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ORIGINAL ARTICLE

Transcriptomic differences of leukemic stem cells with profiles CD99 high and t(8;21)

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Abstract

Background: *Leukemic stem cells (LSC) are characterized by excessive proliferation, self-renewal capacity, and chemoresistance, these attributes contribute to the complexity of leukemia treatment.* **Objective:** *To identify differences in the transcriptome of two LSC profiles.* **Methods:** *RNA sequencing analysis was conducted on CD99 high and t(8;21) LSC profiles, with the results subsequently correlated with the overall survival of patients with myeloid leukemia.* **Results:** *The CD99 high profile was found to be regulated by senescence mechanisms, RNA modulation, WNT, RHO GTPASES, and interleukin 7 signaling pathways, whereas the t(8;21) cells by small molecule transport, RNA polymerase II, and G protein alpha and GPCR-mediated signaling pathways. Furthermore, high expression of 19 genes of the t(8;21) profile and 4 of the high CD99 profile correlated with poor survival in patients with myeloid leukemia treated with chemotherapy.* **Conclusion:** *This study provides novel information leading to the conclusion that the LSC profiles analyzed in this work exhibit divergent transcriptomic regulation that may benefit chemoresistant capacity in the context of myeloid leukemia.*

Keywords: *Leukemic stem cells. CD99. t(8;21). Chemoresistance. Prognosis.*

Diferencias transcriptómicas de células madre leucémicas con perfiles CD99 alto y t(8;21)

Resumen

Antecedentes: *Las células madre leucémicas se caracterizan por una proliferación excesiva, capacidad de autorrenovación y quimiorresistencia, lo que dificulta el tratamiento de la leucemia.* **Objetivo:** *Identificar diferencias en el transcriptoma de dos perfiles de células madre leucémicas.* **Método:** *Se realizó análisis de secuenciación de ARN de los perfiles de células madre leucémicas CD99 alto y t(8;21), como también correlación de esos resultados con la supervivencia general de pacientes con leucemia mieloide.* **Resultados:** *Se encontró que el perfil CD99 alto presenta regulación mediada por mecanismos de senescencia, modulación de ARN, las vías de señalización WNT, RHO GTPASES e interleucina 7, mientras que las células t(8;21) por transporte de moléculas pequeñas, ARN polimerasa II y vías de señalización mediadas por proteínas G alfa y GPCR. Además, la expresión alta de 19 genes del perfil t(8;21) y cuatro del perfil high-CD99 se correlacionó con una supervivencia pobre en pacientes con leucemia mieloide tratados con quimioterapia.* **Conclusión:** *Este estudio aporta información novedosa que permite concluir que los perfiles de células madre leucémicas analizadas en este trabajo presentan regulación transcriptómica divergente que puede beneficiar la capacidad quimiorresistente en el contexto de la leucemia mieloide.*

Palabras clave: *Células madre leucémicas. CD99. t(8;21). Quimiorresistencia. Pronóstico.*

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Introduction

Leukemic stem cells (LSCs) are chemoresistant and possess a high capacity for proliferation and self-renewal, surpassing hematopoietic stem cells (HSC) in cell population expansión[1]. One of the best-characterized profiles of LSC is the t(8;21) translocation, which is a common chromosomal abnormality in acute myeloid leukemia (AML) and results in the formation of the RUNX1-ETO fusion protein, which has been shown to interfere with the hematopoietic master regulator RUNX1[2]. In addition, other profiles have been observed, including the LSC subset which does not present the t(8;21) but exhibits CD99 high expression. The transmembrane protein CD99 is involved in several cellular processes, such as the regulation of apoptosis and cell adhesion. Furthermore, it has been demonstrated that AML patients with FLT3 translocation and CD99 high expression exhibit resistance to chemotherapy and targeted therapies[3].

This research project aimed to analyze the transcriptome of the LSC t(8;21) and CD99 high profiles, to identify differences in the modulation of the genes within each profile and their relationship with survival in AML patients treated with cytarabine + mitoxantrone or fludarabine + ARA-C and G-CSF.

Methods

This is a case-control study in which we independently compared RNA sequences of LSC profiles, CD99 high (eight samples) and t(8;21) (four samples), with CD34+, CD38-, and Lin- phenotypes against non-leukemic HSC samples with CD34+, CD38- phenotype (five samples). All datasets were obtained from the Gene Expression Omnibus with accession numbers GSE86506, GSE226592, GSE111410, GSE86506[4], GSE226592[5], and GSE111410[6] respectively.

Then, the normalized fragments per kilobase of transcript per million mapped reads of LSC-CD99 high or LSC-t(8; 21) derived from the above analysis were used for enrichment analysis, which was performed using gene set enrichment analysis software, accessible at the following link: https://www.gsea-msigdb.org/gsea/index.jsp (accessed May 25, 2024)[7]. Finally, the correlation between genes that restricted their expression to only one of the LSC profiles analyzed here with $p \leq 0.05$, and the overall survival of patients receiving chemotherapy (cytarabine + mitoxantrone or fludarabine + ARA-C and G-CSF), was performed using the online Kaplan-Meier analysis tool, available at (https://kmplot.com/analysis/index.php?p=service&cancer=aml, accessed on June 18, 2024). The specific statistical methods used are described in the supplementary data, annex 1.

Results

Gene sets enriched in the REACTOME database

Enrichment analysis performed to provide information about changes in the transcriptomics of LSC profiles identified pathways that exhibited exclusive positive enrichment in CD99-high or t(8;21) LSCs; however, no negatively enriched pathways were identified under these conditions. As illustrated in figure 1, CD99-high LSC demonstrated positive enrichment in 11 signaling pathways with the highest enrichment score observed for the DNA methylation pathway. While in the LSC t(8;21) profile, upregulation of six signaling pathways was found, including RNA polymerase II transcription, which had the highest enrichment of all pathways present in figure 1.

Unique differentially expressed genes (DEGs) in LSC t(8;21) or CD99-high

To provide further insight into the transcriptional differences between the two groups of LSC that were the subject of this study, we identified genes with a fold change of ≥ 1.5 and a $p \leq 0.05$ that were expressed at high levels in either CD99-high or t(8;21) LSCs. As illustrated in figure 2, 69 genes were identified in CD99-high LSC, whereas 341 genes were identified in t(8;21) LSC.

Relationship between genes unique to LSC CD99 high or t(8;21) and survival of AML patients undergoing chemotherapy

After identifying DEGs in the CD99-high, and LSC t(8;21) groups, the objective was to ascertain the relationship between the elevated expression of these genes and the reduced probability of survival in AML patients treated with cytarabine + mitoxantrone or fludarabine + ARA-C + G-CSF. However, in the absence of correlation, we evaluated all single genes in each profile with a significance threshold of $p < 0.05$.

Thus, we found that elevated expression of *CA10, KRT82,* and *KLHL1* genes, derived from the CD99-high LSC profile, was associated with a worse survival probability when AML patients were treated with cytarabine

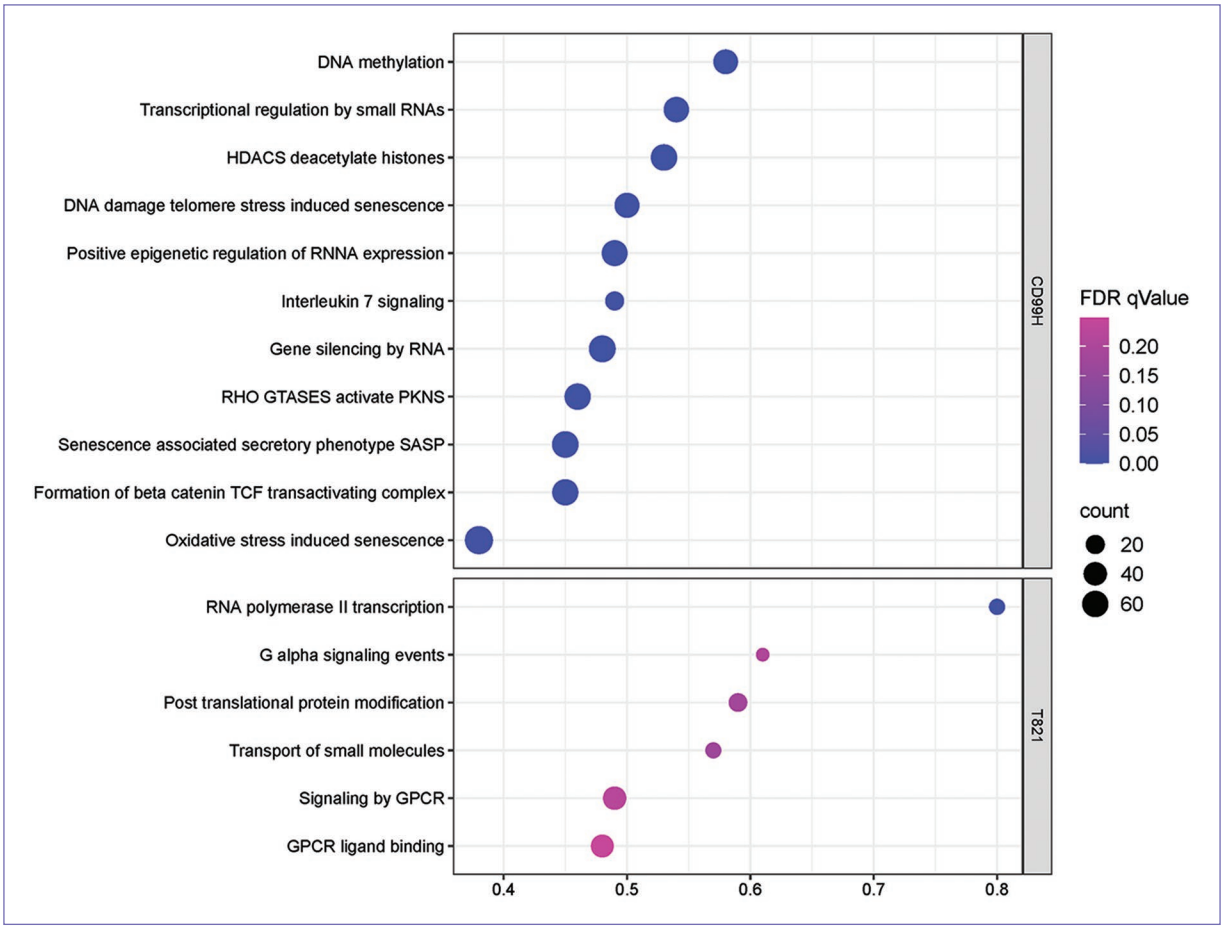

Figure 1. Positively enriched signaling pathways in the LSCs-high CD99 and t(8;21) profiles. The color scale represents the false discovery rate (FDR), and the circles indicate the number of enriched genes in each set. The x-axis of the graph represents the enrichment score for each gene set, ranging from 0 to 1.

and mitoxantrone, while OPCML presented the same pattern in patients treated with fludarabine + ARA-C + G-CSF (Fig. 3). Conversely, elevated expression of *ZP4, KCNA1, HHLA1, KLHL40, NT5C1A, PTH2, CHRM1, MAGEB6, TMEM174,* and *TUSC7* genes present in the LSC t(8;21) profile, correlated with reduced survival probability in patients treated with cytarabine + mitoxantrone or fludarabine + ARA-C + G-CSF (Fig. 4). In contrast, patients treated with fludarabine + ARA-C + G-CSF exhibited a reduced survival probability when *FGG, KISS1, GDF2, TRAG3, GALK2, PPY, HAO2, PLA2G2A, BPESC1,* and *UGT2A1* expression was elevated, as illustrated in figure 5.

Discussion

LSCs are characterized by uncontrolled proliferation, self-renewal, chemoresistance, and heterogeneity, which makes their study difficult[8]. In this work, we analyzed the transcriptome of two profiles of LSC: CD99 high and translocation t(8;21), with the aim of providing information that will help to understand molecular aspects of LSC and their implication in the survival of patients with AML.

These results revealed positively enriched pathways restricted to each profile of LSC; however, none of the negatively enriched pathways were exclusive to a single profile. We found 11 pathways in CD99 high LSC, as shown in figure 1, three of them related to processes involving RNA. Regarding transcriptional regulation by small RNAs, one study has demonstrated that small RNAs, including miR-125b and miR-29a, can promote features of LSC and induce AML in a murine model[9].

About to the epigenetic regulatory pathway of ribosomal RNAs, mutations in *RUNX1* result in a reduction in ribosomal gene levels, converting HSCs into

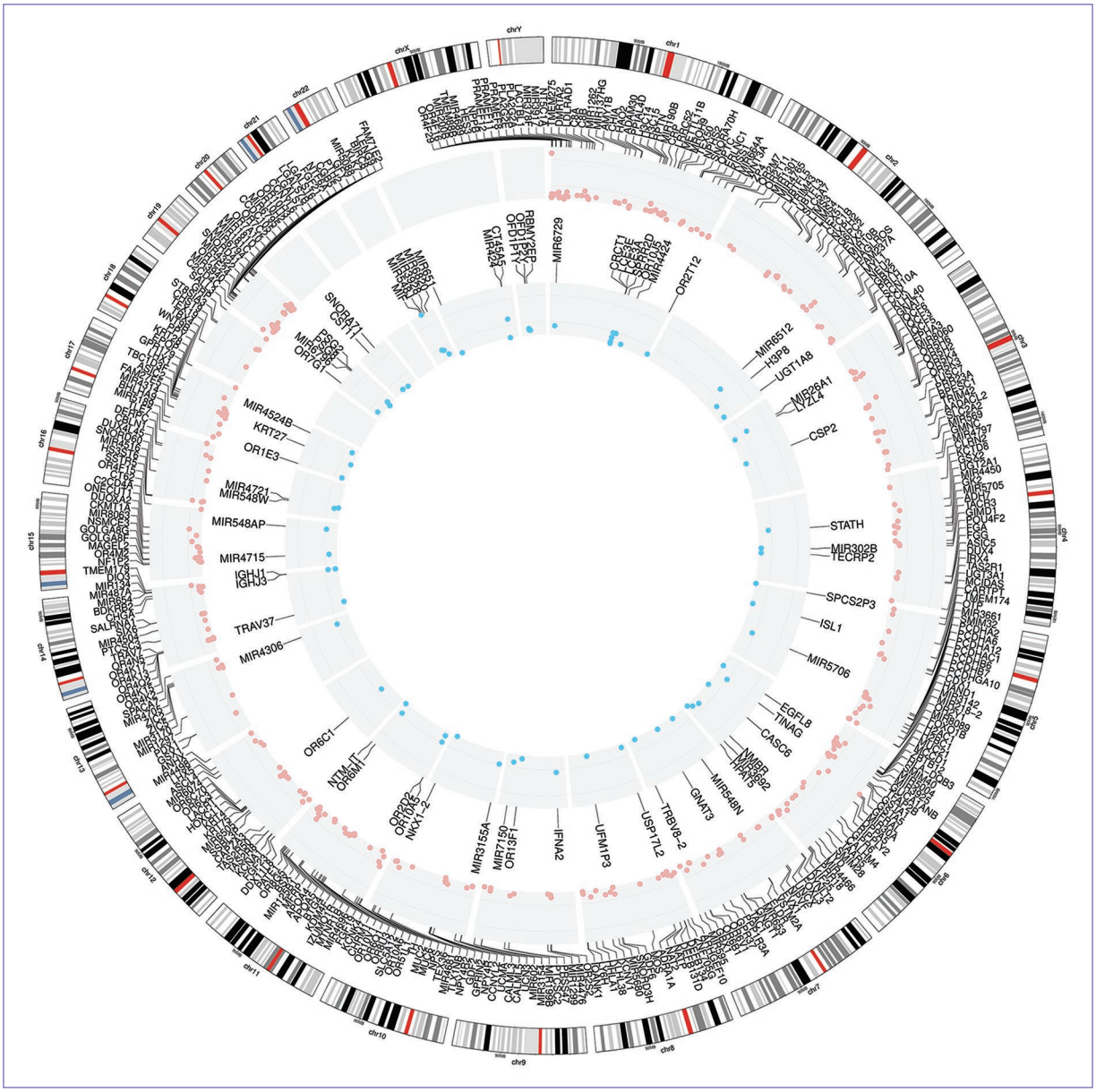

Figure 2. Multitrack plot of differentially expressed genes in LCSs-high CD99 or t(8;21) profiles. The inner ring indicates gene names with high expression in LCSs-high CD99 (represented by blue circles), and the next ring indicates gene names with high expression in LSCs t(8;21) (represented by pink circles). The outer ring represents a clockwise chromosome ideogram, with the size of each chromosome indicated in megabases (Mb).

pre-LSC[10]. Conversely, with regard to RNA gene silencing, it has been observed that the short hairpin RNA HOTAIR inhibits p15 expression, facilitating the self-renewal of LSC[11].

Another group of pathways in CD99 high LSC was related to the process of cellular senescence. Several studies suggest that HSC depletion is directly related to telomere shortening, which in turn leads to replicative cellular senescence and reprograms LSC, resulting in the acquisition of HSC characteristics[12,13]. Moreover, our findings suggest that senescence in CD99-high LSC may also be triggered by oxidative stress. This finding is consistent with previously published observations indicating that LSCs obtain a significant portion of their energy from mitochondrial oxidative respiration[14]. Furthermore, oxidative stress induces an SASP-mediated senescence phenotype, which was another pathway identified in CD99 high

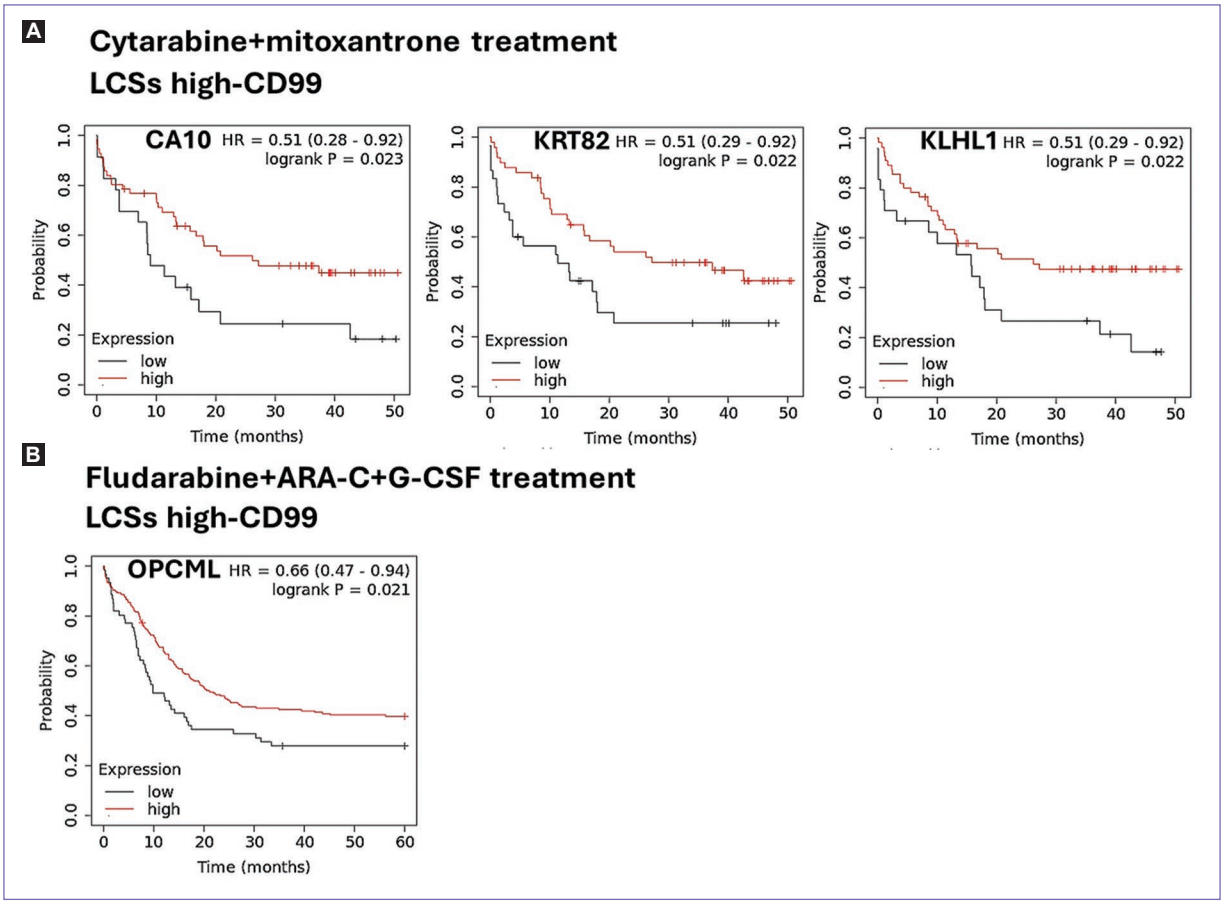

Figure 3. Overall survival analysis of genes derived from the LSCs high-CD99 profile using samples from AML patients. **A:** acute myeloid leukemia patients treated with cytarabine + mitoxantrone. **B:** acute myeloid leukemia patients treated with or fludarabine + ARA-C+G-CSF.

LSC and may contribute to tumor resistance through cell damage[15,16].

In addition, we observed positive DNA enrichment, and DNA methyltransferase and histone methyltransferases such as EZH2 and G9a have been shown to silence the expression of key genes for cell differentiation, thus facilitating the transformation of myeloid progenitors into LSC[17]. As for histone methylation and deacetylation (HDAC), HDACs, as cytostatic agents, induce acetylation of histone and non-histone proteins, which can result in cell cycle arrest, promotion of differentiation or apoptosis[18]. These aspects are crucial for preventing differentiation and maintaining LSC.

In the case of "Rho GTPases activate PKNS," even tyrosine kinase inhibitors (TKIs) have been developed, which have helped to improve the response to chemotherapy in patients with leukemia and other cancers. However, in murine models of chronic myeloid leukemia, LSC has been observed to exhibit resistant to TKIs by adapting to the microenvironment through mitochondrial respiration and maintenance of dormancy[19]. It can be hypothesized that this is a chemoresistance mechanism that is specifically utilized by CD99-high LSC. With regard to "Formation of beta-catenin TCF transactivating complex," there is strong evidence that the overactivation of WNT pathways is present in the transformation of pre-LSC into AML[20].

The last pathway restricted to this LSC profile was interleukin-7 (IL-7) signaling. Despite the lack of a direct link to LSC has not been established, but the IL-7 receptor is considered an early biomarker in T-acute lymphoblastic leukemia, with potential in leukemia-initiating cells[21]. Nevertheless, the role of IL-7 in the promotion of CD99 high LSC needs to be investigated.

By analyzing the LSC t profile (8;21), we identified five positively enriched pathways. One of these pathways is RNA polymerase II-mediated transcription, associated with rapid tumor cell replication. Prior research

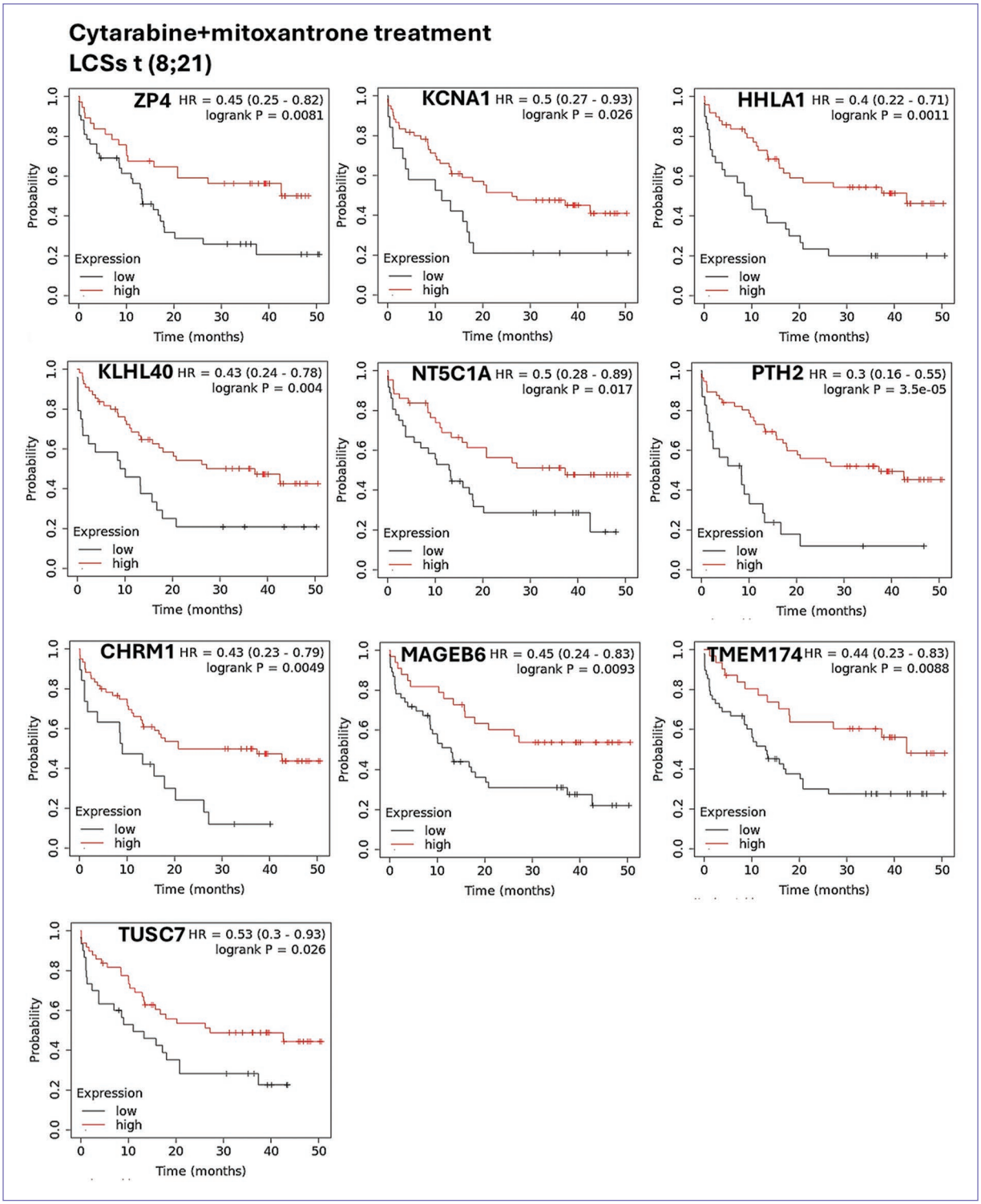

Figure 4. Overall survival analysis of genes derived from LSCs t(8;21) profile, using samples from AML patients treated with cytarabine + mitoxantrone.

has demonstrated that RNA polymerase II inhibitors induce apoptosis in the KG1a cell line (promyeloblasts), suggesting a role in the loss of latency and promotion of myeloid leukemia[22,23]. Another significant finding was the identification of G protein alpha-mediated signaling. The current evidence suggests that the phosphoinositide

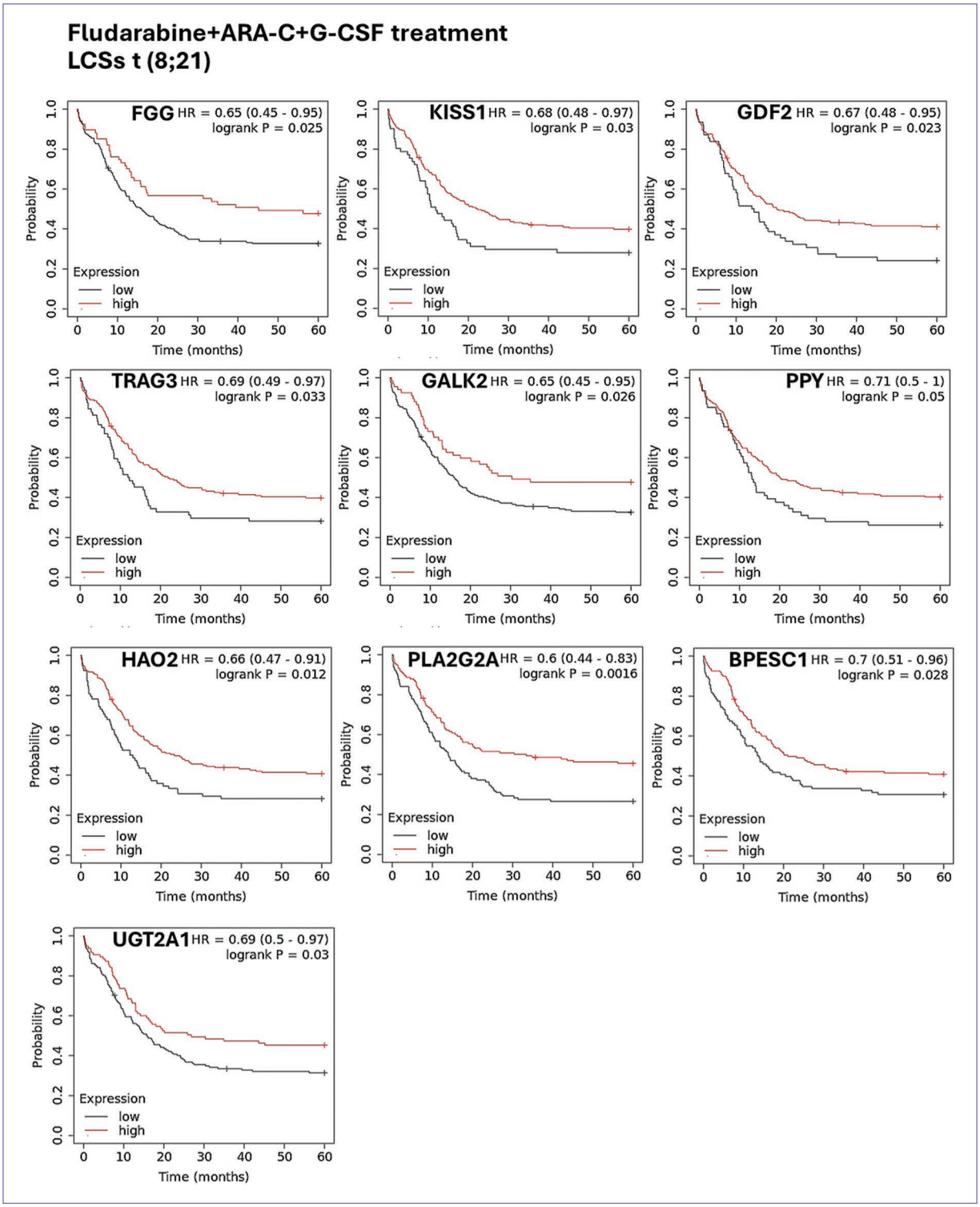

Figure 5. Overall survival analysis of genes derived from LSCs t(8;21) profile, using samples from AML patients treated with fludarabine + ARA-C+G-CSF.

3-kinase pathway, which is activated by G protein-coupled receptors, contributes to the regulation of LSC survival and proliferation[24].

With respect to the protein post-translational modification pathway, it has been observed that increased sumoylation is crucial for the maintenance and function

of HSCs and similar mechanisms could apply to LSC[25,26]. However, further research is required to elucidate the impact of these modifications on LSC and could even be considered an interesting perspective for the development of therapeutic strategies. One of the most interesting findings in the profiling of LSC (8;21) was the positive modulation of the "small molecule transport" pathway, which includes amino acids and lipids. This transport appears to confer to LSC the ability to adapt to stress conditions, such as chemotherapy[27,28].

On the other hand, when identifying the genes with statistically significant expression unique to each LSC profile, we found that the t-profile (8;21) exhibited a greater number of overexpressed genes not found in the profile of CD99 high (341 vs. 69). This is illustrated in figure 2. This finding, in conjunction with the enrichment analysis, suggests that these LSC profiles may have differential characteristics in their molecular modulation. Finally, the survival analyses performed with genes restricted to each LSC profile (Figs. 3-5), revealed that elevated expression of some of these genes is correlated with a lower probability of survival in AML patients treated with cytarabine and mitoxantrone, or fludarabine, ARA-C, and G-CSF.

This suggests that patients with poor response to treatments or relapse may exhibit elevated LSC t(8; 21) or CD99 elevated and that these specific genes contribute to the tumor microenvironment, implying a poor response and resistance to chemotherapy. However, this hypothesis should be extensively evaluated in future experimental studies, and to ascertain its potential prognostic utility.

Conclusion

This work demonstrates that LSC with t(8;21) or CD99-high translocation exhibit distinct transcriptomic profiles, reflected in the positive regulation of specific molecular pathways that have previously been associated with LSC, but not previously observed in a limited set of profiles. Furthermore, the correlation between elevated expression of specific genes in CD99-high or t(8;21) LSC profiles and a poor probability of survival in AML patients treated with cytarabine and mitoxantrone, or with fludarabine, ARA-C, and G-CSF, suggests that these LSC profiles may influence treatment response and chemoresistance. These findings underscore the necessity for further studies to validate the clinical relevance of these pathways and genes in the context of LSC and to investigate their potential as therapeutic targets and prognostic biomarkers in myeloid leukemia.

Limitations of the study

Although our initial aim was to analyze all possible LSC profiles in public databases, it was only possible to access data derived from the profiles we analyzed in this work. Furthermore, since these findings are strictly *in silico* analysis, we hope to be able to validate them experimentally in the future.

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Conflicts of interest

As sole author, I declare that I have no conflicts of interest.

Ethical disclosures

Protection of humans and animals. The author declares that no experiments were performed on humans or animals for this research.

Confidentiality of data. The author declares that no patient data appear in this article. In addition, the author has acknowledged and followed the recommendations according to the SAGER guidelines depending on the type and nature of the study.

Right to privacy and informed consent. The author declares that no patient data appear in this article.

Use of artificial intelligence to generate texts. The author declares that she has not used any type of generative artificial intelligence in the writing of this manuscript or for the creation of figures, graphs, tables, or their corresponding captions or legends.

Supplementary data

Supplementary data is available at DOI: 10.24875/j.gamo.24000066. This material is provided by the corresponding author and posted online for the benefit of the reader. The content of the supplementary data is the sole responsibility of the authors.

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