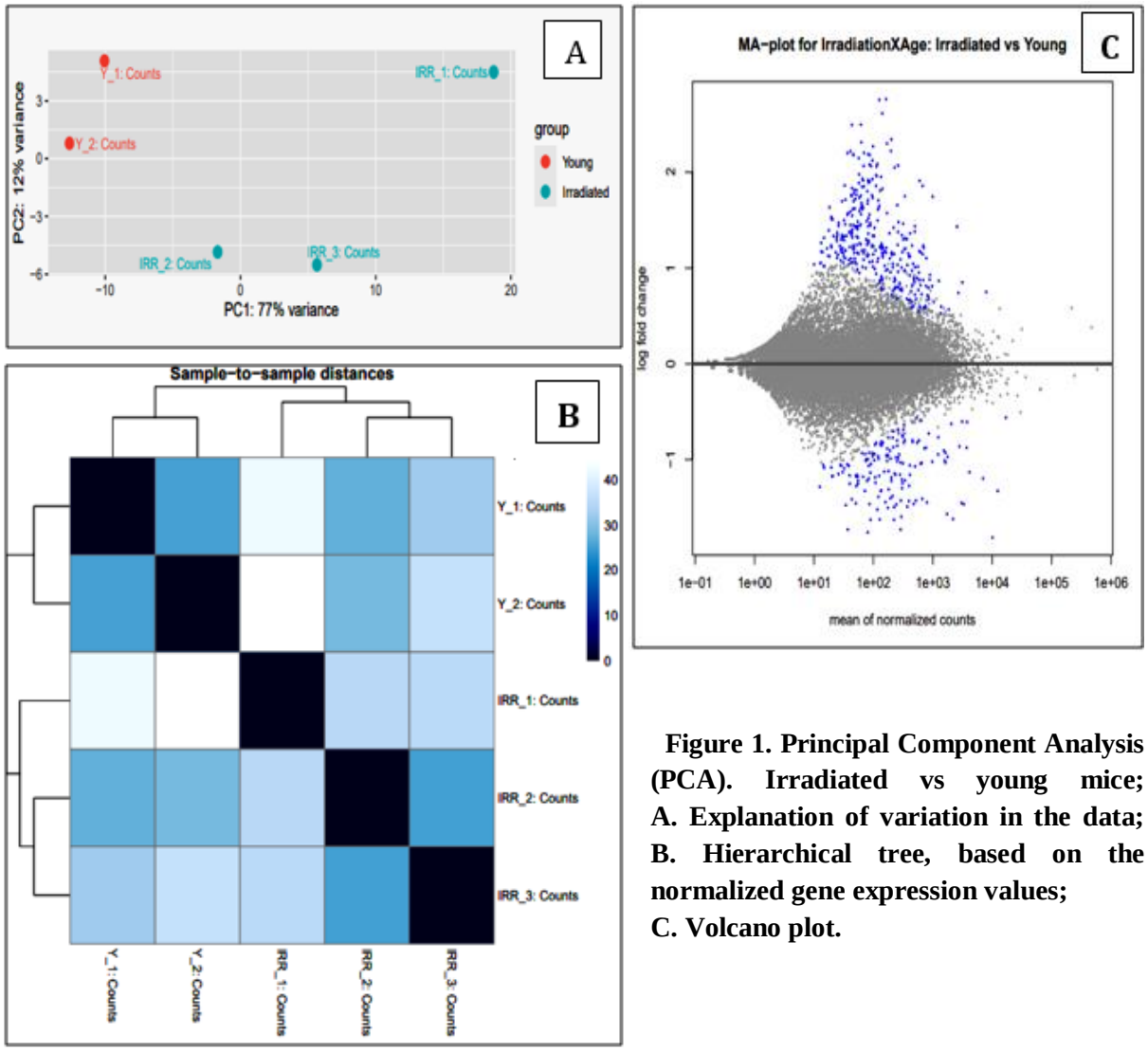

Figure 1. Principal Component Analysis (PCA). Irradiated vs young mice; A. Explanation of variation in the data; B. Hierarchical tree, based on the normalized gene expression values; C. Volcano plot.

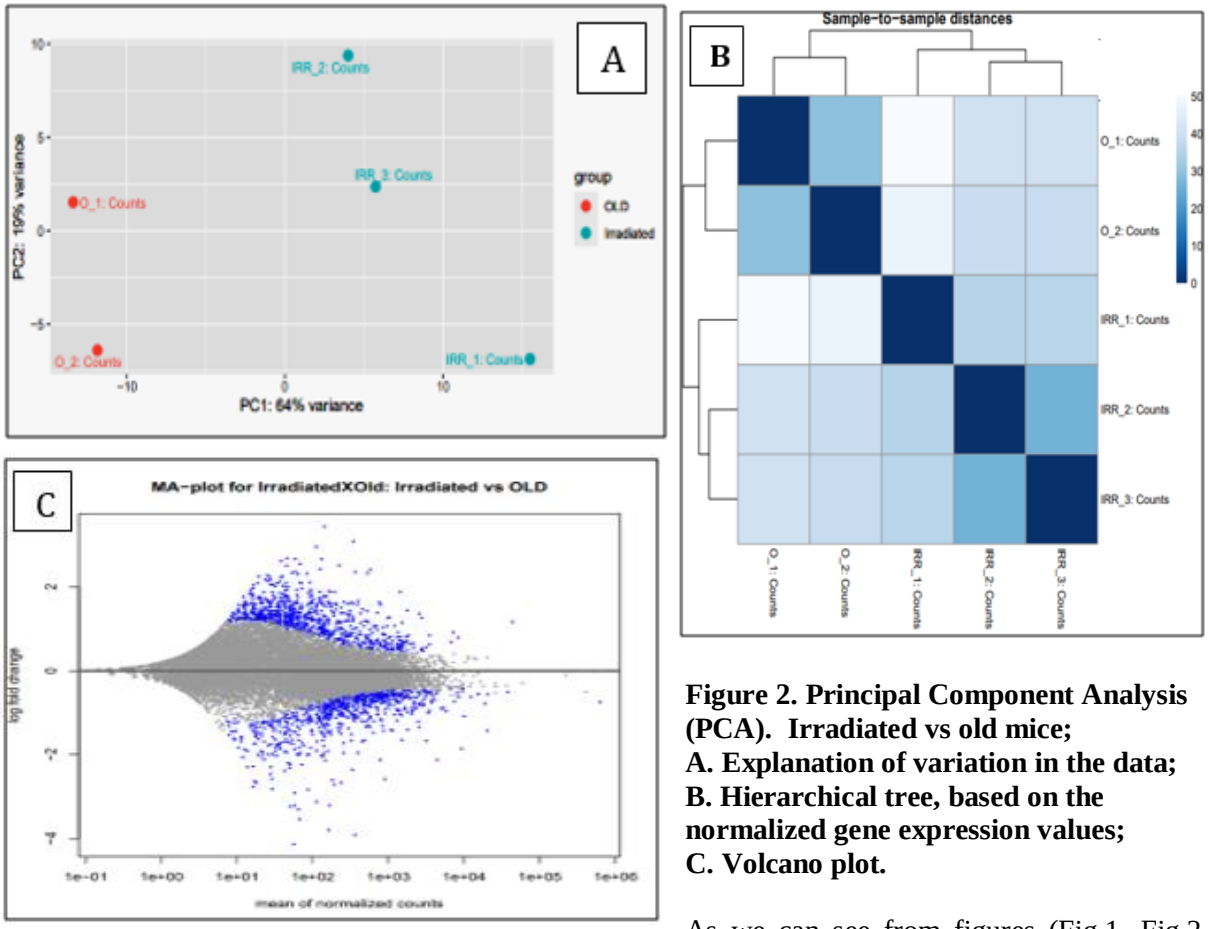

Figure 2. Principal Component Analysis (PCA). Irradiated vs old mice;
A. Explanation of variation in the data;
B. Hierarchical tree, based on the normalized gene expression values;
C. Volcano plot.

As we can see from figures (Fig.1, Fig.2, Fig.3), the comparison of differences in gene expression under the influence of radiation in young and old mice indicates that older mice have more genes with different level of expression. There are almost twice more genes with low expression level in the group of old mice, than in group of young mice.

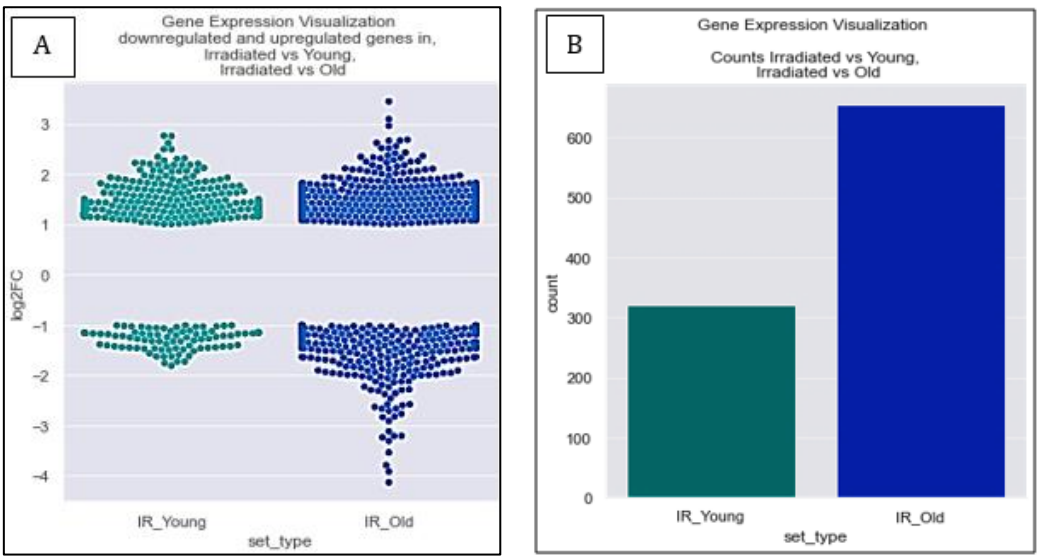

Figure 3. Visualization of gene expression differences (Log2Fold Change) between Irradiated against young and irradiated against old groups of mice.
A. visualization of downregulated and upregulated genes in two groups;
B. Amount of differently expressed genes.

Also in the group of older mice are more upregulated and downregulated genes with high range of changes (abs(Log2(FC))≥2) than in young mice. But absolute level of expression mostly is lower than 2 (abs(Log2(FC)) < 2)(Fig.3) in both cases. If we subset only data with high level of expression, we can indicate that more than 3 genes at once are changed in 2, 10, 11 and 13 chromosomes in case of young mice and more than 5 genes in chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 16, 19 and X in case of old mice (Tab.2). It indicates that old mice have more valuably changed genes and more chromosomes are affected by the influence of radiation.

Table 2. Most differently expressed genes in young and old mice groups

Chromosome	Old mice genes (|Log2(FC)| >= 2)	Young mice genes (|Log2(FC)| >= 2)
chr1	Fcgr2b ; Slamf6 ; Atp1b1 ; Ramp1 ; Gm38204 ; Gm10522	Kif14 ; Nuf2 ;
chr10	Myb ; Tet1 ; Lrrc75b ; Adamts14 ; Slc41a2 ;	Cdk1 ; Lilr4b ; Lilrb4 ;
chr11	Ccl5 ; Eml6 ; Tanc2 ; Alox8 ; Dusp3 ; 2010300F17Rik ;	Mpo ; Aurkb ; Spag5 ;
chr12	Ifi27l2a ;	Rrm2 ; Ncapg2 ;
chr13	Itga1 ; C130051F05Rik ; Tppp ; Gm48624 ; Cenpk ; Gcm2	Hist1h3b ; Hist1h2bb ; Hist1h4f ;
chr14	Bmpr1a ; Spry2 ; Nt5dc2 ; Esco2 ;	/---/
chr15	Rbfox2 ;	Espl1 ;
chr16	Pros1 ; Mx2 ; App ; Cd86 ; Fstl1 ;	/---/
chr17	Afdn ; H2-Eb1 ;	Ccnf ; Uhrf1 ;
chr18	Dsg2 ;	/---/
chr19	Gm6545 ; Anxa1 ; Gm47242 ;	/---/
chr2	Olfm1 ; Ptprj ; Kif5c ; Dapl1 ; Slc43a3 ; Gm44027 ; Nr4a2 ; Sulf2 ;	Tpx2 ; Mcm10 ; Ube2c ; Nusap1 ;
chr3	Gm37589 ; Kcna2 ; A930002I21Rik ;	/---/
chr4	Dmrta1 ; Camk2n1 ; Phf13 ; Id3 ; Adgrb2 ;	Clspn ;
chr5	Oas2 ; Gbp10 ; Drc1 ; Gm42986 ;	Ncapg ;
chr6	Kcnj8 ; Klra9 ; Igkv4-55 ; Igkv5-39 ; Trbv29 ;	/---/
chr7	Fcgrt ; Dennd5a ; Sbf2 ; Tspan32 ; Sox6 ; Trpm1 ;	Sox6 ; Cd22 ;
chr8	Gypa ; Nr3c2 ;	Neil3 ;
chr9	S1pr5 ; Izumo1r ; Phxr4 ; Gm48114 ; Gm38111 ;	
chrX	Tsc22d3 ; Gm5124 ; Cenpi ; Gata1 ;	Kif4 ;

This finding can be explained by superimposing of certain number of genetic mutations (errors) associated with aging, on the effect of radiation-induced damages. This fact manifests itself as high expression levels of differently expressed genes corresponding with level of damage, while the radiosensitivity of young organisms determined by radiosensitivity of critical systems.

We carried out comparative analysis of radiation-induced changes in gene expression of young and old mice with genes associated with the aging process in mice (the database published in the GenAge Database of Aging-Related Genes from Human Aging Genomic Resources). Fig.6.

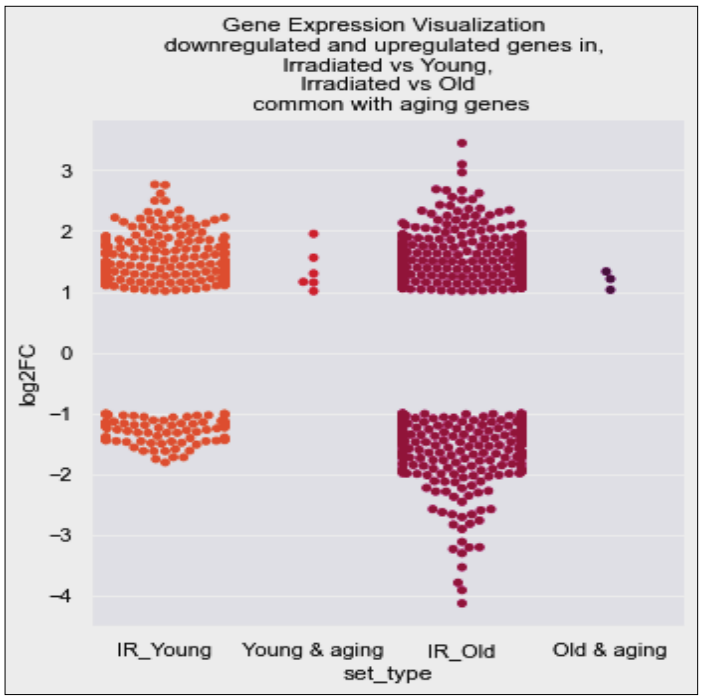

Figure 6. Differently expressed genes in irradiated and young mice, irradiated and old mice, and genes from both groups, which are common with aging associated genes database.

Despite the greater number of differently expressed genes in group of old mice, they have only three genes, common with genes from the database associated with aging. In young mice, there are six common genes. The table (Tab. 1.) represents these proteins with their expression levels and type of impact on the lifespan according to the database.

Table 1. Differently expressed genes from old and young groups, common with aging associated genes.

Group type (Research)	**Gene name**	**Chromosome (Research)**	**log2FC (Research)**	**Description (Web database information)**	**lifespan effect (Web database information)**	**longevity influence (Web database information)**
Young & aging	Bub1b	chr2	1.9509	Budding uninhibited by benzimidazoles 1 homolog, beta (S. cerevisiae)	Increase and Decrease	Pro-Longevity
Old & aging	Bub1b	chr2	1.331002	Budding uninhibited by benzimidazoles 1 homolog, beta (S. cerevisiae)	Increase and Decrease	Pro-Longevity
Young & aging	Hells	chr19	1.1492015	Proliferation associated SNF-2-like gene	Decrease	Pro-Longevity
Old & aging	Hells	chr19	1.0283135	Proliferation associated SNF-2-like gene	Decrease	Pro-Longevity
Young & aging	Lmna	chr3	1.5582066	lamin A	Increase and Decrease	Unclear
Young & aging	Foxm1	chr6	1.297876	Forkhead box M1	Decrease	Pro-Longevity
Young & aging	Txn1	chr4	1.0061757	Thioredoxin 1	Increase	Pro-Longevity
Young & aging	Brca1	chr11	1.1598353	Breast cancer 1	Decrease	Pro-Longevity
Old & aging	Brca1	chr11	1.2083834	Breast cancer 1	Decrease	Pro-Longevity

Indicated genes are in more or less degree responsible for organism development and changes in their expression can cause serious pathologies. *Bub1b* gene is encoding spindle assembly checkpoint protein BubR1, the reduction of BubR1 expression in mouse is associated with increased aneuploidy, senescence and infertility [16]. In human low expression of BUB1B contributes to initiation and progression of human colon adenocarcinomas and lung cancer [17]. In our case we can see slight growth of expression in irradiated animals compared to old and young mice. Lymphoid-specific helicase – HELLS is an important component of chromatin remodeling, which takes part in DNA remodeling [18]. Increased expression of HELLS, as in our case is regulated by the oncogenic transcriptional regulator YAP1 downstream of Smoothened, the positive transducer of SHH signaling [19]. A-type lamins are encoded by the lamin A gene (*LMNA*), mutations in *Lmna* is linked to progeroid diseases Hutchinson-Gilford progeria and atypical Werner's syndromes, striated muscle diseases, muscular dystrophies and dilated cardiomyopathies, lipodystrophies affecting adipose tissue deposition, diseases affecting skeletal development, and a peripheral neuropathy. Nuclear lamina has important roles in regulating DNA synthesis, RNA transcription, and in the organization of chromatin [20]. FoxM1 is a member of the forkhead family of transcription factors [21]. In human overexpression of Forkhead Box M1 (FoxM1 or FoxM1b) transcription factor is observed in a number of aggressive carcinomas [22]. Thioredoxin (TXN), encoded by *Txn1* is a critical antioxidant in the defense against oxidative stress, and regulates dithiol/disulfide balance of interacting proteins. Development of the midbrain in juvenile rats critically needs *Txn1* [23]. Early embryonic lethality can be caused by disruption of *Txn1* gene [24]. Mutations in *Brca1* are causing genome abnormalities and instabilities towards cancer, and affecting many oncogenic genes and pathways including DNA damage repair and oncogenesis [25].

We have a suggestion that this set of genes is most vulnerable to radiation in terms of the effect of radiation-induced aging and requires more sophisticated investigation.

CONCLUSIONS

The investigation of radiobiological effect of ionizing radiation exposure in mice by means of bioinformatics approach based on the criterion of different gene expression, makes it possible to identify a set of genes where visible changes in the level of expression was observed under the influence of radiation. This information may allow us to identify the specific gene set responsible for the radiological regulation of life longevity for inherited aging model.

With a high degree of probability our results may allow us to develop an effective predictive model of aging in mice, to facilitate study of inherited age-related changes. Taking into the account the commonality of investigated genes with human gene analogues, our studies can acquire valuable practical application along with the theoretical significance in the field of biomedicine.

REFERENCES:

[1]. Tinetti ME, McAvay GJ, Murphy TE, Gross CP, Lin H, Allore HG. Contribution of individual diseases to death in older adults with multiple diseases. J Am Geriatr Soc. 2012 Aug;60(8):1448-56. doi: 10.1111/j.1532-5415.2012.04077x. Epub 2012 Jun 26. PMID: 22734792; PMCID: PMC3419332.

[2]. Jian Tong, Tom K. Hei. Aging and age-related health effects of ionizing Radiation Radiation Medicine and Protection, 2020, Volume 1, Issue 1 (Cover date: March 2020) Pages 15-23

[3]. Mohammed Al-Jumayli, Stephen L. Brown, Indrin J Chetty, Martine, Benjamin. The Biological Process of Aging and the Impact of Ionizing Radiation

[4]. Noda A, et al: Unrepairable DNA double-strand breaks that are generated

by ionising radiation determine the fate of normal human cells. J Cell Sci 125:5280-5287, 2012

[5]. Rossiello F, et al: Irreparable telomeric DNA damage and persistent DDR signalling as a shared causative mechanism of cellular senescence and ageing. Curr Opin Genet Dev 26:89-95, 2014

[6.] Suzuki M, Boothman DA: Stress-induced premature senescence (SIPS)-influence of SIPS on radiotherapy. J Radiat Res 2008. 0801220010-0801220010

[7]. Gilbert SF: Developmental Biology. Sunderland, MA: Sinauer Associates, 2014.

[8]. Kalmakhelidze, Sophio & Museridze, D & Gogebashvili, Mikheil & Lomauri, K & Gabunia, T & Sanikidze, Tamara. (2022). Effects of ionizing radiation on cognitive parameters in white mice. Georgian medical news. 187-192.

[9]. Yashin Anatoliy I., Wu Deqing, Arbeeva Liubov S., Arbeev Konstantin G., Kulminski Alexander M., Akushevich Igor, Kovtun Mikhail, Culminskaya Irina, Stallard Eric, Li Miaozhu, Ukraintseva Svetlana V., Genetics of aging, health, and survival: dynamic regulation of human longevity related traits, Frontiers in Genetics, V-6,2015, https://www.frontiersin.org/articles/ 10.3389/fgene.2015.00122, DOI=10.3389/fgene.2015.00122, ISSN=1664-8021

[10]. Paulo MC de Oliveira, Suzana Moss de Oliveira, Americo T Bernardes, Dietrich Stauffer, Siblings of centenarians live longer: a computer simulation, The Lancet Journal, Published: September 12, 1998DOI:https://doi.org/10.1016/S0140-6736(05)60056-6

[11]. Ruby, J. G., Wright, K. M., Rand, K. A., Kermany, A., Noto, K., Curtis, D., Varner, N., Garrigan, D., Slinkov, D., Dorfman, I., Granka, J. M., Byrnes, J., Myres, N., & Ball, C. (2018). Estimates of the Heritability of Human Longevity Are Substantially Inflated due to Assortative Mating. *Genetics*, *210*(3), 1109–1124. https://doi.org/10.1534/ genetics.118.301613

[12]. Anjum A, Jaggi S, Varghese E, Lall S, Bhowmik A, Rai A. Identification of Differentially Expressed Genes in RNA-seq Data of Arabidopsis thaliana: A Compound Distribution Approach. J Comput Biol. 2016 Apr;23(4): 239-47 doi: 10.1089/cmb.2015.0205 Epub 2016 Mar 7. PMID: 26949988; PMCID: PMC4827276

[13]. https://link.springer.com/protocol/10.1007/978-1-0716-1307-8_20

[14]. https://www.ebi.ac.uk/ena/browser/view/PRJEB38394

[15]. Lucille Lopez-Delisle, Customized gtf file from Ensembl version 92 mm10, ZENODO, OpenAIRE, May 14, 2020, DOI: 10.5281/zenodo.3827120

[16]. Baker, D., Jeganathan, K., Cameron, J. *et al.* BubR1 insufficiency causes early onset of aging-associated phenotypes and infertility in mice. *Nat Genet* 36, 744–749 (2004). https://doi.org/10.1038/ng1382

[17]. Fu X, Chen G, Cai ZD, Wang C, Liu ZZ, Lin ZY, Wu YD, Liang YX, Han ZD, Liu JC, Zhong WD. Overexpression of BUB1B contributes to progression of prostate cancer and predicts poor outcome in patients with prostate cancer. Onco Targets Ther. 2016 Apr 15;9:2211-20. doi: 10.2147/OTT.S101994. PMID: 27143916; PMCID: PMC4844448

[18]. Liang Xiao, Li Linji, Fan Yuchao, "Diagnostic, Prognostic, and Immunological Roles of HELLS in Pan-Cancer: A Bioinformatics Analysis", Frontiers in Immunology, V.13, 2022, https://www.frontiersin.org/articles/10.3389/fimmu.2022.870726,
DOI:10.3389/fimmu.2022.870726, ISSN1664-3224

[19]. Robinson MH, Maximov V, Lallani S, Farooq H, Taylor MD, Read RD, Kenney AM. Upregulation of the chromatin remodeler HELLS is mediated by YAP1 in Sonic Hedgehog Medulloblastoma. Sci Rep. 2019 Sep 20;9(1):13611. doi: 10.1038/s41598-019-50088-1. PMID: 31541170; PMCID: PMC6754407

[20]. Stewart CL, Kozlov S, Fong LG, Young SG. Mouse models of the laminopathies. Exp Cell Res. 2007 Jun 10;313(10):2144-56. doi: 10.1016/j.yexcr.2007.03.026. Epub 2007 Mar 31. PMID: 17493612; PMCID: PMC1949387

[21]. Mónica Alvarez-Fernández and René H. Medema, "Novel functions of FoxM1: from molecular mechanisms to cancer therapy", MINI REVIEW article, Front. Oncol., 05 March 2013, Sec. Molecular and Cellular Oncology, https://doi.org/10.3389/fonc.2013.00030

[22]. Tanya V. Kalin, I-Ching Wang, Timothy J. Ackerson, Michael L. Major, Carol J. Detrisac, Vladimir V. Kalinichenko, Alexander Lyubimov, Robert H. Costa, "Increased Levels of the FoxM1 Transcription Factor Accelerate Development and Progression of Prostate Carcinomas in both TRAMP and LADY Transgenic Mice", Cancer Res (2006) 66 (3): 1712–1720. https://doi.org/10.1158/0008-5472.CAN-05-3138

[23].Iori Ohmori, Mamoru Ouchida, Hirohiko Imai, Saeko Ishida, Shinya Toyokuni, Tomoji Mash imo, “Txn1 mutation causes epilepsy associated with vacuolar degeneration in the midbrain”, bioRxiv 2021.10.07.463470; doi: https://doi.org/10.1101/2021.10.07.463470

[24]. Bondareva AA, Capecchi MR, Iverson SV, Li Y, Lopez NI, Lucas O, Merrill GF, Prigge JR, Siders AM, Wakamiya M, Wallin SL, Schmidt EE. Effects of thioredoxin reductase-1 deletion on embryogenesis and transcriptome. Free Radic Biol Med. 2007 Sep 15;43(6):911-23. doi: 10.1016/j.freeradbiomed.2007.05.026. Epub 2007 May 31. PMID: 17697936; PMCID: PMC2099259.

[25]. Wu, X., Guo, M., Cui, J. *et al.* Heterozygotic *Brca1* mutation initiates mouse genome instability at embryonic stage. *Oncogenesis* 11, 41 (2022). https://doi.org/10.1038/s41389-022-00417-3