PROJECT 1

DoS: Giulio Cavalli

Title: Maladaptive metabolic reprogramming of macrophages in the pathogenesis of Erdheim-Chester disease

Curriculum: Cellular and Molecular Biology

Residency Program: Allergy and Clinical Immunology, Internal Medicine

Link to OSR/UniSR personal page: http://www.unisr.it/k-teacher/cavalli-giulio/

Project description:
Erdheim-Chester disease (ECD) is a rare inflammatory myeloid cancer characterized by infiltration of tissues by foamy macrophages (1). Pathologic cells are characterized by activating mutations along the MAPK pathway, most commonly BRAFV600E, and by rampant production of pro-inflammatory cytokines. In addition, ECD macrophages exhibit multiple features of altered cell energy metabolism, including foamy appearance, up-regulation of glycolytic pathways, and activation of autophagy (2). These and several other metabolic changes are likely needed to sustain the increased energy demands of constitutive pro-inflammatory activation.
Scope of this project is to determine the role of maladaptive changes in cell energy metabolism in sustaining survival and inflammatory activation of ECD macrophages. Unraveling these mechanisms is crucial to the understanding of ECD and to the identification of new molecular targets amenable to pharmacologic intervention.
Transduction of healthy human monocytes with a lentiviral vector construct encoding for BRAFV600E, followed by integrated Seahorse flux and metabolomics analyses, will allow evaluating whether activation of the MAPK pathway results in aberrant **metabolic reprogramming** in mutated macrophages (3). In addition, induction and functional consequences of **autophagy** will be revealed by electron and confocal microscopy, western blot, and functional studies. Transduction of human hematopoietic precursors followed by infusion into NSG mice will allow replicating these studies in a relevant *in vivo* model of ECD. In order to provide ultimate proof-of-concept to our hypothesis, we will determine the **therapeutic potential** of inhibiting cell energy metabolism and autophagy in ECD, both in the previously described disease models and in primary macrophages isolated from ECD lesions. Modulation of metabolic changes, cell survival/apoptosis, and cytokine production will be evaluated in vitro using established treatment options for ECD (i.e. vemurafenib, anakinra), as well as novel strategies targeting cell energy metabolism and autophagy (i.e. 2DG, itaconate, hydroxychloroquine). The therapeutic potential of drugs showing most promising activity in vitro will be evaluated ex vivo on ECD tissue samples in bioreactor.

Skills to be acquired by the student:
Development of study hypotheses and experimental design; isolation of primary human cells from blood and tissues, cell culture and treatment studies, lentiviral transduction of primary human cells, flow cytometry, western blot, ELISA, histology and IHC, immunofluorescence, culture of tissue samples in bioreactor; familiarity with Seahorse Flux Analyzer metabolomics studies.

References (max. 3):
Diamond, Dagna, Hyman, Cavalli, et al. Consensus guidelines for the diagnosis and clinical management of Erdheim-Chester disease. Blood. 2014
Cavalli, et al. Interleukin 37 reverses the metabolic cost of inflammation, increases oxidative respiration, and improves exercise tolerance. Proc Natl Acad Sci U S A. 2017
Villa A, Belloni D, Vergani B, Cenci S, Cavalli G, Biavasco R, et al. 3D culture of Erdheim-Chester disease tissues unveils histiocyte metabolism as a new therapeutic target. Ann Rheum Dis. 2018.