P1404 HDAC6 REGULATES HUMAN ERYTHROID DIFFERENTIATION THROUGH MODULATION OF JAK2 SIGNALING

**Topic:** 23. Hematopoiesis, stem cells and microenvironment

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**Background:**

Histone desacetylases (HDAC), through regulation of their acetylation level, modify the physico-chemical properties of proteins and modulate their activity, stability, localization and affinity for partners. Among them, HDAC6 is unusual in its cytoplasmic localization. Its inhibition leads to hyperacetylation of non-histone proteins, inhibiting cell cycle, proliferation and apoptosis. Ricinostat (ACY-1215) is a selective inhibitor of HDAC6 (HDAC6i) that has been shown to be of value in treating multiple myeloma or lymphoma but was associated with secondary off target effects. Among those, anemia is one of the most frequent, arguing for a particular importance of HDAC6 during erythropoiesis. So far, HDAC6 function during human erythropoiesis remains unknown.

**Aims:** We present here an extensive study of HDAC6 expression and function during human erythropoiesis.

**Methods:** We used two cell models: CD34⁺ progenitor cells obtained from apheresis from healthy donor, driven into in vitro erythroid differentiation and the leukemic cell line UT7/EPO. HDAC6 inhibition was obtained chemically using ACY-1215 and using an shRNA-based knockdown.

**Results:** We quantified HDAC6 expression during erythroid differentiation and observed a progressive increase in HDAC6 RNA expression raising from Day 11 to D18. Using indirect immunofluorescence and flow imaging, we observed that HDAC6 expression was mainly cytoplasmic arguing for non-histone cytoplasmic targets. We then cultured CD34⁺-cells into in vitro erythroid differentiation in the presence of ACY-1215. We observed that 0.5µM ACY-1215 gave the best result in terms of HDAC6 inhibition, leading to an increased acetylated α-tubulin without affecting histone H3 acetylation level. ACY-1215 exposure on CD34⁺-cells driven in vitro towards the erythroid lineage led to a decreased cell count, an increased apoptotic rate and a delayed erythroid differentiation with accumulation of weakly hemoglobinized immature erythroblasts. shRNA-mediated HDAC6 knockdown in the erythroleukemic cell line UT7/EPO also decreased GPA expression at cell surface. In primary cells, the delay in erythroid differentiation was accompanied by drastic changes in the transcriptomic profile of primary cells as shown by RNAsseq. We observed 1920 differentially expressed genes including 1134 upregulated and 786 downregulated. Rank-based GSEA analysis showed a (i) significant enrichment of downregulated genes related to bone marrow erythroblast associated with a downregulation of genes involved in heme metabolism, (ii) a significant enrichment of downregulated GATA1 targets and (iii) an enrichment of downregulated genes related to cell cycle. In UT7/EPO and in primary cells, HDAC6i decreased JAK2 phosphorylation in response to EPO as well as STAT5 activation, as assessed using immunoblot and phosphoflow. Using acetylome, we identified 14-3-3ζ, known to interact directly with the JAK2 negative regulator LNK, as a potential HDAC6 target in erythroid cells. We confirmed that 14-3-3ζ was hyperacetylated after ACY-1215 exposure. We analyzed LNK/14-3-3ζ and LNK/JAK2 direct interactions using co-immunoprecipitation assays. ACY-1215 decreased the 14-3-3ζ/LNK interaction while increasing LNK/JAK2 interactions.

**Summary/Conclusion:**

Here, we show that LNK availability to interact with JAK2 in erythroid cells is regulated by HDAC6-dependent 14-3-3ζ hyperacetylation.
3ζ acetylation level. Thus, we identified here a new mechanism of HDAC6-dependent control of human erythropoiesis through 14-3-3ζ acetylation level, LNK availability and JAK2 activation in response to EPO.