



**Karolinska  
Institutet**

**Institutionen för Hematologi och Regenerativ Medicin**

**UNRAVELING NOVEL GENES THAT ARE ESSENTIAL FOR ACUTE MYELOID  
LEUKEMIA AND NORMAL HEMATOPOIESIS**

**AKADEMISK AVHANDLING**

som för avläggande av medicine doktorexamen vid Karolinska institutet offentligen försvaras i **GENE, NEO (Room 5108), plan 5**, Blickagången 16, 141 52 Huddinge

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av

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## ABSTRACT

Acute myeloid leukemia (AML) is an aggressive blood cancer and the most common hematological malignancy among adults. This disease results from an abnormal expansion of myeloid progenitors that are blocked in differentiation. Malignant transformation arises from various genetic and epigenetic changes in hematopoietic stem cells (HSCs) that accumulate with age. The overall aim of this thesis was to discover and characterize novel factors that are essential for AML and normal hematopoiesis.

In study I and study III, our aim was to discover and characterize novel genes that are selectively essential for AML. For this reason, we performed high throughput large-scale shRNA-based screens on AML cell lines and on non-transformed hematopoietic FDCP cell line as control cells. Based on the screening results, we have identified Chromatin Remodelling Factor 4 (CHD4) and General Transcription Factor II-I Repeat Domain-Containing Protein (GTF2IRD1) as the most promising hits, showing a highly significant selective importance in AML cell growth. Loss of function studies including both shRNA or CRISPR-Cas9 technology in normal mouse and human hematopoietic cells and various leukemia cell lines showed that either CHD4 or GTF2IRD1 were selectively required for AML cell growth, however their loss did not significantly affect normal hematopoietic cells. The importance of GTF2IRD1 or CHD4 was found to be conserved in primary AML cells, including leukemia-initiating cells, using niche-like co-culture system. The importance of CHD4 in childhood AML was further supported by the fact that the shRNA-targeted childhood primary AML cells displayed a significantly lower level of engraftment when transplanted into a xenograft mouse model for AML. In addition, the downregulation of GTF2IRD1 in primary AML samples from both childhood and adult samples significantly prevented disease progression, compared to controls. The importance of CHD4 or GTF2IRD1 in AML was found to be associated with cell cycle progression via MYC and its target genes. Finally, using a heterologous reporter system we showed that GTF2IRD1 acts as a transcriptional repressor, *in vitro* and *in vivo*, but not for normal hematopoietic cells. Our data demonstrated that CHD4 is a novel epigenetic factor required for the maintenance of childhood AML.

In study II, we aimed to determine the role of Euchromatic Histone Lysine Methyltransferase 1 (EHMT1) in AML and to investigate the link to its homolog, EHMT2. Our *in vitro* data showed that both EHMT1 and EHMT2 were required for growth of various leukemic cell lines. CRISPR-Cas9 based individual knock-out of EHMT1 and EHMT2 prevented the growth of AML cell lines to a similar degree, while double knock-out of EHMT1/2 did not show any additive or synergistic effect in growth of AML cell lines. Moreover, downregulation of EHMT1 using shRNA caused a remarkable reduction in engraftment of both childhood and adult primary AML cells in recipient mice, suggesting that suppression of EHMT1 can prevent AML disease progression. Although downregulation of EHMT1 or EHMT2 caused a block in the G0 phase of the cell cycle, we did not observe a significant increase in apoptosis. RNA-seq analysis showed that suppression of either EHMT1 or EHMT2 led to both overlapping and non-overlapping changes in gene expression linked to different biological processes.

In study IV, our goal was to identify and characterize novel factors in HSC function and normal hematopoiesis via bioinformatics analysis of various gene expression datasets of hematopoietic cells. From this analysis we identified an epigenetic factor, Nucleosome Assembly Protein 1 Like 3 (NAP1L3), that was consistently more highly expressed in HSCs compared to more mature cells. Loss of NAP1L3 function or overexpression of Nucleosome Assembly Protein 1 Like 3 (NAP1L3) caused a reduction in the number of colony-forming cells and myeloid progenitor cells *in vitro*. shRNA-mediated knock-down of NAP1L3 in umbilical cord blood (UCB) HSCs resulted in disruption of HSC maintenance and proliferation, both *in vitro* and *in vivo*. Moreover, loss of NAP1L3 function led to an impaired repopulation capacity of HSCs *in vivo*. Suppression of NAP1L3 in UCB HSCs results in block of cell cycle progression in the G0 phase and inhibits transcription of gene sets linked to cell cycle progression, including E2F and MYC. In addition, we also observed upregulation of HOXA gene clusters such as HOXA3, HOXA5, HOXA6, and HOXA9 genes upon downregulation of NAP1L3 in UCB HSCs.

In summary; we determined functionally relevant genes (CHD4, EHMT1 and GTF2IRD1) that are specifically required for AML without significant effects on normal hematopoietic cells. We thus suggested that these target genes may serve as potential therapeutic targets in AML treatment. In addition, we demonstrated an important role for NAP1L3 in HSC homeostasis and hematopoietic differentiation.