# Ionising Radiation Induces Promoter DNA Hypomethylation

Subjects: Molecular Biology

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## Definition

How chronic exposures to sublethal doses of pollutants affect wild life is still under schientific debate. In this paper we exposed fertilized zebrafish embryos to low to moderate dose rates of ionizing radiations, a well known physical stressor that induces DNA damages. We assessed the molecular effects induced by ionizing radiations on gastrulation, a key developmental stage during embryogenesis, focusing on the transcriptome and DNA methylation patterns. An hypomethylation of the promoter of genes involved in ectoderm and mesoderm development was observed, and correlated with perturbation of transcriptional activity. Our data suggest that the early developmental perturbations in the morphogenesis of the neuroectoderm and the mesoderm might predict the functional defects in neurogenesis and muscle development observed at later stages.

## 1. Introduction

We investigated how the early step of zebrafish embryonic development can be altered after exposure to IR by studying genome-wide gene expression and DNA methylation. No morphological abnormalities or increased embryonic lethality could be observed at any of the dose rates tested here. This is in agreement with earlier studies that showed that IR exposure at less than 150 mGy does not impact embryonic survival directly [37], but rather induces subtle neuromuscular and motility defects [25].

However, we observed different molecular effects depending on the dose rates, from modification of mitochondrial processes at moderate and high dose rates (> 0.5 mGy/h), to perturbation of important TF involved in morphogenesis of ectoderm and mesoderm at the two highest dose rates (5 and 50 mGy/h). The transcriptional profile obtained at 5 mGy/h was largely overlapping the one at 50 mGy/h, and, as expected, dose rates at 0.5 mGy/h and below induced subtle changes on gene expression. However, a high number of unique genes were found at 50 and 0.5 mGy/h indicating that a particular transcriptional response might occur at these two dose rates. In addition, the expression of several genes varied proportionally with dose rates from 0.5 to 50 mGy/h, while others displayed nonlinear patterns especially around 0.5 mGy/h. These results suggest that, in our study, a linear dose–response with irradiation is not a common feature to all genes and all dose rates. Rather, we observe that a specific set of genes responded linearly with dose rates between 0.5 and 50 mGy/h, but not at lower dose rates. Similar nonlinear response to gamma radiation has already been described in mouse and plants for cytogenetic damages like micronuclei and chromosomic aberrations [38].

## 2. Functional Analysis

Functional analysis made on the transcriptomic data showed a significant enrichment of genetic pathways involved in neuroectoderm and mesoderm development at 5 and 50 mGy/h. More specifically, molecular processes regulating neurogenesis (GO:0060322: head development, GO:0007399: nervous system development and GO:0030902: hindbrain development), somitogenesis (GO:0061053: somite development) and differentiation of blood cells (GO:0061515: myeloid cell differentiation and GO:0043249: erythrocyte differentiation) were significantly enriched at the two highest dose rates. However, these organs are not formed yet in shield embryos (6 hpf). The detailed analysis of the deregulated genes present in these GO terms, highlights the presence of many genes involved in Notch signalling (deltaB, her6, her12 and notch3), as well as morphogens (shha and bmp2b) and TF (sox2, six3b, otx2a and lft1). All these genes are known to be expressed during gastrulation but are also involved in organogenesis later in development (reviewed in [39,40,41]).

We thus interpreted these results as a deregulation of morphogenesis in the corresponding germ layers, neuroectoderm and mesoderm for the central nervous system and somite development, respectively. The deregulation of TF specifically expressed in the ectoderm (msx1a, otx2b, lft2 and her2) and in the mesoderm (tbx16l, fzd2, tbx6 and twist1a), during gastrulation, support the hypothesis that ectoderm and mesoderm were impacted at the molecular level by IR. How these molecular effects affect gastrulation or neurogenesis and somitogenesis at the cellular level was not investigated in the present study. And further studies are required to confirm the observed transcriptional changes at other levels. However, it was observed in our previous study (using the same experimental settings) that IR at 5 and 50 mGy/h altered the expression of genes involved in neurogenesis and somitogenesis in 24, 48 and 96 hpf zebrafish embryos and larvae, and these molecular effects were linked to neuromuscular impairments and larval motility defects in 5 days old larvae [25]. From these complementary sets of data, it can be proposed that IR at 5 and 50 mGy/h deregulates gastrula stage neuroectoderm and mesoderm morphogenesis, which might lead later in development to central nervous system and muscle impairments. Another study demonstrated neuromuscular impairment as well as a decrease of acetylcholinesterase expression during chronic exposure at similar dose rates, reinforcing prior results showing that IR at more than 5 mGy/h can lead to neuromuscular disorders [42]. All these results indicate that the developing central nervous system seems particularly vulnerable to stress and DNA damage [11,43]. In line with this observation, epidemiological studies conducted on Hiroshima and Nagasaki survivors demonstrated brain development defects and reduced cognitive performance in foetus exposed in uterus at doses higher than 0.31 Gy [44,45,46]. Likewise, recent field studies showed similar effects on wild animals, as decreased brain and body size were reported in monkey foetuses obtained in Fukushima Prefecture [47] and brain size of young birds living in the Chernobyl exclusion zone were also found to be smaller than in control area [48]. Taken together, our results indicate that the functional defects in neurogenesis and muscle development observed at 24 hpf and up to 5 days post fertilisation, might have their root, at least in part, in early developmental perturbations in the morphogenesis of the neuroectoderm and the mesoderm.

## 3. Development

In a recent study, Hurem et al. [49] described the effects of IR (at dose rates higher than 0.54 mGy/h) on zebrafish gastrulation. The authors described a deregulation of RA and Notch signalling, as well as other important developmental genes like vegfab, apoA1b, sox2 and vox. In addition, the authors used the Ingenuity software (QIAGEN, Inc., California, USA https://targetexplorer.ingenuity.com/) to describe potential upstream regulators (myc, tp53, tnf and hnf4a) and potential disease networks (brain malformation, growth failure, necrosis and hypoplasia of organs). If the effect on important developmental pathways (in particular RA and Notch signalling) was clear, the authors did not describe how IR can alter the morphogenesis of the three germ layers. In addition, the relationship between upstream regulators and downstream effects did not reveal any clear hypothesis on how the TF expressed during gastrulation were altered and how these effects could translate to detrimental effects at later stages (besides the potential role of myc in the induction of tumorigenesis and tp53 induction of apoptosis). Presumably, the usage of the Ingenuity software precluded such analysis, as its knowledge-base is using annotations from human, rat and mouse [50] but not zebrafish. In the present study, we confirmed the deregulation of Notch and RA signalling at 5 and 50 mGy/h. Furthermore, we found that other pathways such as Wnt and BMP signalling were also altered. The identification of DNA-binding site of key TF expressed in ectoderm and the mesoderm in the DEG promoter consolidates the hypothesis that exposure to IR at dose rates higher than 5 mGy/h deregulates morphogenesis during gastrulation, with potential harmful consequences on neurogenesis and somitogenesis later in development.

In addition to the effects on the transcriptome, our WBGS results demonstrated that embryonic exposure to IR at 5 and 50 mGy/h can alter DNA methylation patterns outside, but also in the vicinity of TSS. Between 20% and 25% of the DMR were located in promoter regions (distance < 3 kb from TSS), while 60% to 70% of the DMR were located inside intronic or intergenic regions. Importantly, the functional annotation of differentially methylated promoters (DMR located at < 3kb from TSS) showed an

enrichment of genes involved in embryonic development, neurogenesis and somitogenesis. These results correlate nicely with the transcriptomic data and collectively point towards a deregulation of genes involved in the morphogenesis of ectoderm and mesoderm upon exposure to 5 and 50 mGy/h. Mechanistically, these data suggest that IR could lead to changes in promoter methylation at the origin of the modulation of gene activity we observed at the transcriptomics level. It was already shown that exposure to high doses of IR decreases the expression levels of DNMTs and MeCP2 leading to global DNA hypomethylation [20,51]. If such mechanisms occur during embryonic development for low doses of IR remains to be confirmed, but no changes in DNMT expression was detected in our data (data not shown). How IR induces promoter hypomethylation during embryogenesis remains to be deciphered, as well as whether these methylation changes modify gene activity directly or not.

The distinction between hypo- and hypermethylated regions showed that most DNA methylation changes in promoters and in CGIs corresponded to a hypomethylation of these DNA regions. Previous studies demonstrated that demethylation of promoters positively modulate transcription [52]. By crossing the WGBS data with the expression data obtained by RNA-seq, we detected that hypomethylated DMR located at less than 500 bp from TSS were linked with transcriptional activation in about half of the cases, the other half of the genes being downregulated after exposure compared to controls. In addition, both H3K4me3 activating and H3K27me3 repressing marks were found in the vicinity of the hypomethylated DMR. Even if developmental genes can be poised for activation and thus harbour both H3K4me3 and H3K27me3 marks [53], these results show that DMR can be located in functional gene promoter. But establishing a clear link between DNA hypomethylation and gene activation remains difficult, as other factors (histone code, nucleosome assembly and TF-binding sites occupancy) affect also the chromatin state. Thus, the interaction of DMR with histone marks remains to be determined, for instance, by producing ChIP against H3K4me3 and H3K27me3 on irradiated embryos. In addition, functional studies on particular target genes will help deciphering the causal relationship between methylation changes, transcriptional regulation and adverse outcomes.

Hypomethylation of DNA has already been observed in previous studies after acute exposure to high dose of IR (0.5 to 5 Gy) either in cell lines [54] or in rodents [55,56,57]. Global DNA hypomethylation can lead to genomic instability [58,59] and the reactivation of transposable elements. We did not observe any changes in global methylation levels, nor in LINE elements or other transposable elements in the zebrafish genome, which suggest that the highest total dose rate used in this study (50 mGy/h during 6 h, i.e., total dose of 300 mGy) is not sufficient to cause genomic instability. Rather, our results indicate that the DMR associated with a role in gene regulation could be part of the stress response induced by IR. A recent study on zebrafish analysed the effects of a 27 days parental exposure at 8.7 mGy/h on DNA methylation changes in nonexposed F1 embryos at 50% of epiboly (5.5 hpf) [60]. Kamstra et al. found 5658 DMRs, predominantly located at regulatory regions (promoters and enhancers), and did not observe differences in the number of hypo- and hypermethylated DMR. As a comparison, we found 1858 and 1208 DMRs after exposure to 5 and 50 mGy/h, respectively. Despite differences in the exposure scenario (parental compared to direct exposure of embryos), these results suggest that longer exposure to IR (27 days compared to the 6 h used in our study) induce more effect on DNA methylation. DMRs and DEGs analyses on the F1-derived progeny pointed to an alteration of axonal guidance signalling. If axonal guidance is not a biological process ongoing in gastrulating embryos, these results can suggest an impairment of neurogenesis later in development (as proposed by the authors), which is in accordance with our present study where fertilised eggs were directly exposed to IR. These results raise the possibility that modification of DNA methylation patterns induced by IR can occur more frequently in the vicinity of developmental genes that could constitute IR-sensitive hotspots.

Our transcriptomic analysis detected potential effects of IR on mitochondrial energetic metabolism at dose rates higher than 0.5 mGy/h, indicating that this organelle could be sensitive to IR. Previous studies detected effects on mitochondrial activity for high doses (> 0.1 Gy) [61]. Our data show that mitochondrial activity could be altered at low dose rates (< 6 mGy/h). The fact that mitochondria possess less efficient DNA repair mechanisms compared to the nuclear genome [62] could, at least in part, explain

our observation. Interestingly, mitigation of oxidative stress and DNA methylation share one biomolecule in their biological pathway, i.e., the S-adenosylmethionine (SAM). Indeed, the intracellular oxidative stress can be reduced in the cells by the synthesis of glutathione, a potent antioxidative molecule [63]. The limiting substrate for glutathione biosynthesis is the cysteine, which is itself synthesised from methionine via transsulphuration [64]. During glutathione synthesis, the methionine is converted into cysteine through reactions that involve SAM. As SAM is also the substrate used by DNMT enzyme to methylate DNA, a competition between DNA methylation and glutathione synthesis can occur in case of limited SAM bioavailability [65]. Thus, the redox status in the cell has an influence on SAM usage and may impact DNA methylation [66]. IR is known to increase the production of reactive oxygen species (ROS) [42] and produce DNA damages even in early gastrula embryos [67]. In the case of prolonged exposure to oxidative stress, glutathione stock can be depleted to protect cell from oxidative stress, which can result in an impairment of DNA methylation [68,69,70]. In our study, we found an impact on genes involved in oxidative stress and in mitochondrial redox processes at all dose rates but especially at dose rates higher than or equal to 5 mGy/h, suggesting that the redox balance during embryogenesis can be modified during exposure to IR. We found, for instance, that gpx4a and gbx4b expression, involved in the reduction of hydrogen peroxide, is increased at 5 and 50 mGy/h, as well as duox, bco2l and nox1 at 50 mGy/h, which are involved in oxidative stress protection. It is thus possible that the increases of oxidative stress after exposure to IR can lead to partial depletion the SAM stock, at the expense of DNA methylation. However, such scenario would lead to global DNA hypomethylation of the genome, which is not observed in our data, but for much higher doses of IR [54,55,56,57,71]. More likely, our data points towards changes in mitochondrial activity as part of the stress response induced by IR, but more data on mitochondrial activity after exposure to low/moderate dose rates of IR (> 0.5 mGy/h) are needed to answer this question.

Our study explored the effects of low to high dose of IR on early embryonic development of zebrafish. Promoter hypomethylation at 5 and 50 mGy/h was associated with significant modulation on gene expression highlighting changes in the expression of gene involved in germ layer development. This observation underlines the role of the epigenetic mechanisms in the understanding of the effects caused by IR. Concordantly, many important developmental pathways like RA, BMP and Wnt signalling were impacted at the transcriptional level at 5 and 50 mGy/h, while few effects were detected at lower dose rates (0.5, 0.05 and 0.005 mGy/h). The transition between the effects induced by low and high dose rates seems thus to be located between 0.5 and 5 mGy/h during embryogenesis (total dose between 3 and 30 mGy, respectively). This is less than the proposed low dose and low dose rate limits defined so far in human (100 and 6 mGy/h [12]), but in the range of the expected dose rates giving rise to observable effects in the fish (0.4 to 4 mGy/h). In addition, we showed that transcription of genes involved in mitochondrial physiology was impacted at dose rates > 0.5 mGy/h. Taken together, our data suggest that the early developmental perturbations in the morphogenesis of the neuroectoderm and the mesoderm might predict the functional defects in neurogenesis and muscle development observed at later stages.

**Keywords**

DNA damages, gastrulation, development

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