DOTTORATO DI RICERCA IN BIOLOGIA CELLULARE E DELLO SVILUPPO

38 CYCLE

Project proposal for a Sapienza PhD scholarship

Title:
Role of protein isoforms of class 5 histone demethylase (KDM5) in transcription regulation, DNA repair and oncogenesis

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Summary (max 300 words)

KDM5 histone demethylases are involved in development and differentiation processes, in DNA damage repair, in gene expression regulation, in cancerogenesis and drug resistance. KDM5B is up regulated in many types of cancer but it may have a different function in different types of cancer. Until now remains unclear what contextual determinants dictate whether KDM5B protein function as oncogene or tumor suppressor. Recently it has been proposed that the relative abundance of different KDM5B isoforms may contribute to tumor progression in melanoma, but the significance of the alternative transcripts of PLU-1 remains unclear. We previously showed that a predicted protein isoform exists in different cancer cell lines which is of particular interest. In fact, this isoform, lacking an N-terminal portion, may act as a negative dominant, maintaining the ability to bind chromatin and coregulators but losing its catalytic demethylase function. At the same time, it is possible that this isoform may perform a new function, since it includes an alternative exon that is not present in the canonical protein isoform, by giving the protein the ability to bind new interactors. Therefore, the understanding of the biological contribution of this and other protein predicted isoforms for tumor progression and chemoresistance in breast cancer and melanoma may be of crucial importance.

Pertinent Publications of the proponent (last 5 years)

- DI NISIO E, LUPO G, **LICURSI V**, NEGRI R (2021). The Role of Histone Lysine Methylation in the Response of Mammalian Cells to Ionizing Radiation. ***Frontiers in Genetics***, 12: 1664-8021.

- PIPPA S, MANNIRONI C, **LICURSI V**, BOMBARDI L, COLOTTI G, CUNDARI E, MOLLICA A, COLUCCIA A, NACCARATO V, LA REGINA G, SILVESTRI R, NEGRI R (2019). Small molecule inhibitors of KDM5 histone demethylases increase the radiosensitivity of breast cancer cells overexpressing Jarid1b. ***MOLECULES*** 24: PII: E1739

- MOCAVINI I, PIPPA S, **LICURSI V**, PACI P, TRISCIUOGLIO D, MANNIRONI C, PRESUTTI C, NEGRI, R (2018). JARID1B expression and its function in DNA damage repair are tightly regulated by miRNAs in breast cancer. ***Cancer Science*** 110:1232-1243.

DESCRIPTION OF THE RESEARCH

Research objectives

In this program we plan to overexpress the truncated isoform in breast cancer cell lines and to analyze its functional effects on bulk chromatin histone methylation, transcription regulation and DNA repair. We will also investigate on the existence and functions of putative isoforms of KDM5A. Finally, the role of the unique KDM5 demethylase JHD2 in DNA repair will be addressed.

Preliminary plan of activities:

1. KDM5B isoforms characterization by Western Blot, IP and mass spec to evaluate the effective presence of different gene products and their relative amount. We already performed a Western blot analysis in three human breast cancer cell lines, two luminal types such as MCF-7 and T47D, and one basal type such as MDA-MB-231. The analysis will now be extended also to the SK-MEL-28 and to other cell lines of human melanoma. We will then proceed to the analysis of patients' biopsies in search of a correlation between the PLU1/NTT isoforms ratio with the role of KDM5B in oncogenesis. The analysis will be performed using two different antibodies. One of these recognizes an epitope in C-terminal portion of all isoforms, the other one recognizes specifically the region of alternative exon-6. Moreover, we will do immuno-precipitation (IP) experiments followed by SDS- PAGE and proteomic analysis to validate the existence of the two expected native protein isoforms.
2. Searching interactors and PTMs of the KDM5B isoforms by protein-tagging, IP and mass spec. After featuring the KDM5B native isoforms, it will be interesting to find their functional interactors. Using recombinant proteins with a protein tag, we will immune precipitate the tagged isoforms followed by to perform a mass spec analysis to find the interactors and the post-translational modifications of each isoform in the different cell lines.
3. Cellular localization of KDM5B isoforms by cell fractionation followed by western blot and immunofluorescence analysis to verify whether the truncation and/or inclusion of the exon-6 alters the nuclear localization of the protein in the different cell lines.
4. Analysis of the effects of NTT overexpression on H3K4 methylation levels of bulk chromatin. For transiently overexpressing NTT, we will transfect MCF7 and MDA-MB-231 with a construct which carries the relative transcript downstream of a constitutive promoter. Quantitative evaulation of H3K4me1,2,3 levels will be performed on cells untreated or transfected with empty vector or with the NTT expressing construct, respectively by quantitative Mass -spectrometry (MS) analysis.
5. Transcriptomic analysis of cells untreated or transfected with empty vector or with the NTT expressing construct. The analysis will be performed by RNA-seq.
6. Genome wide analysis of H3K4 trimethylation cells untreated or transfected with empty vector or with the NTT expressing construct. The analysis will be performed by CUT&RUN assay followed by sequencing.
7. Analysis of radio- and chemo-resistance of MCF7 cells transfected with empty vector or with the NTT expressing construct.
8. Analysis of radio- and chemo-resistance of MCF7 cells treated with catalytic inhibitors of KDM5 enzymes.
9. Bioinformatic analysis of the KDM5B isoforms expressed in different cancer cell lines
10. Define the function of S. cerevisiae H3K4 histone methylases and demethylases (Set1 and Jhd2) in DSB response (mutants and chemical inhibitors).