RIBONUCLEASE INHIBITOR (RNH1) IS A NOVEL REGULATOR IN MYELOPOIESIS & RESOLVES DIFFERENTIATION BLOCKADE IN ACUTE MYELOID LEUKEMIA

Experimental Haematology/ Oncology

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BACKGROUND

Ribonuclease Inhibitor (RNH1), traditionally known for inhibiting pancreatic ribonucleases, has emerged as a key regulator of haematopoiesis, crucial in embryonic development and erythropoiesis. In-vivo mouse studies highlighted this role, whereby Rnh1 deletion in haematopoietic cells caused an anaemic phenotype but increased myelopoiesis. Despite the increase, there was no leukaemogenesis and the myeloid cells were functional, the phenotype likened to a subtle form of emergency myelopoiesis.

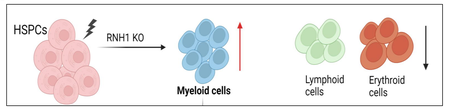


Fig 1: Schematic representation of hematopoietic skewing caused by RNH1 manipulation

This phenotype of augmented myelopoiesis led us to hypothesize that the gene may be playing a pivotal role in controlling the differentiation and proliferation of the myeloid lineage from HSPCs.

Acute myeloid leukemia (AML) is a myeloid malignancy characterized by a differentiation block in myeloid progenitor cells leading to excessive immature blast cells and ultimate bone marrow failure due to the inability to form functional myeloid cells. Therefore, in our work we attempt to understand if RNH1 is involved in the disease pathophysiology, delineate the underlying mechanism of regulation and evaluate if RNH1 may be a potential target to help resolve the myeloid differentiation block and aid in AML therapy.

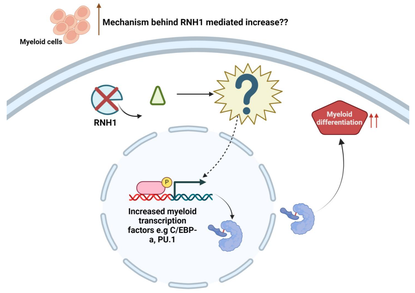


Fig 2: Hypothesized mechanism of myelopoiesis regulation by RNH1

OBJECTIVES

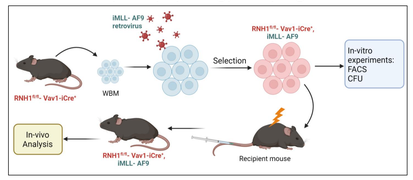
- Understanding role of RNH1 in AML pathophysiology
- Dissecting mechanism behind manipulation of myelopoiesis by RNH1
- Evaluation of RNH1 as a potential therapeutic target in AML, by using AML patient derived cells

CONCLUSION

- RNH1 deletion in AML cell lines increases differentiation, as evidenced by:
  - Increase in mRNA and protein expression of myeloid TFs C/EBPa, C/EBP-b, PU.1
  - Increase in expression of surface markers CD11b, CD14 and CD16
  - Morphological changes with increased cytoplasmic to nuclear ratio
- shRNA mediated RNH1 knockdown in AML blast cells seen to shift them towards more mature myeloid cells.
- Possible epigenetic changes induced by RNH1 as a consequence of its binding to histone deacetylase (HDAC1/2). ATAC-Sequencing and analysis ongoing to understand if resultant epigenetic change is the cause behind RNH1 mediated myeloid differentiation.

FUTURE PROSPECTS

- Validation of observed myeloid differentiation on more number of AML patient derived cells to understand the translational potential.
- In-vivo studies in AML mice to evaluate how the presence and absence of RNH1 affects disease pathophysiology and progression.



RESULTS

RNH1 is Over-Expressed in AML Patients

Analysis of publicly available datasets of AML patients belonging to different diagnostic classifications highlight a significant over-expression of RNH1 as compared to healthy individuals.

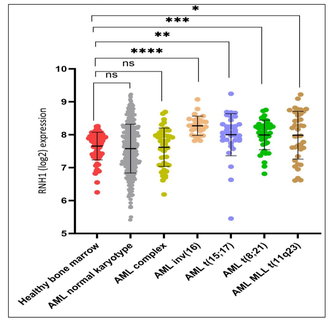


Fig 3: RNA-seq data from BloodSpot study (Nucl. Acids Res., 2018)

Histopathological analysis of AML patient derived bone marrow biopsies show an enhanced expression of RNH1 protein as compared to a healthy BM. This indicates a potential role of RNH1 in AML pathophysiology.

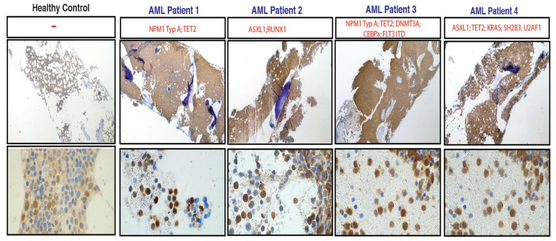


Fig 4: Histopathological analysis of bone marrow biopsies of AML patients and healthy control

Absence of RNH1 enhances myeloid differentiation

The expression of cell surface marker CD11b, which is indicative of myeloid differentiation, was analysed between the WT and RNH1 KO cell lines by FACS. Upon removal of RNH1, the AML cell lines exhibited significant increase in differentiation, which was evidenced by increased expression of CD11b marker, as well as an increase in expression of myeloid differentiation related genes (C/EBP-a, C/EBP-b, CD36) and at the protein level as an enhanced expression of myeloid transcription factors C/EBP-a and PU.1 (WB) in various WT and RNH1 KO AML cell lines.

Absence of RNH1 Enhances Myeloid Differentiation

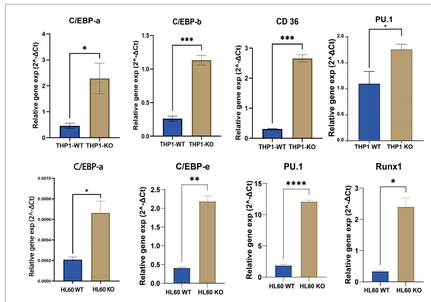


Fig 5: RT-PCR analysis of different myeloid differentiation related genes shows an upregulation in the RNH1 KO AML cells

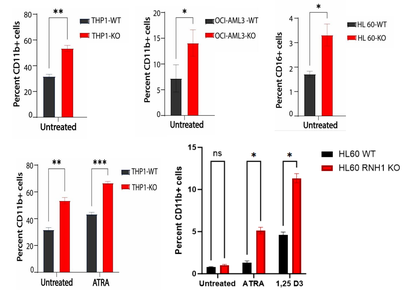


Fig 6: Flow cytometry analysis of expression of pan myeloid surface marker CD11b on WT and RNH1 KO AML cells

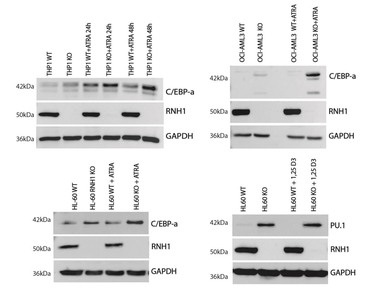


Fig 7: Western blot analysis of myeloid transcription factors in WT and RNH1 KO AML cells

To better understand the translational potential of targeting RNH1 in AML, peripheral and bone marrow biopsies were acquired and mononuclear cells (MNCs) were isolated. Lentivirus-mediated RNH1 knockdown experiments were performed in these blast cells (MNCs). A similar phenotype of increased myeloid differentiation in the RNH1 depleted cells compared to control blasts was observed.

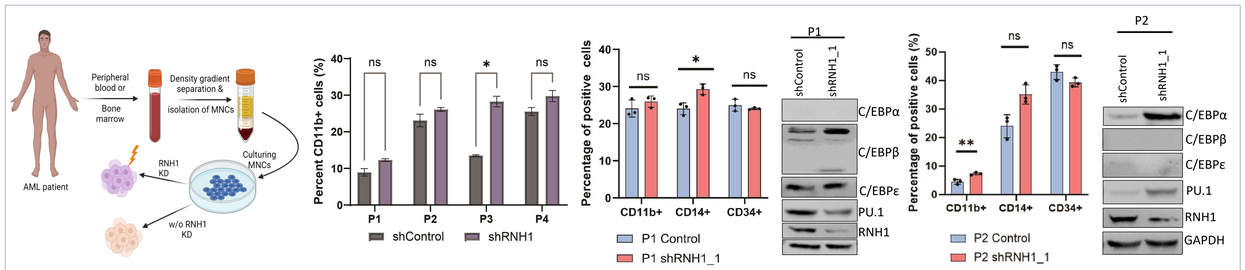


Fig 8: Flow cytometric and Western blot analysis of shRNA mediated RNH1 depletion in AML patient derived PBMCs showing induction of myeloid differentiation

RNH1 dependent Epigenetic Modifications Possible Cause behind Elevated Myeloid Differentiation

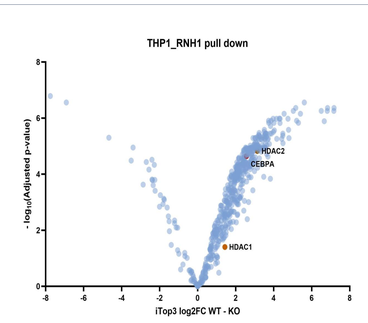


Fig 9: Mass spectrometry analysis of RNH1 binding proteins in THP1 cells shows HDAC1 & 2 and C/EBP-a as interacting partners

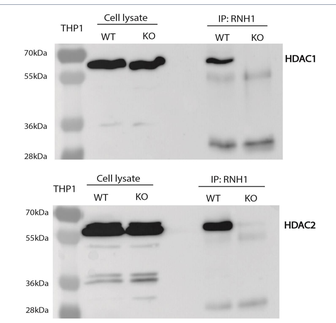


Fig 10: Western blot of co-immunoprecipitation of RNH1 interacting proteins shows RNH1 binds to HDAC1 & 2

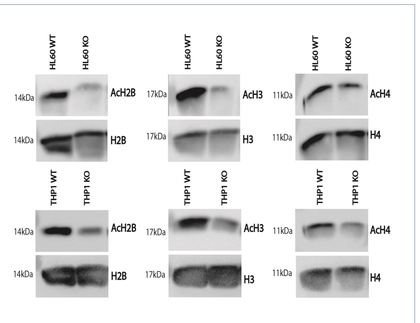


Fig 11:Total and acetylated f histones in chromatin-bound protein extract from THP1 and HL60 WT and RNH1 KO cells

Mass spectrometry analysis identified interaction of RNH1 with histone deacetylases (HDAC1 and 2) and the master myeloid TF CEBP-a, indicating possibility of RNH1 mediated epigenetic regulation of myelopoiesis. Since RNH1 binds with HDAC1/2, in the RNH1 KO cells, the absence of this interaction may lead to an increase in available HDACs in the cell. Alteration in levels of total and acetylated form of histones between WT and RNH1 KO AML cells were also observed which encourages the hypothesis that interaction of RNH1 with HDAC1/2 induces epigenetic regulation of myeloid TFs, resulting in the observed phenotype of differentiation.

ACKNOWLEDGEMENTS

Schweizerischer Nationalfonds

INSELSPITAL UNIVERSITÄTSSPITAL BERN

krebsliga schweiz ligue suisse contre le cancer lega svizzera contro il cancro

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Presented at SOHC 2024 on 20-22 November 2024