



MÁSTER EN INVESTIGACIÓN BIOMÉDICA

Research Project Proposal

Academic year 2022-2023

Project Nº 53 ASIGNADO

Title: A CRISPR/Cas9-based functional screen to identify KRAS-dependent genes sensitizing to radiotherapy

Department/ Laboratory

Oncogenes and Effector Targets (OnTarget) lab, Program in Solid Tumors (CIMA)

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Summary

KRAS is one of the most frequently found oncogene in human cancer and the largest mutated in lung cancer. Aberrant KRAS expression elicits an oncogenic signalling cascade involved in the transformation of epithelial cell which gives rise to the tumor phenotype. The oncogenic phenotype is tightly associated with the hallmarks of cancer. However, oncogenic mutations invariably lead to the development of a series of oncogene-induced stresses such as metabolic, proteotoxic, mitotic, oxidative, and DNA damage/replicative stress. Inhibition of genes sustaining such cellular stress may unveil vulnerabilities that could be exploited to identify novel therapeutic strategies.

Preliminary data from our lab has shown that pharmacological or genetic inhibition of KRAS downregulates the expression of multiple genes involved in the DNA damage response pathway that mutant KRAS cells utilize to cope with replicative stress. Furthermore, pharmacological KRAS inhibition using targeted therapies recently approved in the clinic extraordinarily sensitizes to a series of treatments that force tumor cells to rely on the DNA damage response pathway such as radiotherapy. To understand which KRAS-regulated genes are functionally involved in the sensitization of tumor cells to radiotherapy, we propose the development of a loss-of-function screen that will be followed by in vitro and in vivo follow up experiments.

GOALS

- To carry out an in vitro CRISPR/Cas9-based screen targeting a KRAS-regulated gene signature and KRAS canonical effectors in mutant KRAS lung cancer cells exposed to radiotherapy.
- To functionally validate in vitro the candidates identified in the loss-of-function screen across a panel of mutant KRAS lung cancer cell lines.
- To dissect the impact of validated candidates in vivo.

METHODOLOGY

We will integrate state-of-the-art approaches such as a CRISPR/Cas9 screen, in vitro assays (cytotoxicity, cell cycle, apoptosis, ...) and in vivo experiments (tumor growth, multiplex immunohistochemistry, multiparametric flow cytometry).

Table with 2 columns and 2 rows: yes, X, no

Does the project include the possibility of supervised animal manipulation to complete the training for animal manipulator?