ELSEVIER

Blood 142 (2023) 6064–6065

The 65th ASH Annual Meeting Abstracts

ONLINE PUBLICATION ONLY

618.ACUTE LYMPHOBLASTIC LEUKEMIAS: BIOMARKERS, MOLECULAR MARKERS AND MINIMAL RESIDUAL DISEASE IN DIAGNOSIS AND PROGNOSIS

The Role of Small Nucleolar RNAs As Putative Biomarkers of Chemoresistance in Pediatric Acute Lymphoblastic Leukemia

Olga Krali[1], *Anna Pia Enblad*[1,2], *Kristin Blom*[1], *Claes Andersson*[1], *Britt-Marie Frost*[2], *Josefine Palle*[2], *Arja Harila*[2], *Olli Lohi*[3,4], *Erik Forestier*[5,4], *Jukka Kanerva*[6,4], *Trond Flægstad*[7,4], *Ólafur G Jónsson*[8,4], *Kjeld Schmiegelow*[9,10,4], *Mats Heyman*[11,12,4], *Peter Nygren*[13], *Rolf Larsson*[1], *Gudmar Lönnerholm*[2], *Jessica Nordlund*[1]

[1] Department of Medical Sciences and Science for Life Laboratory, Uppsala University, Uppsala, Sweden
[2] Department of Women's and Children's Health, Uppsala University, Uppsala, Sweden
[3] Tampere Center for Child, Adolescent and Maternal Health Research, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland
[4] For the Nordic Society of Pediatric Hematology and Oncology (NOPHO), Stockholm, Sweden
[5] Department of Medical Biosciences, Umeå University, Umeå, Sweden
[6] Division of Hematology, Oncology and Stem Cell Transplantation, Helsinki University Central Hospital, Helsinki, Finland
[7] Department of Pediatrics, Tromsø University and University Hospital, Tromsø, Norway
[8] Pediatric Hematology-Oncology, Children's Hospital, Barnaspitali Hringsins, Landspitali University Hospital, Reykjavík, Iceland
[9] Department of Pediatrics and Adolescent Medicine, Rigshospitalet, Copenhagen, Denmark
[10] Medical Faculty, Institute of Clinical Medicine, University of Copenhagen, Copenhagen, Denmark
[11] Childhood Cancer Research Unit, Karolinska Institute, Stockholm, Sweden
[12] Astrid Lindgren Children's Hospital, Karolinska University Hospital, Stockholm, Sweden
[13] Department of Immunology, Genetics and Pathology, Uppsala University, Uppsala, Sweden

Background
Many previous studies have focused on the molecular landscape of pediatric acute lymphoblastic leukemia (ALL) with the aim to identify genetic changes related to disease development and outcome. However, the functional role of non-coding RNAs (ncRNAs) in leukemogenesis and treatment response is not fully understood. A group of ncRNAs known as small nucleolar RNAs (snoRNAs) regulates ribosomal biosynthesis, RNA splicing, and performs microRNA-like functions that affect post-translational gene expression. These functionalities can be dysregulated in cancer. The aim of this study was to explore the relationship between snoRNA expression and ex-vivo drug resistance in a cohort of pediatric ALL patients enrolled in the Nordic Society of Pediatric Hematology and Oncology (NOPHO) protocols.

Methods
We analyzed 119 B-ALL patients who were treated according to two consecutive NOPHO protocols (1992 and 2000). B-cells (CD19+) and T-cells (CD3+) from healthy blood donors were used as controls (n = 10). We stratified patients into low-, medium-, and high-resistance groups by ex-vivo drug screening using 10 commonly used chemotherapeutic drugs with the fluorometric microculture cytotoxicity assay (FMCA). RNA sequencing (RNA-seq) data were generated for all patients. Pairwise comparisons (Benjamini-Hochberg (BH) corrected Mann-Whitney U test) were conducted between the low and high resistance groups to detect differentially expressed genes. We selectively retained the differentially expressed snoRNAs for downstream analysis. BH corrected Kruskal-Wallis H and Mann-Whitney U tests were performed to compare the snoRNA expression levels for the three drug resistance groups, and between the ALL and control samples, respectively.

Results
We identified a set of 23 unique significantly upregulated snoRNAs (p-adjusted value < 0.05 and absolute log2FC>1) in the high-resistance group for one or multiple drugs: amsacrine (ams, n = 10), etoposide (eto, n = 10), tioguanine (thio, n = 8) and mitoxantrone (mito, n = 1). Among these genes, five snoRNAs (*SNORA30*, *SNORA33*, *SNORA36C*, *SNORA62* and *SNORA66*) were overexpressed in patients resistant to ams and eto, and one snoRNA (*SNORD27*) in patients resistant to ams and mito. Gene annotation revealed 22 significantly differentially expressed snoRNAs residing within the intronic region of 20

host genes, either protein coding (including ribosomal genes) or long ncRNAs. Three snoRNAs (*SNORD22*-eto, *SNORD26*-ams and *SNORD27*-ams and mito) arise from the same host gene (*SNHG1*). Of the 23 differentially expressed snoRNAs, the majority (n = 18) were not differentially expressed between the ALL and control samples (BH corrected Mann-Whitney U test p-value $\geq$ 0.05).

The expression of the host genes did not differ among the resistance groups (BH corrected Kruskal-Wallis H test p-value $\geq$ 0.05). However, we found that 14 of the host genes were differentially expressed between the ALL samples and controls (BH corrected Mann-Whitney U test p-value $<$ 0.05).

Conclusions

This study is one of the first to focus on the role of snoRNAs and ex-vivo drug resistance in pediatric ALL. Our findings highlight substantial variations in snoRNA expression across the three resistance groups, underscoring the potential role of snoRNAs in modulating drug response. Importantly, our analysis indicates that the differential expression of snoRNAs in the resistance groups cannot be solely attributed to host gene expression, implying that targeting pathways involving host genes might not be the most effective approach. Rather than concentrating on pathways involving host genes, our results suggest that understanding the mechanisms of action of snoRNAs could provide promising avenues for developing novel therapeutic targets to enhance drug response in pediatric ALL.

Disclosures Schmiegelow: *Novo Nordisk Foundation:* Current holder of *stock options* in a privately-held company, Research Funding; *Illumina, Jazz Pharmaceuticals, Servier, Amgen, Medscape:* Honoraria.

https://doi.org/10.1182/blood-2023-185687