PhD Project 2020 - Team Physiology and Cell Fate UMR8227
ED515 Diversité du Vivant - Sorbonne Université
Title : ***Diversity and role of ionic channels along human terminal erythropoiesis***

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.

Membrane ion permeability is a key parameter within these mechanisms, and mammalian red cells have long been a paradigm to study membrane transport, for two main reasons: there are easily available in massive amounts, and their structural simplicity facilitates membrane transport studies. Thus several transporters such as ionic pumps ($3Na^+/2K^+$, Ca^{2+}) or aquaporins have first been described using mammalian erythrocytes. The human red cell also possesses a repertoire of facilitated transporters, cotransporters or antiporters that use the gradients built by the pumps. Nonetheless, among these pathways, ionic channels are far from being fully described and more importantly their physiological role during lifespan is poorly understood.

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 cannels, but also a repertoire of cationic channels whose full description is still lacking. The most described cation channel in RBCs is the Gárdos channel (KCNN4, hSK4), selective for K^+ ions. More recently, the mechanosensitive PIEZO1 channel and the NMDA Receptor, both non selective cationic channels, have been described. Other channels have also been evoked, such as Ca^{2+} or Na^+ channels. If the implication of the two first ones in several pathologies is starting to get clearer, the various physiological functions of all these channels in mature RBCs are still unclear. One of the way to understand it is to look at channel expression and role during terminal erythropoiesis.

In humans, erythropoiesis occurs within the bone marrow, where cells differentiate from the pluripotent HSC (hematopoietic Stem Cell), into first BFU-E and CFU-E progenitors. From CFU-E, cells differentiate in the erythroblastic island, consisting of a central macrophage surrounded by up to 30 erythroid cells at various degrees of maturation. This terminal differentiation is characterized by progressive accumulation of hemoglobin, decrease in cell size and nuclear condensation ultimately resulting in enucleation. At this step, cells are called reticulocytes, which will be eventually released in the circulation and mature within several days into erythrocytes.

It is well known in other cellular models that ion channels are essential for differentiation and morphological changes (1, 2). In the differentiating erythroblast, the ion membrane permeability is known to switch from mainly cationic to anionic at the end of differentiation (3), with the strong expression of Band 3. The channels responsible for this cation permeability, however, are not clearly identified. Several ion channels have been described in erythroblasts, but only the activity of a TRPC3 channel (Transient Receptor Potential Cation channel) in BFU-E cells, stimulated by Erythropoietin (Epo) and leading to Ca^{2+} influx at specific stages (4-6) has been more deeply investigated, notably using electrophysiology techniques. A putative role for Gárdos or PIEZO1 channels during terminal erythropoiesis has never been extensively studied, however it is strongly supported by the recent studies on erythropoiesis of PIEZO1 mutant, which showed a prolonged process in patient differentiating cells (7, 8).

The proposed thesis project therefore aims at determining the roles of ion channels in the process of terminal erythroid differentiation by following three lines of study:

1) Determine the expression repertoire of ion channels and the expression levels of mRNAs during terminal erythropoiesis.
2) Quantify the relative protein expression levels during this differentiation and determine the protein localization.
3) Establish the activity and the roles of these channels during key steps of terminal differentiation.

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