PROJECT

Supervisor:	Antonella NAI
Title:	Identification of new strategies for *in vivo* targeting of erythroid *Tfr2* in β-thalassemia
Curriculum:	Cellular 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/regulation-of-iron-metabolism/antonella-nai.html

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

Background/gap of knowledge

β-thalassemia is an autosomal recessive disease due to mutations in the β-globin gene, characterized by ineffective erythropoiesis (IE), anemia, splenomegaly and secondary iron overload. Despite available treatments, therapeutic options remain inadequte[1]. With over 1 million affected individuals worldwide, there is an urgent need for novel therapeutic strategies.

Transferrin Receptor 2 (TFR2) is a transmembrane protein expressed in the liver, where it regulates systemic iron homeostasis, and in the erythroid compartment, where it is a partner of erythropoietin receptor (EPOR). Hematopoietic TFR2 functions as a brake of EPO signaling, though the underlying mechanism remains unclear. Notably, its inactivation enhances normal erythropoiesis[2] and improves anemia in murine models of non-transfusion dependent[3] and transfusion-dependent β-thalassemia[4].

Overall, these findings position erythroid TFR2 as a promising therapeutic target for the treatment of β-thalassemia. However, a pharmacologic approach capable of efficiently interfering with TFR2 function in erythroid cells remains to be identified.

Rationale and hypothesis

At present, gene therapy is the only viable method for inhibiting a target of interest in erythroid cells. However, its complexity, cost, and patient eligibility limitations restrict its widespread application[5]. In contrast, interfering peptides and RNA degradation technologies are emerging

as promising innovative therapeutic opportunities. This project aims to explore these approaches to target TFR2 in erythroid cells effectively.

Objectives and specific aims

1. The first aim of the project is *to identify the still unknown TFR2-EPOR interacting region and to test whether this interaction is essential for the TFR2-mediated modulation of erythropoiesis*. These studies will lay the groundwork for designing a targeted interfering strategy to selectively disrupt the erythroid function of TFR2 as a negative modulator of EPO signaling, with potential applications in other forms of anemia.

2. The second aim of the project is *to identify novel strategies to improve the delivery of anti-Tfr2 oligonucleotides to hematopoietic cells.* Indeed, achieving effective downregulation of the target of interest within erythroid cells *in vivo* is a challenging task because of poor cellular uptake[6].

Expected outcomes

1. Identification of the domain responsible for TFR2-EPOR binding and elucidation of the relevance of this interaction on the TFR2 erythroid function. Evolution of these studies will be the design of an interfering peptide to be tested as therapeutic agent.

2. Identification and validation of an efficient and specific method for the inactivation *in vivo* of erythroid *Tfr2* by RNA degradation technology, to be proposed as a novel therapeutic strategy for β-thalassemia and other anemias.

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

- *In vitro* and *ex vivo* culture and transfection of cell lines and primary cells
- RNA extraction and real-time PCR; hematological analysis and iron quantification; Western-Blot, FACS and Seahorse analysis
- Management, genotyping and manipulation of mouse models
- Data presentation at national and international congresses
- Manuscript writing

References (max. 15)

1. Njeim R, Naouss B, Bou-Fakhredin R, Haddad A, Taher A. Unmet needs in β-thalassemia and the evolving treatment landscape. Transfus Clin Biol. 2024 Feb;31(1):48-55. doi: 10.1016/j.tracli.2023.12.003.

2. Nai A, Lidonnici MR, Rausa M, Mandelli G, Pagani A, Silvestri L, Ferrari G, Camaschella C. The second transferrin receptor regulates red blood cell production in mice. Blood. 2015 Feb 12;125(7):1170-9. doi: 10.1182/blood-2014-08-596254.

3. Artuso I, Lidonnici MR, Altamura S, Mandelli G, Pettinato M, Muckenthaler MU, Silvestri L, Ferrari G, Camaschella C, Nai A. Transferrin receptor 2 is a potential novel therapeutic target for β-thalassemia: evidence from a murine model. Blood. 2018 Nov 22;132(21):2286-2297. doi: 10.1182/blood-2018-05-852277.

4. Di Modica SM, Tanzi E, Olivari V, Lidonnici MR, Pettinato M, Pagani A, Tiboni F, Furiosi V, Silvestri L, Ferrari G, Rivella S, Nai A. Transferrin receptor 2 (Tfr2) genetic deletion makes transfusion-independent a murine model of transfusion-dependent β-thalassemia. Am J Hematol. 2022 Oct;97(10):1324-1336. doi: 10.1002/ajh.26673.

5. Kohn DB, Chen YY, Spencer MJ. Successes and challenges in clinical gene therapy. Gene Ther. 2023 Nov;30(10-11):738-746. doi: 10.1038/s41434-023-00390-5.

6. Halloy F, Iyer PS, Ćwiek P, Ghidini A, Barman-Aksözen J, Wildner-Verhey van Wijk N, Theocharides APA, Minder EI, Schneider-Yin X, Schümperli D, Hall J. Delivery of oligonucleotides to bone marrow to modulate ferrochelatase splicing in a mouse model of erythropoietic protoporphyria. Nucleic Acids Res. 2020 May 21;48(9):4658-4671. doi: 10.1093/nar/gkaa229.