ELSEVIER

Blood 142 (2023) 2759–2760

The 65th ASH Annual Meeting Abstracts

POSTER ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

NK-2 Homeobox Transcription Factor NKX2-3 Confers Differentiation Block in NPM1-Mutated in Acute Myeloid Leukaemia

John A Chadwick[1], *Bettina Wingelhofer, PhD*[2], *Tim CP Somervaille, PhDFRCP FRCPath*[3]

[1] Cancer Research UK Manchester Institute, Manchester, United Kingdom
[2] Cancer Research UK Manchester Institute, Manchester, United Kingdom
[3] Cancer Research UK Manchester Institute, Manchester, GBR

Dysregulation of HOX homeobox family transcription factor (TF) gene expression is important in the pathogenesis of AML in particular in *NPM1*-mutated and *MLL* rearranged molecular subtypes. However, little is known about the role of NK2-homeobox (NKX2) family TF genes. NKX2 genes have critical roles in normal development of the central nervous system, thyroid, lung and pancreas. We observed high expression of *NKX2-3* in patient AML samples and hypothesised the transcription factor might have a functional role in the disease.

By qPCR, we found that *NKX2-3* is highly expressed in normal HSCs but is rapidly down regulated as cells differentiate. In primary AML samples, it is highly expressed in 21-37% cases, including within the immunophenotypic LSC compartment, in particular in cases with *NPM1* and/or *FLT3*-ITD mutations.

Forced expression of *NKX2-3* in normal murine KIT $^{+}$ bone marrow (BM) HSPCs transiently enhanced their clonogenic activity and impaired differentiation: *NKX2-3*-expressing HSPCs expressed higher levels of E2F or MYC target genes, and leukemia stem cell (LSC) maintenance genes. Congenic transplant experiments revealed that forced expression of *NKX2-3* in normal BM HSPCs was sufficient to enhance multilineage engraftment across four months of follow up without inducing leukemia. In keeping with this, transplantation of *Nkx2-3* $^{-/-}$ knockout BM cells led to significantly inferior engraftment compared with BM cells from litter mate control mice. Thus, while increased expression of *Nkx2-3* enhances engraftment of normal stem cells in transplantation assays, loss of *Nkx2-3* impairs it.

Analysis of *NKX2-3* ^{high} primary human AML cases demonstrated that *HOXA9* was the most highly upregulated transcription factor gene compared with *NKX2-3* ^{low} cases. We expressed both factors singly and together in murine KIT+ BM HSPCs: *Hoxa9/NKX2-3* cells exhibited significantly higher clonogenic activity and reduced morphologic and immunophenotypic differentiation compared with *Hoxa9/MTV* cells. Congenic BM transplant experiments demonstrated that *Hoxa9/NKX2-3* recipients developed leukemias significantly earlier than *Hoxa9/MTV* recipients (median 82 vs 155 days). BM morphology and immunophenotyping confirmed that *Hoxa9/NKX2-3*AMLs exhibited a greater degree of differentiation block than *Hoxa9/MTV* AMLs. Leukemia cells from *Hoxa9/NKX2-3* recipients coordinately expressed higher levels of E2F or MYC target genes, and LSC maintenance genes.

NKX2-3 KD to ~30% of control values reduced proliferation and induced morphologic and immunophenotypic differentiation in primary patient *NPM1*-mutated AML cells. *NKX2-3* KD led to down regulation of E2F and MYC target genes and normal human CD34 $^{+}$ stem cell genes; and up regulation of myeloid differentiation genes. Using a CRISPR ribonucleofection approach to relocate NPM1c to the nucleus we confirmed that *NKX2-3* expression in *NPM1*-mutant primary AML cells is sustained by mutant NPM1c.

ChIPseq in *NPM1*-mutated primary patient and KO52 AML cells identified NKX2-3 binding peaks. As expected, DNA sequences under peak apices were strongly enriched for NKX2-3 binding motifs; there was also significant enrichment for RUNX and ETS family motifs, but not HOX/MEIS/PBX motifs. To identify genes potentially regulated by NKX2-3, genomic coordinates of strong binding peaks were mapped to the single nearest gene. Remarkably, among genes up regulated following *NKX2-3* knockdown in primary human AML cells there was a highly significant enrichment of genes with putative regulatory elements bound by NKX2-3. Likewise, among genes down regulated in *NKX2-3*-expressing KIT+ murine HSPCs, there was a similar significant enrichment of genes with putative regulatory elements bound by NKX2-3. These data suggest that NKX2-3 functions as a transcription repressor at bound regulatory elements in primary AML cells.

In summary, *NKX2-3* is highly expressed in *NPM1* & *FLT3* mutated molecular subtypes of AML and serves to enhance self-renewal and the level of differentiation block through binding to and repressing regulatory elements of myeloid differentiation genes.

Disclosures Somervaille: *Glaxo Smith Kline:* Consultancy; *CellCentric Ltd:* Research Funding; *Oryzon Genomics:* Consultancy; *Imago BioSciences, Inc., a subsidiary of Merck & Co., Inc.:* Research Funding; *Abbvie:* Consultancy; *Novartis:* Consultancy; *Rain Oncology:* Consultancy; *Bristol Myers Squibb:* Consultancy.

https://doi.org/10.1182/blood-2023-182990