EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office via the User Portal:
https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Deadlines for submission of Experimental Reports

Experimental reports must be submitted within the period of 3 months after the end of the experiment.

Experiment Report supporting a new proposal ("relevant report")

If you are submitting a proposal for a new project, or to continue a project for which you have previously been allocated beam time, you must submit a report on each of your previous measurement(s):

- even on those carried out close to the proposal submission deadline (it can be a *"preliminary report"),*
- even for experiments whose scientific area is different form the scientific area of the new proposal,
- carried out on CRG beamlines.

You must then register the report(s) as "relevant report(s)" in the new application form for beam time.

Deadlines for submitting a report supporting a new proposal

- 1st March Proposal Round - **5th March**
- 10th September Proposal Round - **13th September**

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report in English.
- include the experiment number to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

	Experiment title:	Experiment number:
	Brain tumor irradiative therapies and related mRNA and miRNA: a window on potential differential genic and epigenetic responses to MRT	MD-1073
Beamline: ID17	**Date of experiment**: 26 January 2018 / 27 January 2018	**Date of report**: 05/03/2020
Shifts: 6	**Local contact(s)**: Hervig Requardt	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The working hypothesis of the present project is based on an already published data (Bouchet *et al.*, 2013). In this paper the authors matched the gene expression profile of normal brain, before and after MRT radiation (400Gy, 50 micron wide, 211 micron center to center; 10Gy dose valley), and of brain injected with 9L glioblastoma tumor cells, at the level of caudatum nucleus, before and after radiation. The comparison between brain sample before and after MRT revealed how many and which genes are differentially expressed due to MRT in the normal tissue (DEGs brain + MRT, DEGs: Differential Expressed Genes); the comparison between glioblastoma cells before and after MRT revealed how many and which genes of the glioblastoma are differentially expressed and are affected by MRT (DEGs 9L + MRT).

Here, we considered those genes of the glioblastoma which are affected by MRT radiation (n=16 DEGs 9L+MRT). We found that three of these 16 genes are connected by physical interaction, namely PLK1, CCNB1 and CDC20. Looking for validated miRNAs able to control these 16 DEGs, we found 44 miRNAs regulating 8 DEGs of the 16 DEGs 9L+MRT. Three miRNAs of the 44 (namely, miR-215-5p, miR-192-5p and miR-335-5p) have a higher degree centrality (HDC), being degree centrality the capability of a miRNA to regulate more than one target (Cava *et al.*, 2018); these three miRNAs are indeed able to control 3 DEGs, as depicted in the figure 1. Further investigation are required to:

1. validate the identified miRNAs in glioblastoma tumors before and after MRT, in order to understand if they are possible 'responders' to MRT
2. understand the role of these miRNAs within the selected network
3. understand which pathways could be involved in MRT effect in glioblastoma tumors.

Validation procedure

Total RNA of tissue samples (n=26) will be isolated using Trireagent reagent (Sigma) following the manufacturer's recommendations. For serum samples (n=26), miRNeasy Serum/Plasma Kit will be used following manufacturer's recommendation. For miRNA quantification, the obtained RNA will be reverse transcribed using MystiCq microRNA cDNA synthesis kit (Sigma Aldrich), following manufacturer's recommendations. miRNAs will be amplified in Eco real time-PCR (RT-PCR) (Illumina, Euroclone) using Power Up Sybr green mix (Applied Biosystem) in combination with homemade designed primers. miRNAs will be selected by *in silico* analysis of public databases (i.e. GSE50478) containing information on gene expression profile of normal brain before and after MRT radiation and of brain injected with 9L glioblastoma tumor cells, at the level of caudatum nucleus, before and after radiation. miRNAs able to control differentially expressed genes of glioblastoma as a response to MRT treatment will be selected and analysed in our samples by RT-PCR.

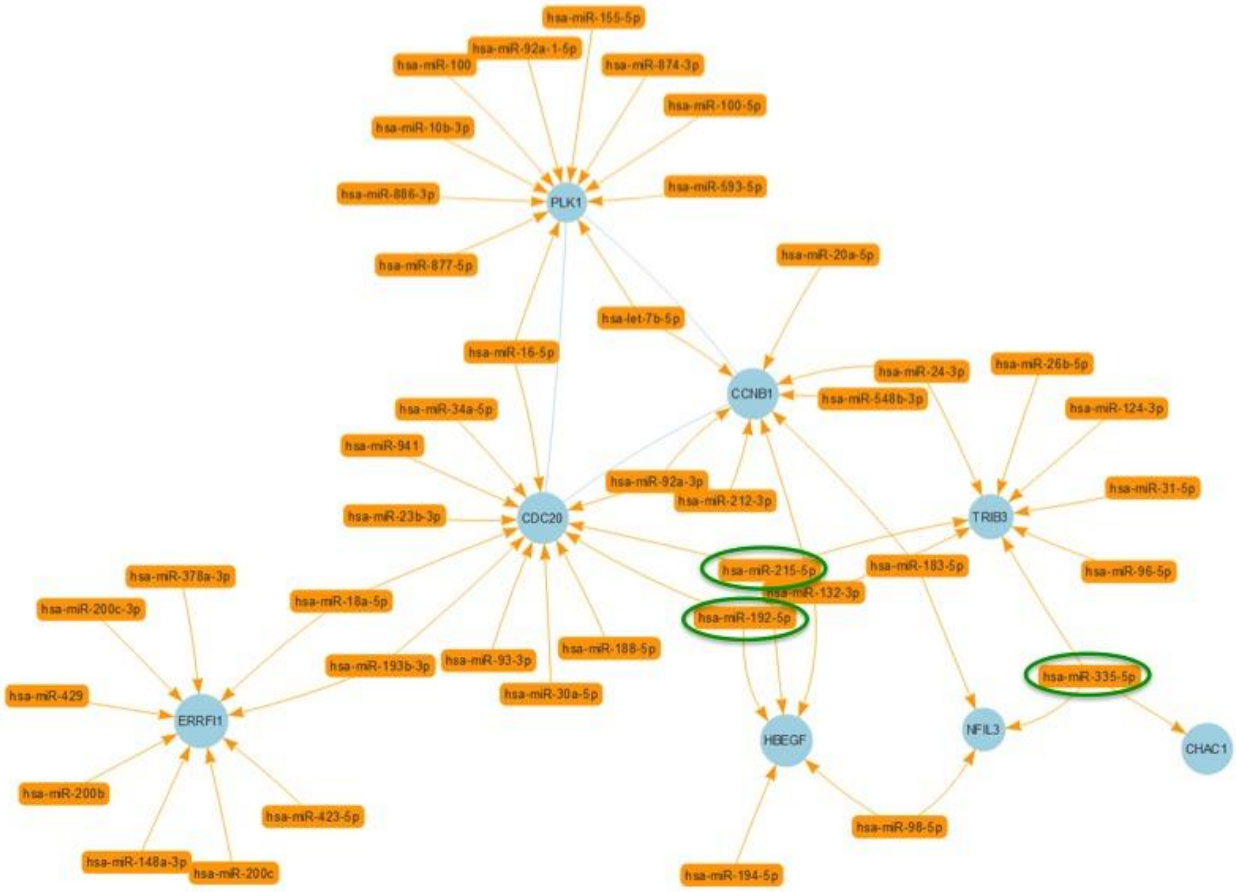

Figure 1: schematic representation of the network created between 8 connected DEGs, altered by MRT in glioblastoma tumors, and their related miRNAs, regulating those DEGs. In green, miRNAs with higher degree centrality (HDC) are highlighted.

References

1. Bouchet A, Sakakini N, El Atifi M, Le Clec'h C, Brauer E, Moisan A, et al. Early gene expression analysis in 9L orthotopic tumor-bearing rats identifies immune modulation in molecular response to synchrotron microbeam radiation therapy. PLoS One. 2013;8(12):e81874.

2. Cava C, Bertoli G, Colaprico A, Olsen C, Bontempi G, Castiglioni I. Integration of multiple networks and pathways identifies cancer driver genes in pan-cancer analysis. BMC Genomics. 2018 Jan 6;19(1):25. doi: 10.1186/s12864-017-4423-x.