PROJECT 2 (optional)

DoS: Paolo Ghia

Title: **Deciphering the the role of EGR2 mutations in Chronic Lymphocytic Leukemia**

Curriculum: **Basic and Applied Immunology and oncology**

Link to OSR/UniSR personal page:
https://research.hsr.it/en/divisions/experimental-oncology/b-cell-neoplasia.html
https://www.unisr.it/docenti/g/ghia-paoloprospero

Project description (*Number of characters*, *including spaces*: 2.000 - 3.000):
Background and rationale

Chronic lymphocytic leukemia (CLL) is the most frequent adult leukemia in the West. No single common genetic lesions have been described in CLL, though recurrent mutations have been observed in a number of genes, each present in ≤10% of cases, often correlating with the clinical outcome of patients and response to therapies. Among these genes is EGR2, a transcription factor involved in the intracellular signalling cascade downstream of the B Cell Receptor. EGR2 mutations are associated with a shorter time to treatment and poor overall survival. We have also observed a higher frequency of EGR2 mutations in patients resistant to the BTK inhibitor Ibrutinib, and in particular in those patients carrying mutations also in the BTK gene, again in the BcR pathway. Recurrent mutations in EGR2 are located in one of the Zinc-finger domain of the protein, but studies conducted so far revealed that mutations modulate, rather than abrogate, EGR2 function. The functional relevance of EGR2 mutations in CLL is to date unexplored, but it is of utmost interest to understand at molecular level the mechanisms trough which mutated EGR2 is involved in more aggressive clinical behaviour and treatment resistance.

Specific aims and methodology

The aim of the project is to investigate the functional consequences of EGR2 mutations in CLL. In particular, we are planning to use CRISPR/Cas9 technology to model in the MEC1 CLL cell line the most recurrent mutations occurring in patients and utilize genome-wide analysis and **next-generation sequencing** technology, at both DNA and RNA level, to investigate:

- gene expression changes
- altered DNA-binding properties
- epigenetic profiles

We will also analyse *in vitro* the functional consequences of EGR2 manipulations on cell proliferation, survival, BcR signalling and response to drugs (***in vitro* models**), and investigate the *in vivo* relevance of mutated EGR2 in CLL by injecting immunodeficient Rag2-/-γc-/- mice with EGR2-mutated MEC1 cells. Tumor engraftment and dissemination in different organs will be evaluated by flow cytometry and immunohistochemistry as well as survival analyses will be performed.

Expected results

The molecular dissection of signalling pathways in CLL have helped to improve our understanding of the mechanisms acting in the pathogenesis of CLL and to design effective targeted therapies. Further definition of the mechanisms acting through the deregulation of EGR2 and its target genes will improve our knowledge on the complex interplay between genetic and microenvironmental elements in shaping the clonal behaviour as well as the clinical outcome.

Skills to be acquired by the student:

At the completion of the PhD project, the student will acquire deep knowledge of the molecular mechanisms responsible for the development of B cell lymphoproliferative disorders, practical expertise in cellular biology including handling, purification and sorting of primary human and murine normal and neoplastic cell, and in molecular biology, including CRISP/CAS technology.

References (max. 3)

1. Young E et al. Leukemia. 2017 Jul;31(7):1547-1554.