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# Parental exposure to gamma radiation causes progressively altered transcriptomes linked to adverse effects in zebrafish offspring☆

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ABSTRACT

Ionizing radiation causes a variety of effects, including DNA damage associated to cancers. However, the effects in progeny from irradiated parents is not well documented. Using zebrafish as a model, we previously found that parental exposure to ionizing radiation is associated with effects in offspring, such as increased hatching rates, deformities, increased DNA damage and reactive oxygen species. Here, we assessed short (one month) and long term effects (one year) on gene expression in embryonic offspring (5.5 h post fertilization) from zebrafish exposed during gametogenesis to gamma radiation (8.7 or 53 mGy/h for 27 days, total dose 5.2 or 31 Gy) using mRNA sequencing. One month after exposure, a global change in gene expression was observed in offspring from the 53 mGy/h group, followed by embryonic death at late gastrula, whereas offspring from the 8.7 mGy/h group was unaffected. Interestingly, one year after exposure newly derived embryos from the 8.7 mGy/h group exhibited 2390 (67.7% downregulated) differentially expressed genes. Overlaps in differentially expressed genes and enriched biological pathways were evident between the 53 mGy/h group one month and 8.7 mGy/h one year after exposure, but were oppositely regulated. Pathways could be linked to effects in adults and offspring, such as DNA damage (via Atm signaling) and reproduction (via Gnrh signaling). Comparison with gene expression analysis in directly exposed embryos indicate *transferrin a* and *cytochrome P450 2x6* as possible biomarkers for radiation response in zebrafish. Our results indicate latent effects following ionizing radiation exposure from the lower dose in parents that can be transmitted to offspring and warrants monitoring effects over subsequent generations.



## 1. Introduction

Gamma radiation, either anthropogenic or naturally occurring, can affect the genetic material directly, by induction of DNA single and double strand breaks and indirectly, via excitation of water molecules and formation of free radicals (Han and Yu, 2012). Exposure to gamma radiation is associated with a wide range of effects, such as genomic instability and tumor formation, as observed in animal models and human cohort studies (Unscear, 2010). Furthermore, studies in animal models provide evidence of effects in subsequent unexposed generations, due to affected germ cells exposed to radiation during gametogenesis (Buisset-Goussen et al., 2014; Soubry et al., 2014).

Recent studies show that zebrafish is a sensitive model in studying effects of ionizing radiation during embryogenesis (Choi and Yu, 2015). More specifically, embryos appear to be sensitive to effects of ionizing radiation at the transcriptional level, which may affect a diverse range of phenotypic outcomes, such as mortality rate, hatching time, embryo length, and malformation rate (Freeman et al., 2014; Hurem et al., 2017b; Jaafar et al., 2013). However, the effects on gene expression and phenotypic traits in

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progeny following parental irradiation during gametogenesis are not well studied.

In a previous study we observed, a 100% mortality in progeny around 8 h post fertilization (hpf; 80% epiboly, late gastrula), after irradiation of parental fish during gametogenesis to 53 mGy/h for 27 days (Hurem et al., 2017a). In the progeny of parents exposed to 8.7 mGy/h reactive oxygen species (ROS) were found to be increased in 72 hpf larvae one month after parental irradiation, but decreased one year after parental irradiation, while lipid peroxidation (LPO) and DNA damage were found to be significantly increased in embryos one year after parental exposure compared to controls (Hurem et al., 2017a). Similarly, a significant increase in DNA damage was reported in offspring of adult zebrafish exposed to 1 Gy of X-rays (Lemos et al., 2017). These results clearly indicate that biological effects of parental exposure to ionizing radiation may be transferred to their progeny.

In order to investigate the effects on the transcriptome, we produced embryos from exposed zebrafish one month and one year after exposure and performed mRNA sequencing. We sampled early gastrula stage embryos (5.5 hpf), a developmental stage where the zygotic genome has been activated, and most of the maternal mRNAs are degraded (Haberle et al., 2014; Aanes et al., 2011). Hence, this stage allows to measure expressed genes in a still relatively undifferentiated homogeneous cell population (Kimmel et al., 1995).

## 2. Material and methods

### 2.1. Zebrafish husbandry and exposures

This study was approved by the institutional animal ethics committee (IACUC) and the Norwegian food inspection authority (NFIA), under permit number 5793. Zebrafish of the AB wild type strain were obtained from the Norwegian university of Life Sciences (NMBU) zebrafish facility and maintained according to standard operating procedures (Hurem et al., 2017a). The NMBU zebrafish facility is licensed by the NFIA and accredited by the association for assessment and accreditation of laboratory animal care (AAALAC, license number: 2014/225976). The exposures of fish, including mating and embryo production were done as described previously (Hurem et al., 2017a). In short, adult zebrafish (6 months of age) were exposed for 27 days to a $^{60}$Co source at 8.7 and 53 mGy/h (total 5.2 and 31 Gy, respectively) (Fig. 1). The chosen doses were comparable to those accumulated by fish during 60 days after the accident in the Chernobyl reactor cooling pond, which were estimated to 10 Gy (Hinton et al., 2007). Control fish were kept separately under similar environmental conditions. One month and one year after exposures, fish were mated by family inbreeds per exposure. F1 embryos were pooled per exposure group, and were incubated in autoclaved system water (28 ± 2 °C).

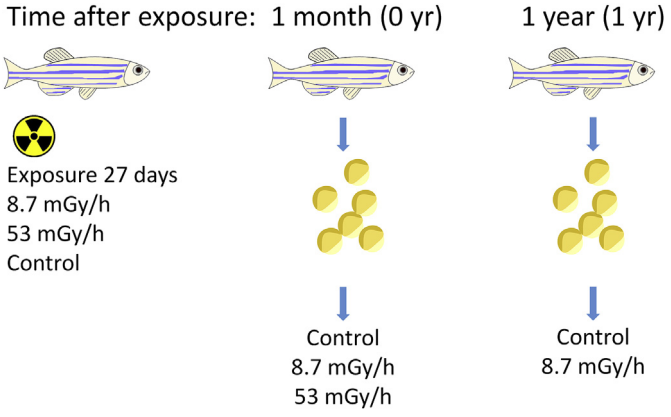


**Fig. 1.** Experimental set-up. Zebrafish were exposed as indicated. Embryos were generated one month (0 yr) and one year after exposure (1 yr) for transcriptomics analysis.

### 2.2. Embryo sampling

F1 embryos were sampled in pools of 100 embryos (3 replicates per exposure), in 12 well plates in 3 mL temperature controlled autoclaved system water (28 ± 2 °C), one month (0 yr) and one year (1 yr) after exposure. This resulted in 5 groups; control 0 yr, 8.7 and 53 mGy/h 0 yr, control 1 yr and 8.7 mGy/h 1 yr (Fig. 1). The 53 mGy/h 1 yr could not be generated due to sterility of parental fish (Hurem et al., 2018). Unfertilized and coagulated and underdeveloped embryos were excluded from analysis. At 50% epiboly stage, embryos were transferred in 1.5 mL tubes (Thermo Fisher Scientific, Waltham, MA) and snap frozen in liquid nitrogen. Samples were stored at −80 °C until further analysis.

### 2.3. RNA purification

Total RNA was isolated with TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA) according to manufactures' instructions. Briefly, 1 mL TRIzol was added to each sample consisting of 100 embryos and homogenized using Magnalyser Beads (Roche Diagnostics, Germany). Each sample was eluted in 40 μL RNase-free water and stored at −80 °C until further analysis. RNA purity and yield was determined using NanoDrop-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE) and RNA integrity number (RIN) was assessed with Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) using RNA Nano LabChip Kit (Agilent Technologies, Santa Clara, CA), which were all of sufficient quality for sequencing (RIN > 9.0). One sample (control 0 yr) got lost during the RNA extraction and we proceeded with duplicate samples of the controls of 0 yr.

### 2.4. mRNA sequencing

Sequencing was outsourced to Novogene (Hong Kong, China). Per sample, a total of 1.5 μg total RNA was used for library preparation. Non-directional libraries were generated using the NEBNext Ultra mRNA kit (New England Biolabs, Ipswich, MA) according to the manufacturers' recommendations. Total RNA was quality checked for integrity with the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA) and agarose gel electrophoresis. Concentration was determined with Qubit analysis (Thermo Fisher Scientific, Waltham, MA). After the QC procedures, mRNA was enriched using oligo (dT) beads, followed by fragmentation and first strand cDNA synthesis using random hexamers and M-MuLV reverse transcriptase. After first-strand synthesis, a second-strand synthesis buffer (Illumina, San Diego, CA) with dNTPs, RNase H and *Escherichia coli* polymerase I was added to generate the second strand. Subsequently, a cDNA library was generated after a round of purification, terminal repair, A-tailing, ligation of sequencing adapters, size selection and PCR enrichment. PCR products were purified with the AMPure XP system (Beckman, US) and library quality was checked on the Agilent Bioanalyzer 2100 system (Agilent Technologies, Santa Clara, CA). Libraries were analyzed using Hiseq 4000 (Illumina, San Diego, CA), using 150 bp paired-end reads, with a depth of 20 million reads per sample.

### 2.5. Bioinformatics

Raw fastq files were adapter trimmed using trim_galore (v0.4.2, Babraham institute, UK) under standard parameters, with extra