EXPRESSION PATTERN AND TARGET PREDICTION OF miR-155 ON CHRONIC MYELOID LEUKEMIA

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INTRODUCTION

microRNAs have an important role in the study of cancer because differential expressions of oncomirs or tumor suppressors can not only be assessed as biomarkers but also as different prognostics reference. The identification of an expression pattern and which genes are regulated by those miRNAs may allow the development of new therapies and a better comprehension of the disease's development, determining if they are cause or consequence in this model. Chronic myeloid leukemia (LMC) is characterized by an excessive proliferation of the myeloid lineage, caused by a chromosomal translocation that generates the fusion gene BCR-ABL, which leads to the choice of the miR-155, as it has been experimentally validated as an important regulator of myelopoiesis.

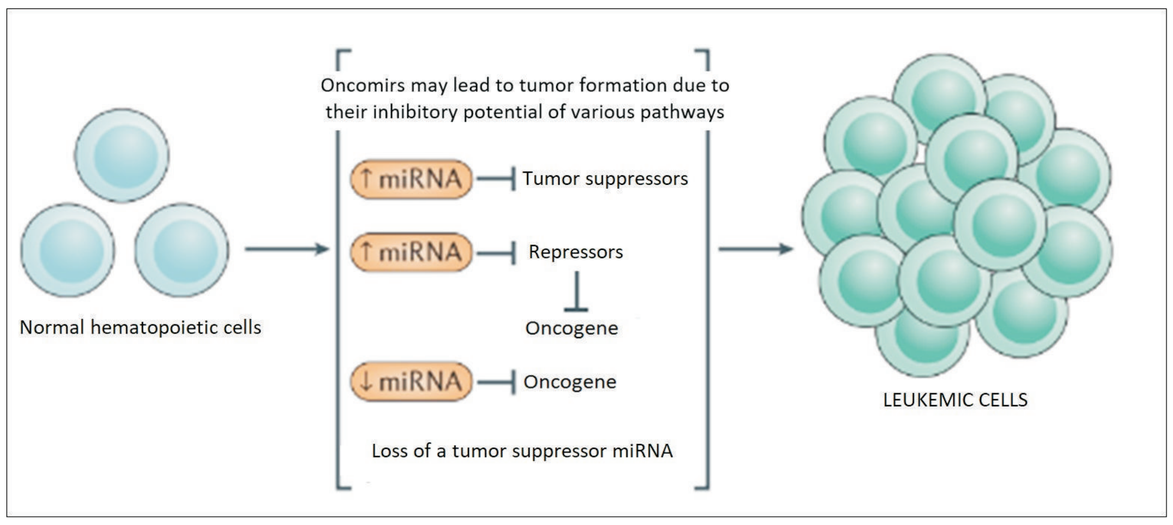


Figure 1. Known miRNAs mechanisms that may lead to tumoral formation. Adapted from Baltimore, B & Mehta, A (2016).

OBJECTIVES

✓ Elaborate an expression pattern of miR-155 in different stages of the treatment;

✓ Compare the differential expression using different controls (donators and cell lineages Kasumi and K562);

✓Determinate target genes using *in silico* analysis;

✓ Assess the expression of these target genes to provide evidence of the correlation to the miRNA.

PATIENTS AND METHODS

	Resistentes	Ao diagnóstico	Respondedores	Resp (não-TMO)
Homens	20	14	9	6
Mulheres	13	21	11	4
Total	33	35	20	10

Figure 2. Data from the patient's cohort.

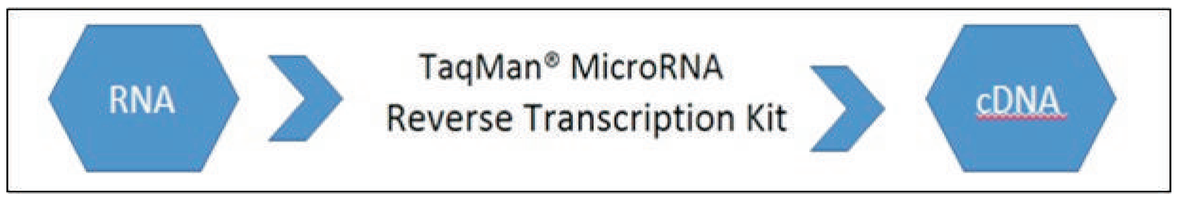


Figure 3. Methodology used for the quantification using peripherical blood.

The patients on the cohort were assessed by the method of Comparative CT on qRT-PCR, using the endogenous sno RNU48, which was chosen after stability assays measured by the softwares GeNorm and NormFinder. Analysis *in silico* were made to stablish genes related to the disease's pathways and the miRNA, which are to be analysed further through qRT-PCR.

RESULTS

To determine the most suited endogenous, we used a mixed cohort of 22 patients, and the statistical assessment showed that the most stable was the sno RNU48, which we so used as endogenous on further analysis.

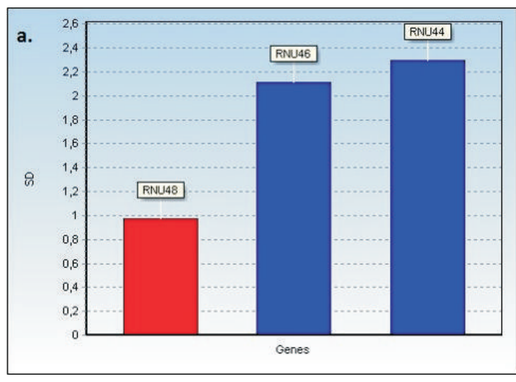


Figure 4. Analysed by NormFinder.

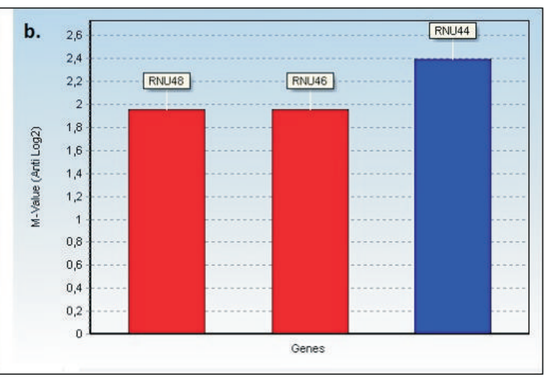


Figure 5. Analysed by GeNorm.

Our results showed overexpression (p<0,0001) of 1 log on both recently diagnosed and unresponsive groups. Analysis using lineage cells as control showed slightly smaller overexpression (p>0,0001). It was also observed significant difference between the responsive and responsive after transplantation groups, suggesting predictive relation, that may be used for treatment decisions.

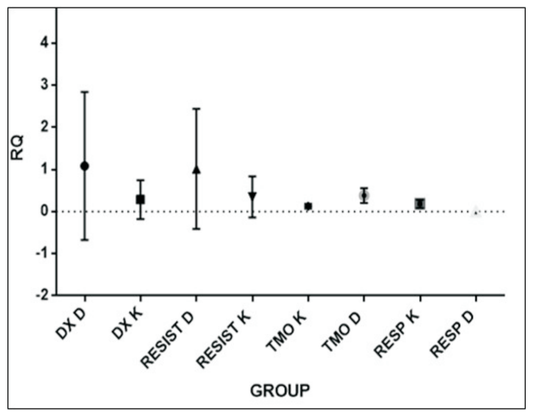


Figure 6. Comparative plot between the controls.

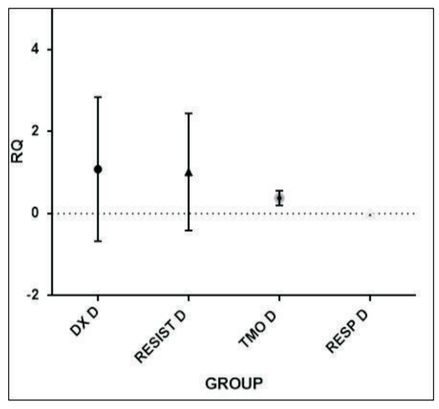
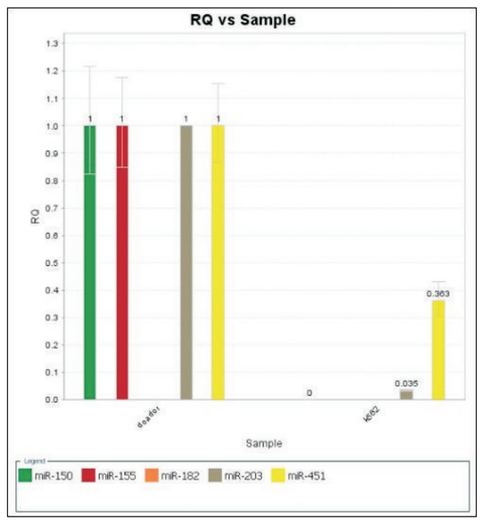


Figure 7. Comparative plot between the groups of patients.



After verifying the expression absence of the referred miRNA on the K562 lineage on our samples, we realized a new assessment to validate these data, comparing to other expression patterns known on literature. The lineage we used showed the expected results for the other miRNAs, again not expressing the target of this study (miR-155).

Figure 8. Expression of the assessed mi-RNAs on K562 lineage.

CONCLUSION

Oppositely to the literature, that uses lineage cells of LMC (K562) to assess miRNA expression, we used blood from patients and donators as samples and controls, what may explain our findings on overexpression, that being an particularity of the lineage microambient that biased the analysis. With our next step to be the analysis of the expression of related to the disease's pathways genes, we intend to show a relation between the overexpression and the role of these genes on the unresponsive group, leading to a better comprehension of the mechanisms of resistance.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA