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Irradiation of rainbow trout at early life stages results in a proteomic legacy in adult gills. Part B; the effect of a second radiation dose, after one year, on the proteomic responses in the irradiated and non-irradiated bystander fish

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ABSTRACT

This study extends the investigation of the legacy effects of exposure to a single radiation dose at one of four early life stages, in adult rainbow trout (Part A), by examining the effects of a second identical dose after one year; i.e. egg 48 h after fertilisation (48 h egg) + 1 year, eyed egg + 1 year, yolk sac larvae (YSL) + 1 year and first feeder + 1 year. This included the induction of a bystander effect in non-irradiated trout which had swam with the irradiated fish. The second radiation dose negated any beneficial proteomic responses following early life stage irradiation only, particularly irradiation of 48 h eggs and eyed eggs (Part A). Instead the responses after early life stage + 1 year irradiation are consistently associated with tumorigenesis, cancer progression, or are otherwise damaging: upregulation of alpha-globin 1 (YSL + 1 year and first feeders + 1 year) and downregulation of histone H1, type II keratin, malate dehydrogenase 2–2, Na/K ATPase alpha subunit isoform 1b, nucleoside diphosphate kinase (48 h egg + 1 year), electron transfer flavoprotein subunit alpha (eyed egg + 1 year), 60 S ribosomal protein L30 (YSL + 1 year) and haemoglobin subunit beta-4 (first feeder + 1 year). Most significantly the second radiation dose also negated the overwhelmingly beneficial bystander effect proteomic responses induced by trout irradiated at an early life stage only (Part A). Instead the bystander effect proteomic changes induced by trout irradiated at an early life stage and again at 1 year have been associated with uncertain, with respect to tumorigenesis, or detrimental effects; upregulation of alpha-globin 1 (YSL + 1 year and first feeder + 1 year) and downregulation of malate dehydrogenase 2–2, nucleoside diphosphate kinase (48 h egg + 1 year), transferrin precursor (eyed egg + 1 year), 60 S ribosomal protein L30 (YSL + 1 year) and serine / threonine-protein phosphatase 2 A 65 kDa (first feeder + 1 year). This difference between the bystander effect induced proteomic changes following early life stage irradiation only and early life stage + 1 year irradiation may indicate a fundamental change in the non-targeted effects of radiation.

1. Introduction

Clearly exposure to multiple or to variable (i.e. chronic and acute) radiation doses is clearly a real possibility. In medaka (*Oryzias latipes*) continual chronic radiation exposure modifies both the response to an acute radiation dose as well as the nature of the bystander effect (reviewed by Mothersill and Seymour, 2001 and refer to Part A) induced, by the irradiated fish, in non-irradiated fish (Smith et al., 2011). Similarly the long term (two year) legacy of rainbow trout, which had been irradiated either as eggs at 48 h after fertilisation, eyed eggs, yolk sac larvae (YSL) or first feeders, was shown to be modified by exposure to a second identical radiation dose at one year (Mothersill et al., 2010). Again this also included modifications to the bystander effect induced, by the irradiated trout, in non-irradiated fish (Mothersill et al., 2010).

The studies mentioned above employed a sensitive reporter cell line (HPV-G; refer to Mothersill et al., 2006) to show how multiple radiation doses induce the production of pro-death or growth promoting factors in the irradiated and bystander fish. However there has been no attempt to use proteomics to identify specific molecular responses to these more complex radiation exposure regimens.

Therefore the aim of this study was an extension of the experiment described in the accompanying Part A of this investigation. The gill proteomes of two year old rainbow trout, which had been irradiated at one of the four early life stages listed above and again at one year, and

Abbreviations: HPV-G, human papilloma virus – G; LCMS, liquid chromatography–mass spectrometry; PPP2R2A, serine / threonine protein phosphatase 2A; YSL, yolk sac larvae

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their bystander fish, were examined to determine if exposure to a second radiation dose, at one year, resulted in different proteomic responses to those induced by early like stage irradiation only, as described in Part A.

2. Materials and methods

2.1. Irradiation, bystander effect induction and fish gill sample collection

Experimental groups of rainbow trout were generated which had been irradiated firstly, either as eggs at 48 h after fertilisation, or eyed eggs, or YSL or first feeders and then irradiated again as one year old fish. At two years old these irradiated trout were paired with non-irradiated two year old bystander fish and gill samples from both the irradiated and bystander fish were collected, as described by Mothersill et al. (2010) and in Part A, *2.1. Irradiation, bystander effect induction and fish gill sample collection*.

Thus the gill lamellae samples involved in this investigation were taken from the same individual fish which had already shown a response to a combination of early life stage irradiation and exposure to a second radiation dose at one year, and from the same individual non-irradiated bystander fish which had swam with these irradiated fish (Mothersill et al., 2010).

2.2. Proteomic analysis; protein digestion and mass spectrometry

Sample preparation, protein digestion, liquid chromatography–mass spectrometry (LCMS) and statistical analysis and presentation of the data was carried out in the same manner as previously described (refer to Part A; *2.2. Proteomic analysis; protein digestion and mass spectrometry*).

3. Results and discussion

3.1. Protein identification and quantitative expression and statistical analysis

Information on peptide coverage and identification significance, are submitted as Supplementary Files; B1 = fish irradiated as eggs at 48 h after fertilisation and again at one year, B2 = fish irradiated as eyed eggs and again at one year, B3 = fish irradiated as YSL and again at one year, B4 = fish irradiated as first feeders and again at one year. These data were collected from n = 11 completely untreated control fish, n = 5 irradiated fish from each irradiated group and n = 5 bystander fish from each irradiated / bystander pairing.

As was the case with Part A of this study the analysis described here was also carried out as a "blind study". LCMS peak area data was only combined into treatment groups once all the samples had been analysed and protein expression was described as "fold-changes" (refer to Part A, *3.1. Protein identification, quantitative expression and statistical analysis*). The fold-changes in the expression of all the detected proteins are given in Supplementary Table B5. This table includes all the detected proteins irrespective of whether they were detected in all the gill samples (i.e. consistently expressed) or only detected in a portion of the samples. The table also includes those proteins which have not yet been named but which have been assigned a NCBI accession number. Again it is recognised that biological and statistical significance are not necessarily the same. However consistently expressed proteins were statistically analysed as described in Part A *3.1. Protein identification, quantitative expression and statistical analysis*.

Figs. 1–4 illustrate the proteomic changes in the gills of two year old rainbow trout irradiated, with a single 0.5 Gy X-ray dose as eggs at 48 h after fertilisation and one year (Fig. 1), eyed eggs and one year (Fig. 2), YSL and one year (Fig. 3) and first feeders and one year (Fig. 4), and also the proteomic changes induced, by the bystander effect, in the gills of non-irradiated adult trout which had been swimming with these

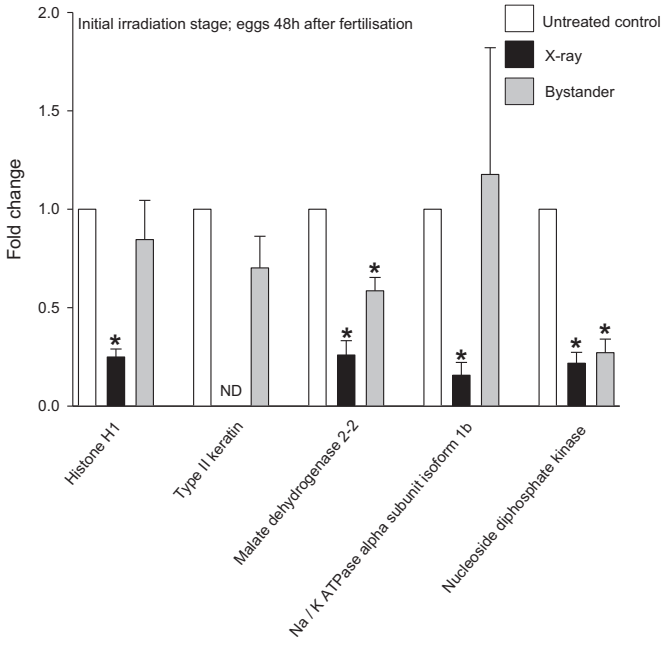

Fig. 1. Proteomic changes in 2 year old adult trout irradiated with a single 0.5 Gy X-ray dose as eggs at 48 h after fertilisation and again at 1 year (black bars) and 2 year old non-irradiated trout which had swam with the irradiated fish (grey bars). * = statistically significant change in protein expression relative to completely untreated control trout ($P < 0.05$). ND = not detected in any sample. NCBI database accession numbers: Histone H1 = gi|121951, Type II keratin = gi|185132221, Malate dehydrogenase 2–2 = gi|213514494, Na/K ATPase alpha subunit isoform 1b = gi|185135314, Nucleoside diphosphate kinase = gi|185135416.

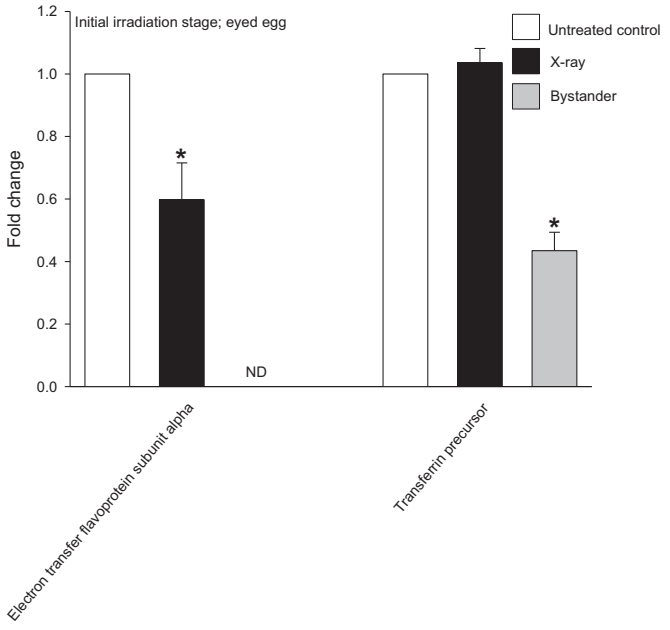

Fig. 2. Proteomic changes in 2 year old adult trout irradiated with a single 0.5 X-ray dose as eyed eggs and again at 1 year (black bars) and 2 year old non-irradiated trout which had swam with the irradiated fish (grey bars). * = statistically significant change in protein expression relative to completely untreated control trout ($P < .05$). ND = not detected in any sample. NCBI database accession numbers: Electron transfer flavoprotein subunit alpha = gi|3870985, Transferrin receptor = gi|218931236.