LncRNA profiling reveals that the deregulation of H19, WT1-AS, TCL6, and LEF1-AS1 is associated with higher-risk myelodysplastic syndrome

Michaela Dostalova Merkerova[1], Katarina Szikszai[1], Zdenek Krejcik[1], Jiri Klema[2], Andrea Hrustincova[1,3], David Kundrat[1], Pavla Pecherkova[1], Jaroslav Cermak[1], Anna Jonasova[5] and Monika Belickova[1]

[1] Institute of Hematology and Blood Transfusion, Prague, Czech Republic, [2] Czech Technical University, Prague, Czech Republic,[3] Faculty of Science, Charles University, Prague, Czech Republic, [4] First Faculty of Medicine, Charles University, Prague, Czech Republic, [5] General University Hospital, Prague, Czech Republic

Introduction

Myelodysplastic syndrome (MDS) is a hematopoietic stem cell disorder with an incompletely known pathogenesis. Long noncoding RNAs (lncRNAs) play multiple roles in hematopoiesis and represent a new class of biomarkers and therapeutic targets, but information on their roles in MDS is limited.

Methods

- Microarray expression profiling and RT-qPCR of lncRNAs and protein-coding genes (PCGs) were performed in 183 CD34+ bone marrow cells of MDS patients and healthy controls.
- Expression profiles were analyzed in relation to different aspects of the disease (i.e., diagnosis, disease subtypes, cytogenetic and mutational aberrations, and risk of progression).
- LncRNA-PCG networks were constructed to link deregulated lncRNAs with regulatory mechanisms associated with MDS.

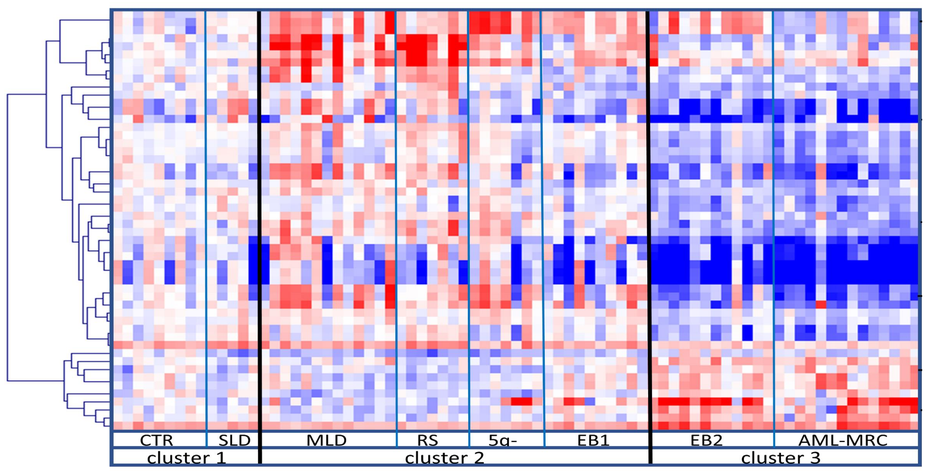
Aim of the study

We aimed to characterize lncRNAs deregulated in MDS that are involved in disease pathogenesis and may function as novel disease biomarkers.

Results

- **Several lncRNAs were strongly associated with disease pathogenesis (e.g., H19, WT1-AS, TCL6, LEF1-AS1, EPB41L4A-AS1, PVT1, GAS5, and ZFAS1).**

Figure 1. Heatmap of differentially expressed lncRNAs among MDS subtypes (FDR < 0.05). The analysis defined three clusters of samples with comparable expression profiles. The expression level is calculated as the binary logarithm of fold change (logFC) compared to the mean expression of controls. Blue - downregulation, red - upregulation, white - unchanged expression.

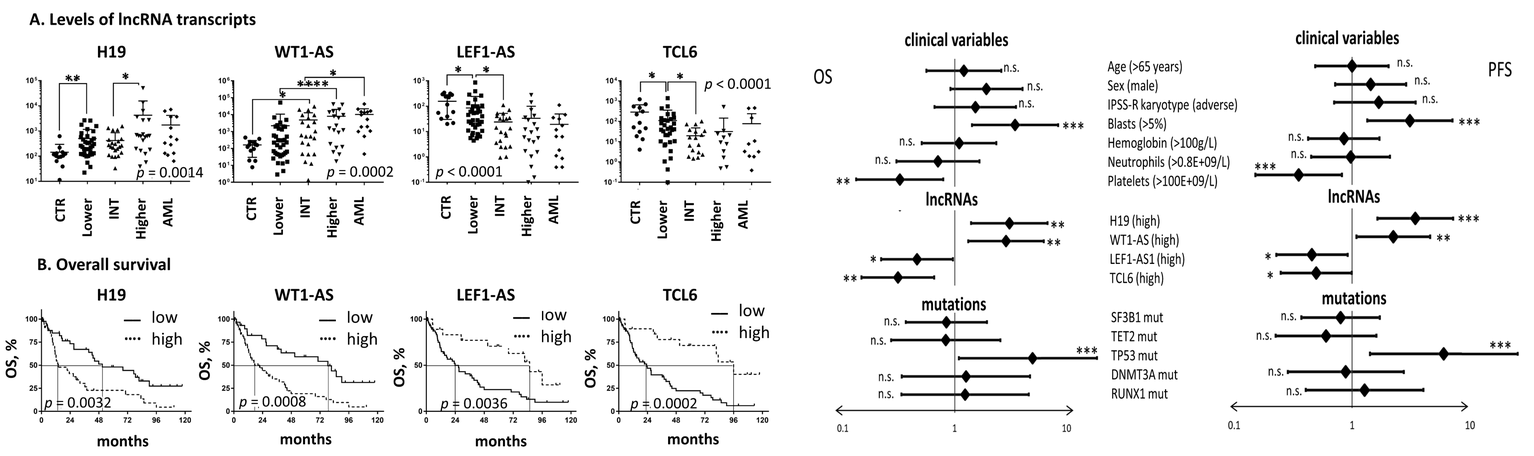


- **Downregulation of LEF1-AS1 and TCL6 and upregulation of H19 and WT1-AS were associated with adverse outcomes in MDS patients. Multivariate analysis revealed that the predominant variables predictive of survival are blast count (p < 0.001), H19 level (p = 0.015), and TP53 mutation (p = 0.004).**

Figure 2. (A) Expression of H19, WT1-AS, LEF1-AS, and TCL6 lncRNAs according to IPSS-R risk score.

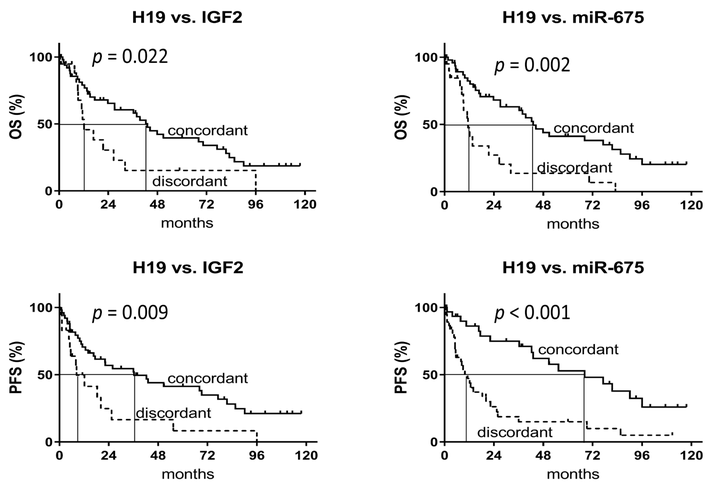
(B) Kaplan-Meier curves for overall survival of MDS patients stratified based on lncRNA levels.

(C) Univariate analysis for overall survival (OS) and progression-free survival (PFS) of MDS patients. Hazard ratios including 95% confidence intervals are plotted and the significance of the results is included (*p < 0.05, **p < 0.01, ***p < 0.001, n.s. - nonsignificant).

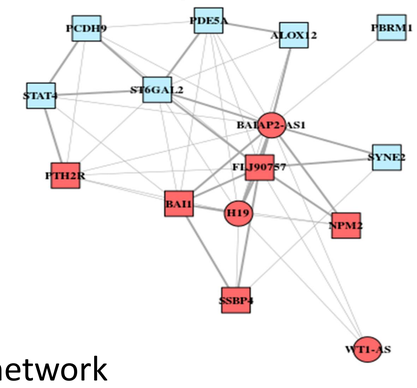


- **Transcriptional regulation in the H19/IGF2 region is disrupted in higher-risk MDS, and discordant expression in this locus is associated with worse outcome.**

Figure 4. Kaplan-Meier curves for overall survival (OS) and progression-free survival (PFS) in MDS patients with concordant vs. discordant expression of the H19/IGF2 and H19/miR-675 pairs.

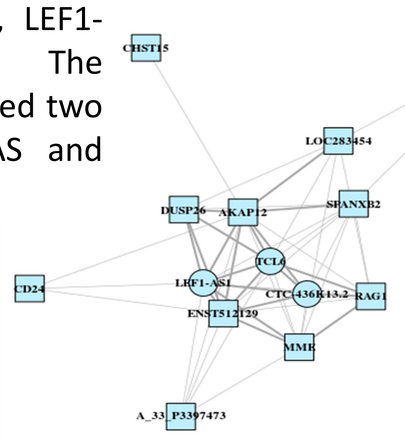


- **Coexpression network data suggested that prognosis-related lncRNAs are predominantly related to cell adhesion and differentiation processes (H19 and WT1-AS) and mechanisms such as chromatin modification, cytokine response, and cell proliferation and death (LEF1-AS1 and TCL6).**



H19/WT1-AS_MODULE	
GO term	***p* value**
Cell junction organization	4.90×10^{-5}
Biological adhesion	8.60×10^{-5}
Positive regulation of cell differentiation	1.99×10^{-5}
Positive regulation of developmental process	3.06×10^{-5}

Figure 5. Coexpression network formed around H19, WT1-AS, LEF1-AS1, and TCL6 lncRNAs. The computational process generated two modules for the H19/WT1-AS and LEF1-AS1/TCL6 lncRNA pairs.



LEF1-AS1/TCL6_MODULE	
GO term	***p* value**
Protein-DNA complex	7.07×10^{-4}
Cell death	1.45×10^{-3}
DNA packaging complex	1.59×10^{-3}
Somatic cell DNA recombination	2.32×10^{-3}
Chromatin modification	3.30×10^{-3}
Positive regulation of cell adhesion	3.48×10^{-3}
Adaptive immune response	3.63×10^{-3}
Regulation of cell proliferation	5.04×10^{-3}
TNF-mediated signaling pathway	5.05×10^{-3}
Cellular response to cytokine stimulus	6.20×10^{-3}
Histone H4 acetylation	6.44×10^{-3}
Leukocyte homeostasis	8.04×10^{-3}
Apoptotic signaling pathway	9.64×10^{-3}
ER-associated ubiquitin dependent protein catabolic process	9.80×10^{-3}

Conclusion

We identified specific lncRNAs contributing to MDS pathogenesis and proposed cellular processes associated with these transcripts.

Of the lncRNAs associated with patient prognosis, the level of H19 transcript might serve as a robust marker comparable to the clinical variables currently used for patient stratification.

Supported by AZV CR (17-31398A), GA CR (20-19162S), and MZ CR (00023736).