Low dose irradiation of the early vertebrate embryo and early onset of tissue aging

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There is considerable overlap between cellular and molecular changes that occur in response to low doses of ionizing radiation and those that occur during aging. Both processes are characterized by accumulation of persistent DNA damage ("wear and tear" on the genome), persistent oxidative stress, and depletion of stem/progenitor cells, leading to loss of repair and regenerative capacity. Here we test a hypothesis that exposure to low-dose ionizing radiation accelerates normal, aging-related tissue changes.

The work was performed using a small, genetically tractable vertebrate model organism, the Japanese medaka fish (Oryzias latipes). Medaka have a long history as a radiobiological model. They have a compact genome with an $LD_{50/30}$ of about 20 Gy. A pioneering long-term study in the 1960s and early 1970s showed decreased lifespan following exposure to single, sublethal doses of radiation (0.25 to 10 Gy) at the embryonic stage of development [1]. To establish a framework for studies of radiation and aging, we first identified quantifiable biomarkers of normal aging in this model. These include, most notably, inflammatory and degenerative changes in the liver and accumulation of senescence-associated beta-galactosidase in the basal layer of the dermis [2].

We report here results of an experiment where medaka embryos were exposed to a single dose of radiation. The design was similar to the early experiment of Egami and Etoh, although in our case, exposure was performed at the pre-early gastrula stage, when there is no morphologic differentiation and none of the organs affected by radiation in adults have begun to form. In addition, experiments were performed in a laboratory setting, rather than the outdoor setting used in the earlier work. Primary endpoints of the present study were median lifespan, histological changes in the liver, and senescence-associated beta-galactosidase in skin and nearby muscle.

Consistent with the earlier findings, radiation exposure resulted in a shortening of median lifespan. Normal lifespan under our conditions (excluding perihatching mortality in the first 30 days of life) was 22.2 months. Exposure to 15 Gy of gamma radiation, which was the highest dose tested, resulted in shortening of lifespan by more than a factor of two, to 10.9 months. More modest effects on lifespan were seen at lower doses in the range of 0.15 to 5 Gy. Interestingly, no radiation-induced cancers were evident, suggesting that medaka may be particularly valuable for investigating long-term, non-cancer effects of radiation exposure.

Analysis of liver pathology was performed in fish that were sampled at 16 months of age. Prior work has shown that the onset of inflammatory and degenerative changes occurs between 16 and 24 months, coinciding with the peak period of age-related mortality [2]. The rationale for sampling irradiated populations at 16 months of age was that we would be able to observe the acceleration of incipient changes that were present in nonirradiated individuals at this age. Indeed, the majority of individuals in the irradiated groups (13/14) showed fibrosis, necrosis, or both (13/14) whereas only a minority of individuals from the nonirradiated control group showed these changes (3/11). Irradiated groups also showed highly significant increases in the prevalence of nuclear pyknosis and steatosis and modest increases in the prevalence of inflammatory infiltrates and macrophage aggregates. A few irradiated individuals showed other changes, including ballooning degeneration, perivenous inflammation, or a cyst, which were not observed in any of the non-exposed fish at this age. In many respects, the livers of 16-month old fish from the irradiated groups resembled those from 24-month old fish examined in our earlier study.

Analysis of senescence-associated beta galactosidase was also performed in fish that were sampled at 16 months of age. Although the stained areas were small and difficult to quantify, they appeared to be much more numerous in samples from the irradiated groups, whereas they were nearly absent from the non-irradiated controls. Interestingly, prominent senescence-associated beta galactosidase staining was also seen skeletal muscle adjacent to the dermis. We did not observe this pattern of beta-galactosidase staining in skeletal muscle of unexposed medaka at any age.

Together, results are strongly indicative that a single exposure to a low dose of ionizing radiation (ranging from about 1% to 25% of the estimated LD50/30) resulted in accelerated appearance of aging-related biomarkers. We have recently initiated a new experiment to compare effects of gamma radiation and HZE particle radiation exposure on radiation-accelerated aging. HZE particle radiation exposure is associated with degenerative changes in the rodent nervous system, and the medaka model will afford the opportunity to determine whether these effects can be generalized to other organs. We are also performing experiments in medaka and zebrafish to examine the effect of irradiation early in life on subsequent changes on the frequency of somatic recombination or the pattern of mRNA expression, with the expectation that these surrogate endpoints can be assessed more rapidly than tissue aging and will provide insight into the mechanism of the radiation-accelerated aging phenomenon.

Literature cited:

1. Egami N, Etoh H: **Effect of x-irradiation during embryonic stage on life span in the fish, Oryzias latipes**. *Exp Gerontol* 1973, **8**(4):219-222.
2. Ding L, Kuhne WW, Hinton DE, Song J, Dynan WS: **Quantifiable biomarkers of normal aging in the Japanese medaka fish (Oryzias latipes)**. *PLoS One* 2010, **5**(10):e13287.