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Attività scientifica svolta nel 1° anno di Dottorato
Anno Accademico 2014/2015

"Identification and validation of new BRAF^{V600E} functional interactors"

- Introduction

Metastatic melanoma remains one of the most therapeutically challenging malignancies. A frequently mutated gene in melanomas is BRAF, carrying mainly the mutation V600E. BRAF is a kinase part of the RAS/RAF/MEK/ERK mitogen activated protein kinase (MAPK) signal transduction pathway. The V600E mutation determines a conformational change responsible for a constitutive activation of the protein. BRAF^{V600E}-specific inhibitors have been shown to outperform conventional chemotherapeutic drugs. However, they are not immune of limitations, which need to be overcome by using drug cocktails.
The aim of this study is to assess the functionality of the human BRAF^{V600E} in yeast cells and then to use yeast as a model system to identify novel BRAF^{V600E} functional interactors that can be targeted for therapeutic purposes.

- Materials and methods

I have learned all the basic techniques necessary for yeast culturing and manipulation. Furthermore, I have cloned BRAF^{V600E} ORF in the pYES2 plasmid under the control of the galactose inducible promoter and I have generated HOG pathway deletion mutants.

- Results

Although yeast does not have a BRAF ortholog, it has the MEK counterpart Pbs2. This protein encodes for a key player in the MAPK pathway that mediates the response to osmostress (HOG pathway). By showing that hBRAF^{V600E} can rescue growth in yeast strains deleted for genes belonging to the HOG pathway, I was able to formally demonstrate that human BRAF is functional in yeast.
In order to identify BRAFV600E functional interactors, I am currently taking advantage of a genetic screening in the *S. cerevisiae* yeast deletion pool (YDP). Specifically, I am looking for the deletion mutants that show altered growth in the presence of BRAF^{V600E}. I will then evaluate which is the relationship between the proteins encoded by these mutants and BRAF^{V600E}, which is their relevance in human melanoma and whether they can represent new therapeutic targets to be used alone and/or in combination with BRAFi.

- **CONFERENCES**:

- EACR-AACR-SIC 2015 Special Conference *(Florence, 2015)*
Lubrano S., Cervelli T., Comelli L., Galli A., Poliseno L.
"Identification of novel functional interactors of BRAF^{V600E} through a genetic screening in yeast" [Poster session]

- 27th International Conference on Yeast Genetics and Molecular Biology *(Levico*

Terme, 2015)
Lubrano S., Comelli L., Galli A., Poliseno L. and Cervelli T.
"Role of the Oncogenic BRAFV600E in the Osmostress Response of Yeast *Saccharomyces Cerevisiae"* [Poster session]

- 19th International Fritz-Bender Foundation Symposium on CANCER BIOLOGY FOR CANCER THERAPEUTICS *(Pisa, 2015)*

- 10th Annual Scientific Conference of the Istituto Toscano Tumori (ITT) *(Pisa, 2015)*

- **COURSES**:

"START UP AND TECHNOLOGY TRANSFER" - University of Siena

- **PUBLICATIONS**:

Marranci A., Tuccoli A., Vitiello M., Mercoledi E., Sarti S., **Lubrano S**., Evangelista M., Fogli A., Valdes C., Russo F., Dal Monte M., Adelaide Caligo M.,Pellegrini M., Capobianco E., Tsinoremas N., Poliseno L.
"Identification of BRAF 3'UTR isoforms in melanoma"
Journal of Investigative Dermatology (2015) 135**,** 1694–1697 doi: 10.1038/jid.2015.47.