International Training Network

EVIDENCE

PhD proposal - ESR 6

Location : Roscoff Biological Station, FRANCE

UMR8227 LBI2M

CNRS – Sorbonne University

Contact : Dr Stéphane Egée (egee@sb-roscoff.fr)

Starting date : Nov-Dec 2020

EVIDENCE (https://evidence.eurice.eu/) is a consortium of 12 European partners, granted by the EU in the frame of the MARIE SKŁODOWSKA-CURIE ACTIONS - Innovative Training Networks (ITN), whose objective is to explore the properties and behaviour of RBCs under flow conditions and in vivo to understand pathophysiology and to design novel diagnostic devices. The project includes 15 PhD grants, distributed among the 12 Partners. The grant ESR6 will be based in Roscoff, France, at the Station Biologique which is an institute belonging to the CNRS and Sorbonne University.

Funding:

The EVIDENCE ITN is an EU funded international consortium of academic research centres, diagnostic labs, blood supply centres, and small companies that combines basic and translational research to improve diagnostic and therapeutic approaches on erythrocytes and during erythropoiesis. More details about the consortium can be found at the EVIDENCE website https://evidence.eurice.eu/.

The MSC ITN programme offers highly competitive and attractive salary. The successful candidate will receive a salary in accordance with the regulations for MSC Fellows/early stage researchers (ESRs). Exact salary will be confirmed upon appointment but will involve a generous Living Allowance and monthly mobility allowance depending on the successful candidate's personal family situation. This PhD position will likely provide experience of erythroid culture, molecular and cell biology, imaging, flow cytometry, microfluidics and electrophysiology. In addition to their individual scientific projects, all fellows benefit from further continuing education, which includes internships and secondments, a variety of training modules as well as transferable skills courses and active participation in workshops and conferences.

Subject:

The hydration state of RBCs as an important parameter that ensures the functions of the cell in the blood flow

Objectives of the PhD:

This project will aim at understanding the functions of ion channels within the RBC membrane, particularly in the context of RBC circulation and end of life. It will rely on a diversity of techniques and approaches (from the diversity of channel expression in progenitors to their involvement in aging

and deformability tests) and also include RBC membrane permeability modelling to decipher the involvement of ion channels at these crucial life steps. It will bring implications useful for a better comprehension of pathologies linked to cation permeability, for storage lesions and for the production of cultured RBCs in Bioreactors.

State of the art:

Erythrocytes are highly specialized and atypical cells, as evolution has led to the complete loss of intracellular organelles in mature red cells to optimize respiratory functions. Nonetheless, human erythrocytes are far from being a simple bag of hemoglobin, and have to maintain their deformability, volume and intern homeostasis to accomplish with the best efficiency their function of gas transporters. Indeed, RBCs are in continuous flow, with high and rapid variations of their environment and frequent deformability stresses. This deformability is linked to their unique cytoskeleton and surface / volume ratio, and to a maintenance of the volume; it is tested very regularly in the spleen. During their 120 days of life, RBCs undergo an increase in cell density, reduction of cell volume and are eventually removed from the circulation in the spleen and recycled by macrophages.

Membrane ion permeability is a key parameter for the hydration and volume preservation, and mammalian red cells have long been a paradigm to study membrane transport. Erythrocyte membranes are characterized by an important anionic permeability, allowing the equilibrium for chloride ions and facilitating the transport of CO_2 via the Jacobs-Stewart cycle. In contrast, cationic permeability represents a threat linked to the colloido-osmotic pressure exerted by the high protein content of the cell. Surprisingly erythrocytes possess not only anionic channels, but also a repertoire of cationic channels whose full description is still lacking, including notably the Gárdos channel (KCNN4, hSK4) (1), selective for K^+ ions, PIEZO-1 (2), a mechanosensitive cation channel or the NMDA Receptor (3). The complete repertoire of ion channel is still under investigation, and so is it for their physiological roles (4, 5). A certitude is that dysregulations of cation channel activity (linked to mutations either on the channels or on partner proteins) are involved in multiple pathologies of the RBC (6, 7) and that these channels are linked to senescence of RBCs (8). The roles of these channels has almost always been studied in static conditions, using electrophysiological techniques.

This project aims to :

1) Fully describe the repertoire of ionic channels from early stages of terminal differentiation to mature RBCs.
2) Understand their physiological role in mature RBCs once they are released within circulation.
3) Unravel their involvement in senescence processes in health and disease conditions.
4) Ultimately all the data will be used to feed a quantitative model (already written and yet improved (9, 10)) for diagnostic predictive purposes, for blood preservation in vitro and for the upscaling of cultured RBC production in bioreactors.

Methodologies:

- To determine the repertoire of ion channels in RBCs, we have started a study focusing on the expression of channel proteins in terminal erythropoiesis RBC. All tools are now ready and we have already acquired first results. This part of the project includes erythropoiesis ex vivo, qPCR, Western Blot, and immunofluorescence assays.

- Monitoring of ion channel activity linked to cell flow: We will perform patch clamp studies / CCCP monitoring of membrane potential before and after several stresses implying cell deformation : microsphiltration (11), Shear flow, spleen-on-chip or microfluidic in collaboration with partners from the Evidence ITN.

- model of membrane permeability : the model was originally developed by Lew and Bookchin (9), and was recently update

Application procedure Applicants should send their complete applications in one single PDF by email to Stéphane Egée (egee@sb-roscoff.fr). Each application should provide a cover letter, a CV detailing scientific experience to date and copy of qualification certificates and provide a contact information of appropriate 2 references. For further details of ESR6 or other projects, please refer to the website: https://evidence.eurice.eu/.

The application closing date is 15th September 2020. Candidates who have been selected for interview will be contacted for Skype interviews by the end of September 2020. PhD have to start before 31st December 2020.

Eligibility :

The candidate must have a Master of Science and not have resided or carried out his main activity (e.g. work, studies) in France for more than 12 months in the 3 years immediately before the recruitment date.

1. Hoffman JF, Joiner W, Nehrke K, Potapova O, Foye K, Wickrema A. The hSK4 (KCNN4) isoform is the Ca2+-activated K+ channel (Gardos channel) in human red blood cells. Proc Natl Acad Sci U S A. 2003 Jun 10;100(12):7366-71. PMID 12773623.

2. Albuisson J, Murthy SE, Bandell M, Coste B, Louis-dit-Picard H, Mathur J, Fénéant-Thibault M, Tertian G, de Jaureguiberry J-P, Syfuss P-Y, Cahalan S, Garçon L, Toutain F, Simon Rohrlich P, Delaunay J, Picard V, Jeunemaitre X, Patapoutian A. Dehydrated hereditary stomatocytosis linked to gain-of-function mutations in mechanically activated PIEZO1 ion channels [Article]. Nat Commun. 2013;4:1884. doi:10.1038/ncomms2899.

3. Makhro A, Hänggi P, Goede JS, Wang J, Brüggemann A, Gassmann M, Schmugge M, Kaestner L, Speer O, Bogdanova A. N-methyl-d-aspartate receptors in human erythroid precursor cells and in circulating red blood cells contribute to the intracellular calcium regulation. Am J Physiol Cell Physiol. 2013 305(11):C1123-C1138. doi:10.1152/ajpcell.00031.2013.

4. Thomas SL, Bouyer G, Cueff A, Egee S, Glogowska E, Ollivaux C. Ion channels in human red blood cell membrane: actors or relics? Blood Cells Mol Dis. 2011 Apr 15;46(4):261-5. doi:S1079-9796(11)00058-1 PMID 21429775.

5. Hertz L, Huisjes R, Llaudet-Planas E, Petkova-Kirova P, Makhro A, Danielczok JG, Egee S, del Mar Mañú-Pereira M, van Wijk R, Vives Corrons J-L, Bogdanova A, Kaestner L. Is Increased Intracellular Calcium in Red Blood Cells a Common Component in the Molecular Mechanism Causing Anemia? [Perspective]. Frontiers in Physiology. 2017 2017-September-06;8(673). doi:10.3389/fphys.2017.00673.

6. Gallagher PG. Disorders of erythrocyte hydration. Blood. 2017. doi:10.1182/blood-2017-04-590810.

7. Kaestner L, Bogdanova A, Egee S. Calcium Channels and Calcium-Regulated Channels in Human Red Blood Cells. In: Islam MS, editor. Calcium Signaling. Cham: Springer International Publishing; 2020. p. 625-648. doi:10.1007/978-3-030-12457-1_25

8. Lew VL, Tiffert T. On the Mechanism of Human Red Blood Cell Longevity: Roles of Calcium, the Sodium Pump, PIEZO1, and Gardos Channels [Mini Review]. Frontiers in Physiology. 2017 2017-December-12;8(977). doi:10.3389/fphys.2017.00977.

9. Lew VL, Bookchin RM. Volume, pH, and ion-content regulation in human red cells: analysis of transient behavior with an integrated model. J Membr Biol. 1986;92(1):57-74. PMID 3746891.

10. Rogers S, Lew VL. The circulatory physiopathology of human red blood cells investigated with a multiplatform model of cellular homeostasis. III. Senescence changes during the full circulatory lifespan. bioRxiv. 2020:2020.03.07.981803. doi:10.1101/2020.03.07.981803.

11. Lavazec C, Deplaine G, Safeukui I, Perrot S, Milon G, Mercereau-Puijalon O, David PH, Buffet P. Microsphiltration: A Microsphere Matrix to Explore Erythrocyte Deformability. In: Ménard R, editor. Malaria: Methods and Protocols. Totowa, NJ: Humana Press; 2013. p. 291-297.