PROJECT 1

DoS: **Marta Muzio**

Title: Exploring NFKBIZ-centered pathways in normal and malignant B-cells

Curriculum: BAIO

Link to OSR personal page:

https://research.hsr.it/en/divisions/experimental-oncology/cell-signaling.html

Project description:

Toll-like receptors (TLR) are key regulators of innate immunity; moreover, they bridge the innate and the adaptive immune responses by acting as co-stimulatory signals for B-lymphocytes. TLR are expressed both by normal and malignant B cells with a certain degree of specificity; accordingly, they can modulate the activation of the NFKB network in **lymphoid malignancies** (1). However, the exact role of TLR signaling in B cell lymphoid tumors and chronic lymphocytic leukemia (CLL) deserves further investigation. We previously showed that TLR stimulation *in vitro* can activate several signaling pathways in malignant B-lymphocytes isolated from CLL or lymphoma patients and can protect cells from spontaneous and/or drug induced apoptosis (2,3). Nevertheless, heterogeneity of response to TLR stimulation was observed between normal and malignant B-cells, and among different CLL cases, but the molecular basis for this heterogeneity is ill defined. We recently characterized distinct microRNAs, cytokines and the **atypical NFKBIZ** (co)-transcription factor as markers of TLR activation in specific CLL cases (3,4). NFKBIZ has been primarily studied for its role in inflammation, but its function in the B-cell and tumor context is still elusive. The aim of this PhD project is to comprehensively characterize the molecular framework of TLR signaling molecules, with particular emphasis on the role of NFKBIZ, in normal and malignant B-cells.

To achieve this, we will:

1. Analyze how NFKBIZ is differentially expressed and regulated in normal and leukemic B-cells.

2. Dissect the molecular functions of NFKBIZ in B-cells, and their pathogenic effects in chronic lymphocytic leukemia, in the modulation of NFKB signaling pathways, gene expression and metabolic activation.

Molecular, genetic and biochemical techniques will be applied *in vitro* and *in vivo* in murine models.

Skills to be acquired by the student:

Cellular and molecular biology techniques including genomics and transcriptomics (gene editing, CUT&Tag and RNA-seq).

Independent critical thinking; experimental planning and design; data analysis and data/progress reports; seminar presentations to the scientific community.

References

1) Ntoufa S, Vilia MG, Stamatopoulos K, Ghia P, Muzio M. ***Toll-like receptors signaling: A complex network for NF-κB activation in B-cell lymphoid malignancies***. *Semin Cancer Biol.* 2016;39:15-25.

2) Fonte E, Agathangelidis A, Reverberi D, Ntoufa S, Scarfò L, Ranghetti P, Cutrona G, Tedeschi A, Xochelli A, Caligaris-Cappio F, Ponzoni M, Belessi C, Davis Z, Piris MA, Oscier D, Ghia P, Stamatopoulos K, Muzio M. ***Toll-like receptor stimulation in splenic marginal zone lymphoma can modulate cell signaling, activation and proliferation.*** Haematologica. 2015 Nov;100(11):1460-8.

3) Fonte E, Vilia MG, Reverberi D, Sana I, Scarfò L, Ranghetti P, Orfanelli U, Cenci S, Cutrona G, Ghia P, Muzio M. ***Toll-like receptor 9 stimulation can induce IκBζ expression and IgM secretion in chronic lymphocytic leukemia cells***. Haematologica. 2017 Nov;102(11):1901-1912.

4) Fonte E, Apollonio B, Scarfò L, Ranghetti P, Fazi C, Ghia P, Caligaris-Cappio F, Muzio M. ***In vitro sensitivity of CLL cells to fludarabine may be modulated by the stimulation of toll-like receptors***. *Clin Cancer Res.* 2013; 19(2):367-79.