PROGETTO

Supervisore::	RENATO OSTUNI
Titolo/*Title*:	EPIGENETIC REGULATORS OF INFLAMMATORY RESPONSES
Curriculum:	TERAPIA GENICA E CELLULARE
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/genomics-of-the-innate-immune-system.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Macrophages are key cells of the innate immune system that populate all host tissues and patrol their microanatomical environment. Upon sensing an environmental challenge, macrophages undergo pervasive rewiring of their transcriptional and epigenomic landscape resulting in the rapid induction of hundreds of pro-inflammatory genes. Several chromatin modifications have been linked to gene activation or repression in response to external stimuli. However, the specific chromatin modifying enzymes underlying temporal control of inflammatory responses remain to be elucidated.

Rationale and hypothesis

Stimulation of macrophages with the bacterial molecule lipopolysaccharide (LPS) represents a paradigmatic system to study how extracellular cues impact on chromatin modulation and selective gene transcription. Type I interferons (IFN I) are pleiotropic cytokines that govern antimicrobial immune responses in response to LPS and viral antigens, via the downstream activation of NF-κB, AP-1, and IRF3 transcription factors. IFN I can promote an antiviral cell state and cause tissue damage, inducing the expression of tens of antiviral genes, namely interferon-stimulated genes (ISGs). Therefore, production of these cytokines must be tightly controlled at the epigenetic level to preserve tissue integrity while enabling cytotoxic immunity.

Objectives and specific aims

The Hoxb8 Mx1-Gfp immortalized hematopoietic cell line, in which the *Mx1* promoter controls the IFN I-dependent expression of GFP, have been used to screen a library of compounds targeting enzymes involved in chromatin modifications and remodeling. This screening led to the

identification of a class of inhibitors that suppress IFN I activity. Building on this finding, the candidate will dissect the molecular consequence of the chemical inhibition. Primary mouse macrophages treated with LPS in the presence of the drugs will be evaluated for their transcriptional and epigenetic state by using molecular biology techniques (RNA extraction, RT-qPCR) and genome-wide high-throughput technologies (RNA-Seq, ChIP-Seq). Changes in transcription and chromatin landscape of immune genes will give insights into the biological role of the candidate target. To investigate further the mechanisms linking LPS to the target, the candidate will generate knock-out macrophages via CRISPR-Cas9 and explore the target interactome via mass spectrometry. Finally, the candidate will test whether the inhibitors can reduce IFN signatures in human cells, paving the way for their translation into clinical settings where an exaggerated IFN production is causative of a disease state.

Expected outcomes

The discovery of compounds with a strong blocking activity on IFN I responses in activated macrophages will be complemented with a deeper understanding of the molecular link existing between LPS and the candidate target. We foresee to unveil a previously unknown role of the targeted enzyme in inflammatory contexts.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

The candidate will develop critical thinking skills and appropriate levels of scientific independence and organizational/presentational abilities. She/he will gain experience with transcriptomic and genomic techniques, both bulk and single-cell, as well as with primary cell culture and differentiation. Through close interaction with computational biologists, the student will become familiar with data analysis tools and learn to operate productively in an interdisciplinary context.

Bibliografia (max. 15)

Determinants, mechanisms, and functional outcomes of myeloid cell diversity in cancer. Caronni N, et al Immunol Rev. 2021

Adaptation and memory in immune responses. Natoli G§, Ostuni R§. Nat Immunol. 2019

IL-1b+ macrophages fuel pathogenic inflammation in pancreatic cancer. Caronni N*§, La Terza F*, Vittoria FM*, Barbiera G* et al. Nature. 2023

Cellular and transcriptional dynamics of human neutrophils at steady state and upon stress. Montaldo E*§, Lusito E*, Bianchessi V*, Caronni N* et al. Nat Immunol. 2022

A PGE2-MEF2A axis enables context-dependent control of inflammatory gene expression. Cilenti F*, Barbiera G* et al. Immunity. 2021

Opposing macrophage polarization programs show extensive epigenomic and transcriptional cross-talk. Piccolo V*, Curina A* et al. Nat Immunol. 2017

Latent enhancers activated by stimulation in differentiated cells. Ostuni R§*, Piccolo V, * Barozzi I*, Polletti S * et al. Cell. 2013

CD14 controls the LPS-induced endocytosis of Toll-like receptor 4. Zanoni I et al. Cell. 2011