Unravelling the biology of juvenile myelomonocytic leukaemia using transcriptomics

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SUMMARY

Juvenile myelomonocytic leukaemia is a rare and aggressive blood cancer occurring in early childhood. Research in the past decades mainly focused on identifying aberrations at the DNA level. Although our molecular knowledge about juvenile myelomonocytic leukaemia biology has steadily increased over the last years, haematopoietic stem cell transplantation is currently the only curative option. Unfortunately, the relapse rate after stem cell transplantation remains high and almost half of the children do not survive the disease, indicating that new therapeutic strategies are urgently required. To further elucidate the biology of the disease, we investigated gene expression levels of both coding and non-coding RNA molecules. This led to the identification of *LIN28B* and its co-regulated genes as central players in juvenile myelomonocytic leukaemia biology and opens the door for the development of new targeted therapeutics.
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INTRODUCTION

Juvenile myelomonocytic leukaemia (JMML) is a rare and aggressive stem cell disease of early childhood.[1] RAS activation constitutes the core component of oncogenic signalling. In addition, leukemic blasts in one-fourth of JMML patients present with monosomy 7, and more than half of patients show elevated age-adjusted foetal haemoglobin (HbF) levels. Haematopoietic stem cell transplantation is the current standard-of-care and results in an event-free survival rate of 50-60%, indicating that novel molecular-driven therapeutic options are urgently needed. Cancer research in the past decades mainly focused on aberrations in protein-coding genes at the DNA level. However, only 2% of the genome codes for proteins, whereas up to 75% of the human DNA is capable of being transcribed into RNA.[2] The majority of RNA transcripts should thus be classified as non-coding RNA and an overwhelming amount of research already showed their involvement in health and disease. Long non-coding RNAs (lncRNAs) are one of the most recently discovered classes of RNA genes with a minimum length of 200 nucleotides. Several lncRNAs have been discovered that play a crucial role in oncogenesis and point to the fact that non-coding regions should not be neglected when studying cancer biology.

LIN28B OVEREXPRESSION DEFINES A NOVEL FOETAL-LIKE SUBGROUP OF JMML

Using gene expression profiling in a series of 82 patient samples, we identified a previously unrecognised molecular subgroup characterised by high *LIN28B* expression.[3] *LIN28B* overexpression was significantly correlated with higher HbF levels, whereas patients with monosomy 7 seldom showed enhanced *LIN28B* expression (*Figure 1*). This finding gives

a biological explanation of why patients with monosomy 7 are rarely diagnosed with high age-adjusted HbF levels. In addition, this new foetal-like JMML subgroup presented with reduced levels of most members of the *let-7* microRNA family and showed characteristic overexpression of genes involved in foetal haematopoiesis and stem cell self-renewal. Lastly, high *LIN28B* expression was associated with poor clinical outcome in our JMML patient series but was not independent from other prognostic factors such as age and age-adjusted HbF levels. In conclusion, we identified elevated *LIN28B* expression as a hallmark of a novel foetal-like subgroup in JMML.

Interestingly, *LIN28B* is normally only active before birth. After birth, *LIN28B* is turned off but is known to be reactivated in different types of adult cancer. This specificity is an important feature for the development of new therapies. Indeed, the Holy Grail in cancer treatment is the development of drugs that only target the diseased cells but not the healthy cells. Since *LIN28B* is only active in cancer cells after birth, the possibilities for developing targeted therapies increase – also in JMML.

LIN28B IS OVEREXPRESSED IN SPECIFIC SUBTYPES OF PAEDIATRIC LEUKAEMIA AND REGULATES LNCRNA *H19*

Given the involvement of *LIN28B* in a variety of solid paediatric cancers, we conducted a meta-analysis of *LIN28B* levels using publicly available gene expression data of 1,361 paediatric leukaemia samples. Interestingly, this analysis revealed *LIN28B* overexpression in 102 childhood leukaemia patients (7.5%), suggesting oncogenic activity for *LIN28B* in the context of paediatric haematological diseases (*Figure 2*). As the mode of action of *LIN28B* during normal and malignant haematopoiesis is currently still largely unknown, we subsequently analysed the transcriptional consequences of *LIN28B* modulation on normal and malignant haematopoietic cells and identified the long non-coding RNA (lncRNA) *H19* as the first *LIN28B*-regulated lncRNA.[4]

THE LNCRNA LANDSCAPE IN JMML

After the identification that *LIN28B* expression is linked to the expression of lncRNA *H19,* we investigated expression profiles of 23,042 lncRNAs in a cohort of 44 JMML patients. We identified several lncRNAs that are differentially expressed between patients and healthy donors and between specific JMML subgroups. A total of 295 differentially expressed lncRNAs were identified between patient samples and healthy controls. Remarkably, through advanced bioinformatics we showed that the most important lncRNAs are strongly related to myeloid cell biology and networks deregulated in MLL-
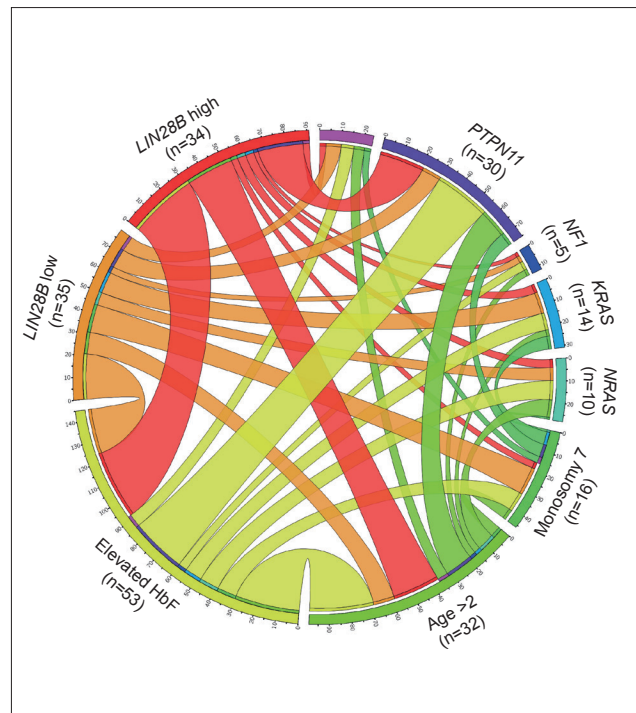

FIGURE 1. Circos plot representing the relationships among *LIN28B* expression, HbF level, age at diagnosis, monosomy 7, and *RAS* mutational status in 69 JMML patients with complete data available. Quadr. neg., quadruple negative. Figure originally published in *Blood*.[3]

rearranged paediatric leukaemia, and thus warrant further research. Diverse JMML subgroups show a distinct lncRNA expression profile, although the number of significantly differentially expressed genes is less than when comparing patients to healthy donors. However, interesting lncRNAs putatively involved in distinct biological processes, such as the regulation of foetal haemoglobin levels in JMML patients, could be identified. We hypothesise that a relatively straightforward mutational landscape interacts with a more complex landscape of lncRNAs in the pathogenesis of JMML. This paves the way for further functional research on the role of lncRNAs in JMML biology, and their diagnostic or therapeutic application.

CONCLUSIONS

To improve the survival rates, new therapeutics for JMML need to be generated and tested. Bottom-up therapeutic development requires a profound biological understanding of the disease, which is currently lacking. In the aftermath of The Human Genome Project, researchers realised that there is more to disease than DNA alone. Indeed, cancer development is a complex interplay between DNA, RNA, proteins, metabolites, methylation, and the environment.
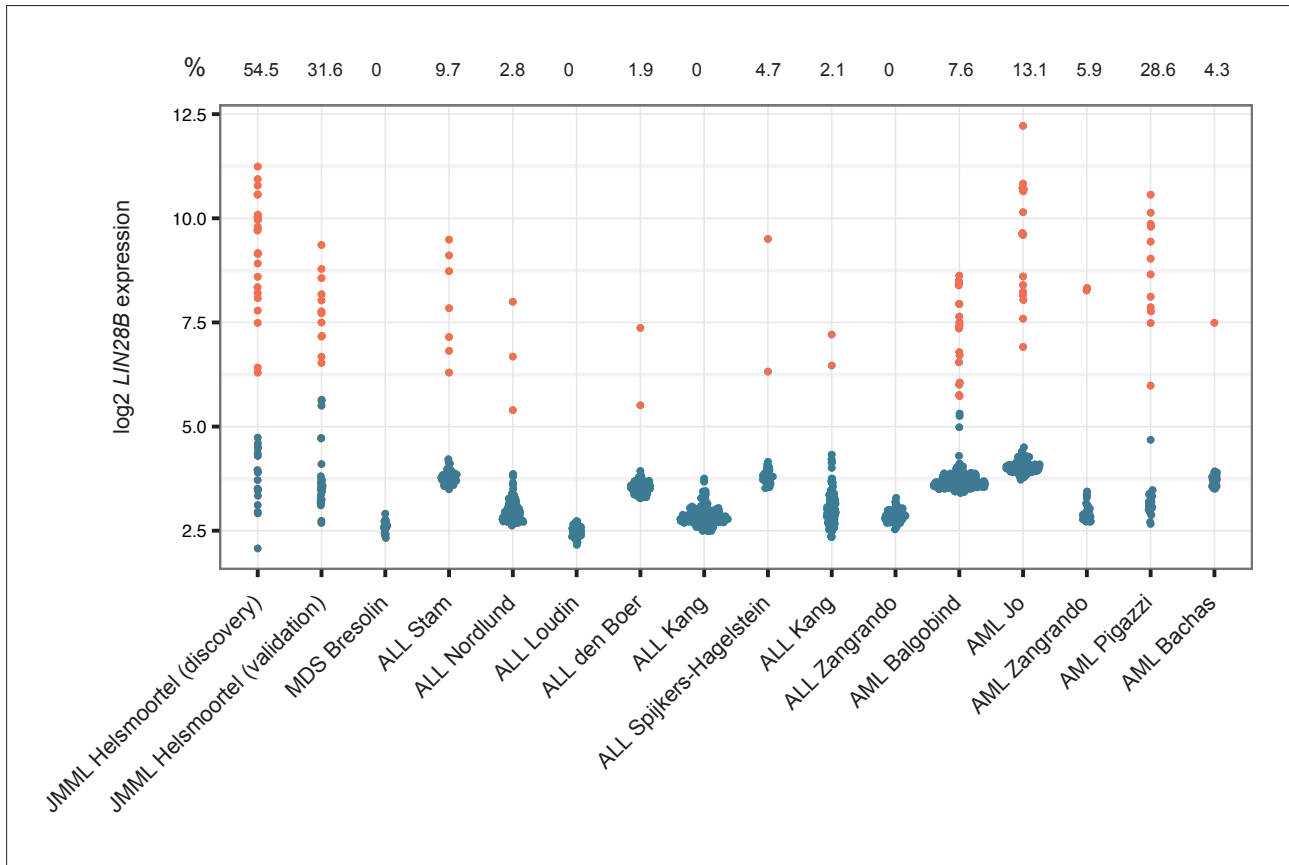
FIGURE 2. Log2 *LIN28B* expression values in fourteen publicly available paediatric leukemia datasets visualised using SinaPlot. Each dot is a patient and red dots represent values above the microarray average. The numbers on top represent the percentage of patients with *LIN28B* expression higher than the average of the study. Figure originally published in *Haematologica*.[4]

Because few studies analysed the coding and non-coding RNA expression in JMML, we aimed to unravel the biology of the disease using transcriptomics.

The generated results can fuel research in different scientific domains. First, the role of *LIN28B* overexpression in paediatric leukaemia should be further examined and its therapeutic applicability evaluated. Second, the identified upstream and downstream effectors of *LIN28B*, both coding and non-coding, can now be examined further. Finally, other researchers can use the datasets to look at them from a different perspective, since all data are publicly available.

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KEY MESSAGES FOR CLINICAL PRACTICE

1 *LIN28B* expression levels stratify JMML patients into groups with different clinical outcomes.

2 The identification of high *LIN28B* levels in different leukaemia entities, together with its absence in normal tissue after birth, make *LIN28B* an excellent target for development of novel, specific therapeutics.