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POSTER ABSTRACTS

113.SICKLE CELL DISEASE, SICKLE CELL TRAIT AND OTHER HEMOGLOBINOPATHIES, EXCLUDING THALASSEMIAS: BASIC AND TRANSLATIONAL

Hydroxyurea's Impact on Erythropoiesis, Cell Death and HbF Expression in the Hudep-2 Model

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Background : Sickle cell disease (SCD) is an inherited disorder caused by a mutation in the beta-globin gene, leading to the synthesis of sickle hemoglobin called HbS. Under deoxygenated conditions, HbS polymerizes and forms fibers that weakens red blood cells (RBC) resulting in hemolysis and vaso-occlusion. Recently, ineffective erythropoiesis in SCD has been reported, characterized by higher apoptosis levels during late erythropoiesis. Interestingly, fetal hemoglobin (HbF)-expressing erythroblasts during erythroid differentiation seem to be protected from the apoptosis.
Hydroxyurea (HU) is the first major approved drug for SCD in US and Europe; its proven efficacy in decreasing SCD morbidity and mortality is mainly mediated by the induction of HbF expression that is well-known as a modulator of disease severity through its ability to interfere with HbS polymerization. Despite being used for decades, HU action mechanism still remains to be fully elucidated. More precisely, HU impact on RBC maturation process is poorly documented as well as its mode of action on gamma-globin gene expression.
Aim : Our aim is to investigate the effect of HU on erythropoiesis kinetics, HbF induction and apoptosis.
Methods : We used HUDEP-2 (Human Umbilical cord Derived Erythroid Progenitors-2) cell line as a model for erythropoiesis. Cells were differentiated in normoxia (20% oxygen) or hypoxia (5% oxygen) - to mimic bone marrow environment, and were treated or not with HU allowing the comparison of HU effect both in normoxia and hypoxia. Analysis was performed at day D1, D3, D6, D8 and D10 of differentiation using flow cytometry, microscopy (MGG staining) and High-Performance Liquid Chromatography (HPLC). Kinetics of differentiation were followed by microscopy with MGG coloration and by flow cytometry using GPA, CD49d, Band3 and HbF antibodies. Apoptosis was assessed using annexin V staining.
Results : Wedid first assess HU effect on HbF expression during erythropoiesis by flow cytometry and HPLC. Both methods showed that HU induced HbF expression from D1 to D6 of differentiation and that this induction is higher when the cells are in hypoxic conditions. We also investigated the apoptotic rate during erythropoiesis. Interestingly, HU treatment does not induce apoptosis in differentiating erythroblast, but decreased proliferation rates by 2-fold change in normoxia. Hypoxic conditions also decreased proliferative rate similarly to HU in normoxia but interestingly, in hypoxic conditions, HU did not furthermore decrease cell proliferation.
Then, we investigated HU effect on the differentiation kinetics. Based on the differential expression of CD49d and Band3 along the differentiation, the percentage of each stage of differentiation (proerythroblast, basophilic, polychromatic and orthochromatic) was estimated by flow cytometry in GPA $^{+}$ cells at D1, D3, D6, D8 and D10 of the differentiation. Figure 1 depicted flow cytometry representative results from our 4 culture conditions at D1. Our results did show that HU accelerates HUDEP-2 differentiation as soon as D1 of culture (normoxia D1 NT vs HU: ProE 53.3% vs 44%, Baso 29.9% vs 33.1%, Poly 15.2% vs 18% and Ortho 3.3% vs 3.8%) until the end of the differentiation (D8 and 10) in normoxia. This acceleration is accentuated in hypoxic conditions (Hypoxia D1 NT vs HU: ProE 34.7% vs 17.9%, Baso 42.2% vs 50.3%, Poly 17.7% vs 25.8% and Ortho 3.8% vs 4.7%) with a cumulative effect of hypoxia and HU. All the flow cytometry results were confirmed by MGG staining and quantification by microscopy. For the same day of differentiation, HU-treated HUDEP-2 are in later stages than HU-non treated cells.
Conclusion : Our study is the first to investigate the impact of HU on RBC maturation - erythropoiesis. HU treatment increases HbF expression without impacting apoptosis. The treatment tends to promote erythroid differentiation by accelerating matu-

ration processes with stronger effect in hypoxic conditions that mimic the *in vivo* low oxygen environment of the bone marrow, strongly suggesting that HU has other molecular targets than inducing HbF. All these results will be confirmed in CD34 $^{+}$ erythroid progenitors from patients with SCD. Our results give new insights on the effect of HU on erythropoiesis that would ultimately lead to a better understanding of its mechanism of action and to the identification of new therapeutical targets to counteract SCD ineffective erythropoiesis.

Disclosures No relevant conflicts of interest to declare.

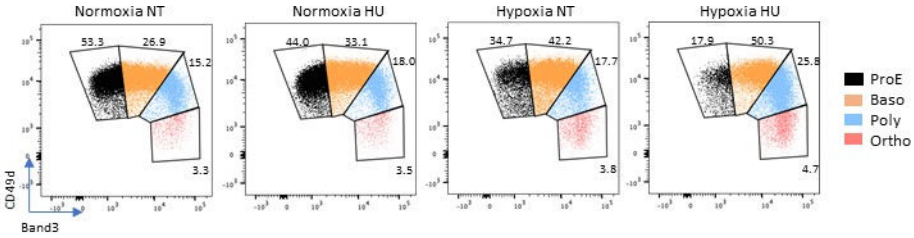

Figure 1: Erythroblast stages repartition at D1 of erythroid differentiation with respect to the expression of Band3 (x-axis) and CD49d (y-axis) by flow cytometry. Dot plot are representative of each condition at D1 and numbers displayed correspond to the mean (n=8) of experiments at D1 for each stage. ProE = ProErythroblast, Baso = Basophilic Erythroblast, Poly = Polychromatic Erythroblast, Ortho = Orthochromatic Erythroblast, NT = non treated, HU = treated with hydroxyurea

Figure 1

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