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Abstract

The lens of the eye is known to be more radiosensitive than previously thought but, despite a substantial reduction in occupational dose limits based on recent epidemiological information and reanalyses, the mechanisms of low dose radiation cataract induction are still unclear. This is an important current public health issue, for instance for medical radiation workers, many of whom will need to amend their working practices despite a clear understanding of the underlying process and ultimate effects of chronic, low dose, ionising radiation exposure.

The LDLensRad project aims to bring together experts from across Europe to answer a number of key research questions on this topic, including: how does low dose radiation cause cataracts; is there a dose rate effect, and how does genetic background influence cataract development after radiation exposure. CONCERT Deliverable 9.44, 1.1.1 of the project, describes progress to date in the molecular studies focused on establishing the role of non-coding miRNAs in radiation-induced cataract development through miRNome analysis based on next generation sequencing (NGS) performed at ENEA with *Ptch1*$^{+/-}$ mouse lenses (thus far) used in the study.

The analysis to date clearly suggests that genetic background has an influence on deregulated miRNAs, particularly in 2 Gy-irradiated *Ptch1*$^{+/-}$ mouse lenses, regardless of dose rate. These data strongly support the key role of genetic background in the control of lens opacity through miRNAs regulation.

Progress summary

1 INTRODUCTION

MicroRNAs (miRNAs) are short segments (19–25 nucleotides) of nonprotein-coding single-stranded RNA that interfere with target gene expression acting primarily at the post-translational level. miRNAs control a wide variety of cellular functions such as apoptosis, cell proliferation, differentiation, metabolism, stem cell renewal and stress response.

It is now well accepted that a single miRNA has the potential to mediate translation of hundreds of targets and, conversely, several miRNAs can regulate the expression of one gene. While most of the miRNA studies are related to cancers, accumulating evidence has demonstrated that miRNAs are also involved in the pathogenesis of ocular diseases, including cataract.

In order to establish the role of non-coding miRNAs in radiation-induced cataract development, miRNome analysis -based on next generation sequencing (NGS) has been performed at ENEA.

2 METHODS

Ptch1$^{+/-}$ mice, a relevant mouse model of radiation-induced cataract, bred on two different genetic backgrounds (CD1 and C57Bl/6J), were whole-body irradiated with 2 Gy of γ-rays (^{60}Co) at 10 weeks of age or left untreated. WT mice of both strains were also included in the study. Radiation doses were delivered with two dose rates, *i.e.* 0.3 or 0.063 Gy/min.

Four and 24 hours after irradiation, lenses were collected and total RNA was extracted using miRNeasy kit according to the manufacturer's instructions. Total RNA (1μg) was converted into miRNA NGS libraries using NEBNEXT library generation kit following manufacturer's instructions. Samples were sequenced on the Illumina NextSeq 500 System. All sequencing data analysis was performed using the R platform (http://www.r-project.org/) and the open-source Bioconductor libraries. Data were filtered based on sequence counts (i.e. > 8 reads per million in at least six samples) and pairwise comparisons of differential miRNA expression were performed using the edgeR package. All miRNAs with P <0.05 were considered.

The selected lists of statistically significant miRNAs were analyzed in Cytoscape (Version 3.6.1) through the application ClueGo (V. 2.5.1) and CluePedia (V. 1.5.1). The initial sets of miRNAs were enriched based on the miRTarBase database in order to obtain the top20 predicted target genes for each miRNA. The obtained networks of genes and miRNAs were then analyzed based on the Reactome pathways database to identify the relevant pathways and functions potentially perturbed by the altered miRNAs.

3 RESULTS

As a first step, a pilot study focused on selecting the most informative time point to perform miRNome analysis at short term after irradiation was carried out. For this aim, lenses from CD1 mice were collected 4 and 24 hrs. after irradiation with 2 Gy of γ-rays (dose rate 0.3 Gy/min). Because at 4 hrs post-irradiation, pathway analysis of deregulated miRNAs revealed that the predominant affected functions converge on DNA repair machinery, the time point 24 h post-irradiation has been selected to extend the analysis for all experimental groups (CD1-*Ptch1*$^{+/-}$, C57Bl6-*Ptch1*$^{+/-}$ and their WT counterparts).

The NGS profiling was successfully completed and bioinformatics analysis has been carried out using miRNAs obtained from CD1-*Ptch1*$^{+/-}$ and C57Bl6-*Ptch1*$^{+/-}$ mouse lenses (Fig. 1). A similar number of significantly deregulated miRNAs was found in CD1-*Ptch1*$^{+/-}$ mouse lenses irradiated with both dose

rates when compared to the unirradiated condition (40 and 41, respectively), with 18 up-regulated miRNAs in common between groups. In C57Bl6-*Ptch1*$^{+/-}$ mouse lenses, 24 significantly deregulated miRNAs were detected at 0.3 Gy/min and 37 at 0.063 Gy/min, with only 11 commonly deregulated miRNAs (5 of which were up-regulated and 6 down-regulated).

Moreover, the final comparison of the common deregulated miRNAs (intersection) showed only 3 matching miRNAs, 1 of which was deregulated in the same direction and 2 were contra-regulated. The nearly complete lack of overlap of miRNAs expression profiles in irradiated CD1-*Ptch1*$^{+/-}$ and C57Bl6-*Ptch1*$^{+/-}$ suggests a strong genetic background-dependence of miRNAs radiation response in the lens.

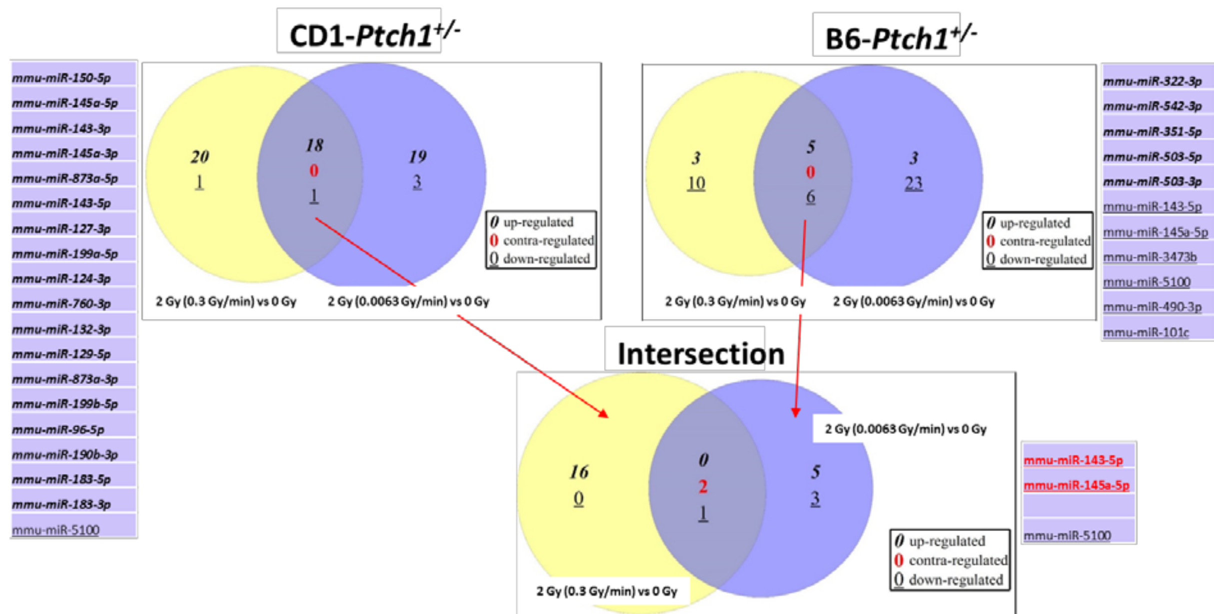

Fig. 1. Venn diagrams of statistically significant deregulated miRNAs.

As shown in Fig. 2A, pathway analysis, predicted by commonly deregulated miRNAs, after irradiation of CD1-*Ptch1*$^{+/-}$ lenses with both dose rate, highlighted altered functions related to SCF-KIT signaling and the regulation of Toll Like Receptors (TLRs). In B6-*Ptch1*$^{+/-}$ mouse lenses different altered functions have been predicted after pathway analysis, suggesting a strong genetic background influence (Fig. 2B).

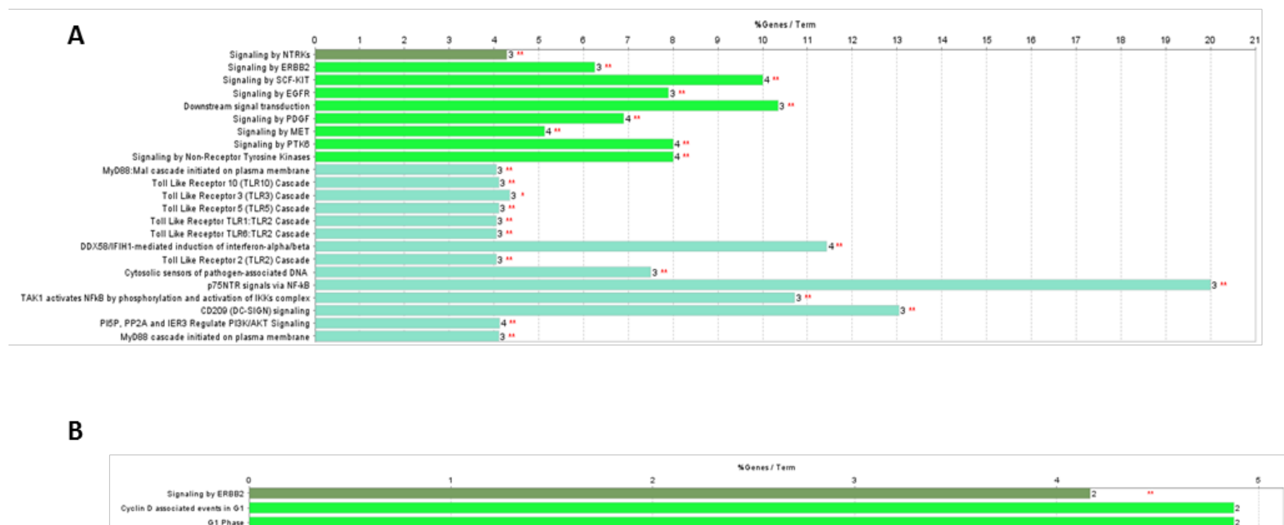

Fig. 2. Pathways deregulated by miRNAs obtained from CD1-*Ptch1*$^{+/-}$ (A) and B6-*Ptch1*$^{+/-}$ (B) lenses and in common after irradiation with both dose rates.

4 DISCUSSION

NGS and bioinformatics analysis reveal that genetic background produce significant differences in the deregulated miRNA lists and in the predicted perturbed biological functions of 2 Gy-irradiated Ptch1+/- mouse lenses, regardless of dose rate.

These data strongly support the key role of genetic background in the control of lens opacity through miRNAs regulation. Validation with qPCR of deregulated miRNAs as well as analysis of perturbed pathways by IHC is ongoing.