PROGETTO

Supervisore::	Andrea Ditadi
Titolo/*Title*:	Gene expression dynamics of blood cell emegence
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/human-hematopoietic-development-and-disease-modeling.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

During embryonic development, blood cells emerge from specialized endothelial cells, named hemogenic endothelial cells (HECs). As HECs are rare and only transiently found in early developing embryos, it remains difficult to distinguish them from endothelial cells. HECs are thought to generate blood cells via an endothelial-to-hematopoietic transition (EHT), in which HECs gradually lose their endothelial characteristics and become hematopoietic cells[1,2]. Little is known about EHT and the molecular dissection of this transition will uncover new regulators of HSCs development and inform experimental approaches to generate HSCs *in vitro*.

Rationale and hypothesis

Gene expression analyses of HECs and of cells undergoing EHT have revealed striking similarity to non-hemogenic vascular endothelial cells (nH-VECs). In fact, these analyses have revealed around 150 differentially expressed genes[3], most of them related to migration and cytoskeletal rearrangements as well as a general upregulation of ribosomal genes[4]. Given this, the dramatic differences between the cellular fate of HECs vs nH-VECs can be explained by the following three major non-mutually exclusive hypothesis:

1)HEC cell fate is controlled at a post-transcriptional level;

2)HEC and nH-VEC display different chromatin accessibility at target genes of transcription factors expressed in both populations.

3)HECs is regulated at the level proteome remodeling.

For each of these hypothesis we have already generated preliminary data that validate them

Objectives and specific aims

1) Dissecting gene expression dynamics during EHT

2) Interrogating the post-transcriptional regulation of HEC transcriptome

3) Establishing the role of chromatin regulators during EHT

Expected outcomes

The combined identification of signaling pathways initiating the lineage transition and of regulators of EHT will provide critical information for the design of optimized protocol in order maximize the hematopoietic output from HECs, which is pivotal for the potential clinical application of hPSC-derived blood cells. Altogether through this aim we want to write the definitive "recipe for blood".

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

Analysis of stemness, pluripotency and developmental stages; analysis of signaling pathways; cell and tissue cultures with mouse and human pluripoternt stem cells, mouse embryos and primary samples; flow cytometry and cell sorting; molecular biology; data analysis; system biology literacy; scientific communication.

Bibliografia (max. 15)

1. Rybtsov, S. et al. Hierarchical organization and early hematopoietic specification of the developing HSC lineage in the AGM region. The Journal of Experimental Medicine 208, 1305–1315 (2011).

2. Rybtsov, S. et al. Tracing the Origin of the HSC Hierarchy Reveals an SCF-Dependent, IL-3-Independent CD43- Embryonic Precursor. Stem Cell Reports 3, 489–501 (2014).

3. Gao, P. et al. Transcriptional regulatory network controlling the ontogeny of hematopoietic stem cells. Gene Dev 34, 950–964 (2020).

4. Zeng, Y. et al. Tracing the first hematopoietic stem cell generation in human embryo by single-cell RNA sequencing. Cell Res 29, 881–894 (2019).

5. Scarfò, R. et al. CD32 allows capturing blood cells emergence in slow motion during human embryonic development. Biorxiv (2023) doi:10.1101/2023.03.23.530597.