Deciphering molecular heterogeneity in pediatric AML using a cancer vs. normal transcriptomic approach

Depreter, Barbara; De Moerloose, Barbara; Vandepoele, Karl; Uyttebroeck, Anne; Van Damme, An; Terras, Eva; Denys, Barbara; Dedeken, Laurence; Dresse, Marie-Françoise; Van der Werff Ten Bosch, Jutte; Hofmans, Mattias; Philippé, Jan; Lammens, Tim

Published in:
Pediatric Research

DOI:
10.1038/s41390-020-01199-3

Publication date:
2021

Document Version:
Other version

Deciphering Molecular Heterogeneity in Pediatric AML Using a Cancer vs Normal Transcriptomic Approach

BARBARA DEPRETER,[1,2] Mattias Hofmans,[1,2] Eva Terras,[1] Anne Uyttebroeck,[3] An Van Damme,[4] Alina Ferster,[5] Jutte Van der Werff Ten Bosch,[6] Marie-Françoise Dresse,[7] Karl Vandepoele,[2,8] Barbara De Moerloose,[1,2,9] Jan Philippé[2,7,9*] and Tim Lammens[1,2*]

*Shared last authors

[1]Department of Internal Medicine and Pediatrics, Ghent University, Ghent, Belgium
[2]Cancer Research Institute Ghent, Ghent, Belgium
[3]Department of Pediatrics, University Hospital Gasthuisberg, Louvain, Belgium
[4]Department of Pediatric Hematology Oncology, University Hospital Saint-Luc, Brussels, Belgium
[5]Department of Pediatric Hematology Oncology, Queen Fabiola Children's University Hospital, Brussels, Belgium.
[6]Department of Pediatric Hematology Oncology, University Hospital Brussel, Brussels, Belgium
[7]Department of Pediatric Hematology Oncology, University Hospital Liège, Liège, Belgium
[8]Department of Laboratory Medicine, Ghent University Hospital, Ghent, Belgium
[9]Department of Pediatric Hematology-Oncology and Stem Cell Transplantation, Ghent University Hospital, Ghent, Belgium
[10]Department of Diagnostic Sciences, Ghent University, Ghent, Belgium

Introduction and Aim

Although cytogenetics and response-guided therapy have considerably improved prognostication of pediatric AML (pedAML) patients, still 30-40% of the good responders relapse. Further delineation of the transcriptome of AML subpopulations, e.g. leukemic stem cells (LSCs), might result in a better understanding of pedAML biology and provide novel biomarkers for diagnostics, risk stratification, follow-up and targeted therapy.

Methods

Fluorescence-activated cell sorting (FACS) was used to isolate CD34+CD38- and CD34+CD38+ cells from pedAML patients/healthy controls (cord blood (CB), normal bone marrow (NBM)), defined as LSC/hematopoietic stem cell (HSC) and leukemic blast (L-blast)/control blast (C-blast), respectively. Sorting multiple phenotypes, both BM and blood, yielded 42 LSC and 35 L-blast fractions. Gene expression profiles (GEP) of LSCs and L-blasts were identified by a Cancer vs Normal (CvN) approach, whereas paired analysis of LSC vs L-blast aimed to identify LSC-specific aberrations. Micro-array analysis (4 pedAML, 3 CB) was followed by targeted quantitative PCR (qPCR) validation of the highest differentially expressed genes (DEGs) in LSC (n=52), L-blast (n=42) and between LSC and L-blast (n=15) (25 pedAML, 11 CB/9 NBM). DEGs were functionally analysed by protein association (STRING) and by gene set enrichment analysis (GSEA-Cytoscape). An overview of the workflow is shown in Fig. 1A.

Results

LSC vs HSC micro-array analysis revealed 83 up- and 212 downregulated targets (Fig. 1B). qPCR confirmed 8 and 11 of the 52 tested targets to be highly significantly up- and downregulated, respectively, in LSC (n=42) compared to HSC (n=20) (P<.001). Overexpressed targets contained well-known oncogenes (e.g. *CFD*, *ANXA2*, *EMP1*), next to genes with undefined roles in AML (e.g. *PLIN2*, *CRIP1*). Six out of the top 30 downregulated DEGs were described as tumor suppressor genes (TSGs) in

solid tumors. Functional protein associations showed enriched cancer pathways, osteoclast differentiation, apoptosis and breast cancer, whereas Th17 cell differentiation and Rap1/MAPK signaling were suppressed in LSCs. LSC-enriched gene sets included inflammation, apoptosis, immune suppression and adipogenesis, while HSC-related signatures were anti-correlated.

L-blasts showed 157 and 332 up- and downregulated DEGs (Fig. 1C), and 8/42 were confirmed as significantly upregulated by qPCR (L-blast=35 vs C-blast=19, P<.001) e.g. *DUSP6*, *HOMER3* and *EMP1*. Functional analysis showed L-blasts to be associated with increased apoptosis and oxidative stress (FoxO signaling, cytokine-cytokine receptor interactions) compared to their normal counterparts.

Gene sets enriched in LSCs vs L-blasts addressed inflammatory responses, adipogenesis, TNF signaling and response. Pathway analysis showed repression of cell cycle genes, consistent with LSC quiescence. qPCR validation of 15 out of the 117 upregulated targets (Fig. 1D), of which 5/15 had a neural link (*PCDHB2, GPRIN3, SLC22A23, CDR1, RPGRIP1L*), did not confirm significant dysregulated expression (P>.05). Interestingly, the set of DEGs between LSCs and L-blasts shared only few genes with those differentially expressed between HSC and C-blast (28/306; 4 up- and 24 downregulated). This low intersection (7.7%) is in strong contrast to the previously reported 34% in adult AML (Gal et al. 2006). Moreover, none of those genes were detected in adult AML (except for CD38 downregulation). Remarkably, 3/4 mutual upregulated genes (*IGF2*, *GPRIN3*, *PROS1*, *CYTH4*) are involved in neural crossroads and have no relation to stemness nor AML.

Conclusion

Combining FACS with CvN transcriptomic profiling, followed by qPCR validation, identified novel DEGs in pedAML subpopulations. The majority of the most significant upregulated targets in LSCs (n=8) and L-blasts (n=8) showed no previous link to pedAML. Identification of 11 novel downregulated targets, often described as TSGs in solid tumors, warrants further studies whether hypomethylating therapy could result into LSC eradication in pedAML. LSCs reflected low cell cycle activity and elevated inflammatory response, hypoxia, metabolic dysregulation and signaling compared to HSC. GEP of LSCs vs L-blasts revealed a distinct molecular landscape compared to adult AML, and suggested a possible link between distortion of neural and hematopoietic systems in leukemogenesis.