PROGETTO

Supervisore: Samuele Ferrari

Titolo/*Title:* Addressing the Unknowns of VEXAS syndrome by Single-cell Multiomic Clonal Tracking and Targeted Genome Editing

Curriculum: Gene and Cell Therapy

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/gene-transfer-technologies-and-new-gene-therapy-strategies/samuele-ferrari.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

Life expectancy of humans substantially improved during the last decades thanks to tremendous achievements in health care[1]. Yet, people older than 65 will increase by 30% by 2050 in the European Union, posing the need to face the implications of age shift for healthy aging[2]. VEXAS syndrome (Vacuoles, E1 enzyme, X-linked, Autoinflammatory, Somatic) is a recently described autoinflammatory disease affecting 1:4,000 males >50 years of age, whose pathogenic hallmarks may serve as prototype of disorder due to clonal imbalance of hematopoiesis in aging individuals[3,4]. Clonal expansion of VEXAS hematopoietic clones leads to treatment-refractory hematologic and rheumatologic manifestations that compromise quality of life and lead to death in 50% VEXAS patients within five years from diagnosis[5,6]. Acquired mutations in the *UBA1* gene are known to cause VEXAS syndrome. Yet, the biology underlying clonal expansion and pathogenesis of VEXAS syndrome, and its contribution to poor prognosis and protean clinical manifestations remain unclear because of the complexity of the disease, the lack of suitable animal models, and the fragility and paucity of patient's cells.

Rationale and hypothesis

Clonal hematopoiesis (CH) is a virtually ineludible condition in aging individuals, is clinically diagnosed in >10-20% over age 70 and is associated to higher risk of blood cancers and other disorders, such as cardiovascular diseases[7,8]. We hypothesize that VEXAS syndrome exemplifies a peculiar type of CH and represents a paradigm to investigate and preclinically model the vicious interplay between clonal expansion and inflammation during aging. The complexity of VEXAS syndrome pathophysiology requires transformative approaches and methodologies able to resolve the intimate mechanisms governing it. To this goal, the candidate will leverage on i) cutting-edge multiomic technologies with single-cell resolution enabling lineage tracing studies and reconstruction of clonal architecture in patients samples[9] and ii) precision genome editing[10,11] to develop humanized xenograft models of VEXAS syndrome.

Objectives and specific aims

The overarching goal of the project is to resolve mechanisms and clonal evolution dynamics of the hematopoietic system that contribute to VEXAS syndrome pathophysiology through two specific aims:

1) Reconstruction of clonal hierarchy and cell state in VEXAS patients hematopoiesis by single-cell regulatory multiomics with mitochondrial mutations profiling (ReDeeM).

2) Development and characterization of humanized xenograft models by generating VEXAS-like hematopoietic stem cells (HSCs) through base and prime editing in healthy donors' cells.

Expected outcomes

We expect that the combination of these orthogonal approaches will allow to:

i) identify the key regulatory pathways driving VEXAS syndrome's pathogenesis

ii) map clonal trajectories and relationships that are peculiar of VEXAS hematopoiesis

iii) develop disease models that may recapitulate key features of VEXAS pathology and may prospectively enable testing of new therapies

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

The PhD candidate will work in a stimulating and collaborative environment, under daily supervision by a senior lab member, progressively taking the lead on the project and learning how to design and perform experiments and interpret the results. Unique technical skills to be acquired include: design of gene editing strategies and application in human cells; manipulation and functional characterization of hematopoietic stem cells; -omic studies; experiments in mouse models. The candidate will regularly present and discuss the results of the project with supervisor and peers.

Bibliografia (max. 15)

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4. Beck, D. B. *et al.* Somatic Mutations in UBA1 and Severe Adult-Onset Autoinflammatory Disease . *New England Journal of Medicine* **383**, 2628–2638 (2020).
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6. Koster, M. J. *et al.* Clinical Heterogeneity of the VEXAS Syndrome. *Mayo Clin Proc* **96**, 2653–2659 (2021).
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8. Jaiswal, S. & Ebert, B. L. Clonal hematopoiesis in human aging and disease. *Science (1979)* **366**, (2019).
9. Weng, C. *et al.* Deciphering cell states and genealogies of human haematopoiesis. *Nature* (2024) doi:10.1038/S41586-024-07066-Z.
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11. Fiumara, M. *et al.* Genotoxic effects of base and prime editing in human hematopoietic stem cells. *Nat Biotechnol* (2023) doi:10.1038/s41587-023-01915-4.