PROJECT

Supervisor: Mara Salomè

Title: Targeted strategies for DUX4-r leukemia

Curriculum: Cell and Molecular Biology

Link to the personal page of the University or relevant hospital site website: https://research.hsr.it/en/divisions/genetics-and-cell-biology/gene-expression-regulation.html

Description of the Project (max 3,000 characters including spaces)

Background/gap of knowledge

Cancer is the second leading cause of death in Europe, and acute lymphoblastic leukemia (ALL) is the most common pediatric cancer and the most frequent cause of cancer-related death before the age of 20. In 10% of cases, ALL is caused by rearrangements of the transcription factor double homeobox 4 (DUX4) giving rise to the oncogenic driver DUX4-r, which maintains the DNA binding domain (DBD) of DUX4[1,2]. DUX4-r transcriptional ability is required for leukemia initiation and maintenance[3]. Since no targeted treatment is available, non-responders/relapsed patients face a dismal prognosis. Hence, novel therapeutic strategies are urgently needed.

Rationale and hypothesis

We and other showed that the ability to bind DNA and activate gene expression is required for DUX4-r leukemogenic potential[4]. Therefore, identifying inhibitors of DUX4-r transcriptional activity and test their efficacy in ALL could help the development of effective therapeutic strategies in a personalized medicine perspective. Through proteomics, we identified Matrin 3 (MATR3)[5] for its ability to bind directly to DUX4-r DBD and inhibit its transcriptional potential. Next, through structure/function studies, we identified a Short Peptide Inhibitor (SPI) based on MATR3 that is sufficient to bind DUX4-r. Notably, we used this knowledge to generate drug-like PROteolisis TArgeting Chimera (PROTAC)[6] recombinant SPI (rSPI) molecules able to induce DUX4-r protein degradation.

Objectives and specific aims

The aim of this study is to test the inhibitory potential of DUX4-r targeting strategies in cellular and animal models of DUX4-r+ ALL.

The candidate will compare the activity and specificity of rSPI candidates and test the effects on DUX4-r target genes expression, cell adhesion, migration, and proliferation in DUX4-r+ and DUX4-r- lines (O1). Then, the candidate will validate the results on a DUX4-r PDX in vitro platform, and use advanced sequencing technologies to test the effects of lead candidates on the DUX4-r oncogenic signature (O2). Finally, safety and efficacy of the best rSPI molecules will be assessed on DUX4-r PDXs in vivo. The candidate will assess the effects of rSPI on ALL engraftment, disease latency and dissemination to lymphoid organs, and on the DUX4-r transcriptional signature (O3).

Expected outcomes

Collectively, this study will provide molecular understanding of the DUX4-r/rSPI molecular and functional interaction. Moreover, the data will support the possibility to generate drug-like mimicking peptides able, within a single molecule, to inhibit both DUX4-r expression and its activity.

Skills that the student should acquire (max. 600 characters including spaces):

The student will acquire and refine cell and molecular biology skills, cell culture and primary cell culture techniques, flow cytometry and imaging techniques. Moreover, the candidate will gain experience in patient derived xenograft animal models of DUX4-r leukemia.

References (max. 15)

1. Zhang, J. *et al.* Deregulation of DUX4 and ERG in acute lymphoblastic leukemia. *Nat. Genet.* **48**, 1481–1489 (2016).
2. Yasuda, T. *et al.* Recurrent DUX4 fusions in B cell acute lymphoblastic leukemia of adolescents and young adults. *Nat Genet.* **48**, 569–574 (2016).
3. Carlet, M. *et al.* In vivo inducible reverse genetics in patients' tumors to identify individual therapeutic targets. *Nat. Commun.* **12**, (2021).
4. Campolungo, D., Salomé, M., Biferali, B., Tascini, A. S. & Gabellini, D. DUX4-r exerts a neomorphic activity that depends on GTF2I in acute lymphoblastic leukemia. *Sci. Adv.* **9**, adi3771 (2023).
5. Runfola, V. *et al.* MATR3 is an endogenous inhibitor of DUX4 in FSHD muscular dystrophy. *Cell Rep.* **42**, 113120 (2023).
6. Tsai, J. M., Nowak, R. P., Ebert, B. L. & Fischer, E. S. Targeted protein degradation: from mechanisms to clinic. *Nat. Rev. Mol. Cell Biol.* (2024) doi:10.1038/s41580-024-00729-9.