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“Links between persistent DNA damage, genome instability, and aging.”
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The goal of this study was to examine long-term effects of low-dose radiation exposure. One of the hypotheses was that radiation exposure would accelerate the normal aging process. The study was jointly funded by NASA and examined both low-LET radiation (γ-rays) and high-LET radiation (1000 MeV/nucleon ^{56}Fe ions) at doses of 0.1 Gy and up.

The work used the Japanese medaka fish (Oryzias latipes), as a vertebrate model organism that can be maintained in large numbers at low cost for lifetime studies. Like other small laboratory fish, Japanese medaka share many anatomical and histological characteristics with other vertebrates, and a variety of genetic and genomic resources are available. Some work also used the zebrafish (Danio rerio), another widely used laboratory model organism.

Japanese medaka fish studies.

To identify potential age-related biomarkers, a pilot study was performed using control, non-irradiated medaka fish. Liver, heart, brain, skeletal muscle, dermis, ocular lens, and retina were examined in young adults (6 months), mature adults (16 months), and older adults (24 months, which is beyond the 22-month median lifespan). The liver showed a number of prominent, quantifiable age-related changes and was selected as the organ for further study. Some age-related changes were also seen in heart, dermis, and retina. Age-related changes in histology were not evident in the other tissues.

For the large-scale irradiation study, medaka embryos were irradiated at doses ranging from 0.1 Gy to 27 Gy of γ-rays and 0.1 to 9 Gy of high-LET charged particle radiation (1000 MeV/nucleon ^{56}Fe ions). With the exception of the highest dose group for each radiation type, all of the groups, including non-irradiated controls, showed very similar mortality curves. Median survival was in the range of 17-18 months, slightly less than in the pilot experiment.

The population was sampled at intervals from 8 to 28 months post-irradiation, and liver tissue was subjected to histological and molecular analysis. Results showed that charged particle radiation and aging contributed synergistically to accumulation of lipid peroxidation products, which are a marker of chronic oxidative stress. This was mirrored by a decline in PPARGC1A mRNA, which encodes a transcriptional co-activator required for expression of oxidative stress defense genes and for mitochondrial maintenance. Consistent with chronic oxidative stress, mitochondria had an elongated and enlarged ultrastructure. Depending on the endpoint, effects of γ-rays in the same dose range were either lesser or not detected. Together, results indicate that a single exposure to high-LET, but not low-LET radiation, early in life, leads to increased oxidative stress throughout the normal lifespan of the individual.

Zebrafish study.

In a separate study, long-term effects of ionizing radiation on gene expression were studied in the zebrafish. As in the medaka, irradiation was performed using embryos, and changes in the

messenger RNA profile were analyzed in the liver of adult fish. Radiation doses were 0.1 and 1.0 Gy of γ-rays and analysis was performed at 16 weeks. Results were compared to acutely irradiated 16-week-old individuals. Analysis at the gene and pathway level indicated that the long-term response includes the induction of cytokine and inflammatory regulators and transcription and growth factors. The acute response includes the induction of p53 target genes and modulation of the hypoxia-induced transcription factor-C/EBP axis. Results help define genes and pathways affected in the long-term, low and moderate dose radiation response and differentiate them from those affected in an acute response in the same tissue.

Other work.

Project personnel contributed to data analysis in real-time studies of the DNA double-strand break response in mammalian cells.

Publications.

1. Ding, LL, Kuhne, WW, Hinton, DE, Song, J, and **Dynan, WS**, "Quantifiable Biomarkers of Normal Aging in the Japanese Medaka Fish (Oryzias latipes). PLoS One. 5:e13287 (2010). PMCID: PMC2952620.
2. Jaafar, L, Podolsky, RH, and **Dynan, WS, "**Long-term effects of ionizing radiation on gene expression in a zebrafish model." PLoS One. 8:e69445 (2013). PMCID: 3728239.
3. Zheng, X, Zhang, X, Ding, L, Lee, JR, Weinberger, PM, and **Dynan, WS**, Synergistic effect of high charge and energy particle radiation and chronological age on biomarkers of oxidative stress and tissue degeneration: a ground-based study using the vertebrate laboratory model organism *Oryzias latipes.* PloS One. 9:e111362 (2014). PMCID: 4222877.
4. Cao, Z, Kuhne, WW, Steeb, J, Merkley, MA, Zhou, Y, Janata, J, and **Dynan, WS**, "Use of a microscope stage-mounted Nickel-63 microirradiator for real-time observation of the DNA double-strand break response," Nucleic Acids Res. 38:e144 (2010). PMCID: PMC291973.