INVESTIGATION OF THE REGULATION BETWEEN MYC AND TOB2 IN RESPONSE TO CYTARABINE IN AML CELL LINES

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INTRODUCTION

Acute myeloid leukemia (AML) comprehends 80% of adult leukemias. The presence of high rate myeloid progenitors and high proliferation rate identifies the disease. The standard treatment for AML patients involves the association of anthracyclines with Cytarabine. Despite this strategy increases survival rate, patients often develop resistance to these drugs and relapse with a more complex karyotype. Although some mechanisms have been described, resistance to treatment has not yet been fully elucidated, mainly because of multifactorial resistance characteristics. Previous data demonstrated that MYC overexpression can be involved in resistance to Cytarabine in AML cell line HL60R (50µM Cytarabine resistant). MYC is a transcription factor that can regulate several pathways, mainly involved with cell cycle progression and inhibition of apoptosis. In this context, the antiproliferative protein TOB2, target of MYC, is mainly related to the cell cycle inhibition.

AIMS

Investigate the participation of the MYC transcription factor and the antiproliferative protein TOB2 in AML cell lines and in response to treatment with Cytarabine.

METHODOS AND RESULTS

Hl60 cell line presents higher expression of TOB2 mRNA in comparison to HL60R, U937 and Kasumi cell lines. Meanwhile, HL60R and HL60 cell lines exhibit higher MYC mRNA levels, compared to the other AML cell lines evaluated by Real time PCR. Western blotting evaluation demonstrated that U937 cell line exhibit high levels of TOB2 protein expression when compared to the other AML cell lines. Silencing MYC by siRNA for 72h hours in HL60R cell line reduced cell proliferation without altering cell cycle distribution, and without increasing annexin V positive cells or DNA fragmentation. In order to overexpress TOB2 in Kasumi and HL60R cell lines, we also developed a transient expression plasmid encoding the human TOB2 gene.

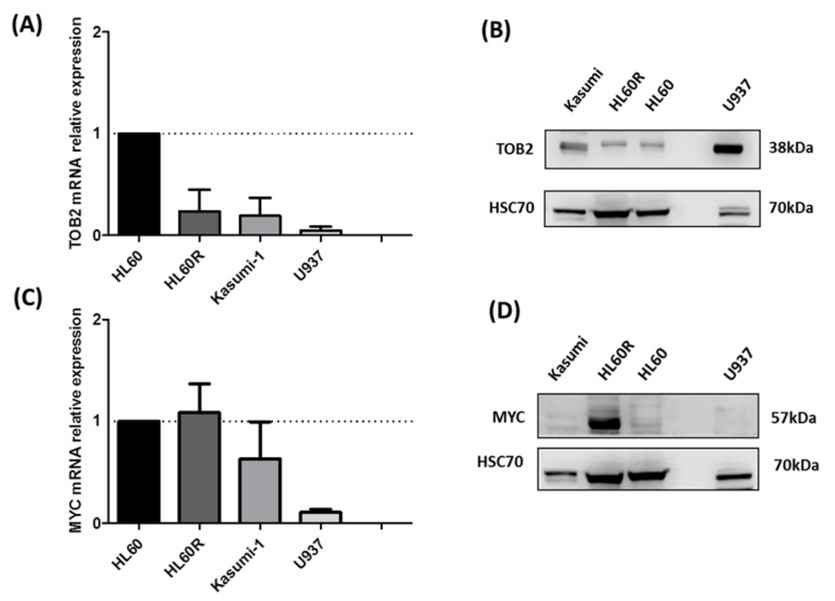

Figure 1: Expression of TOB2 and MYC in AML cell lines wih different responses to Cytarabine. TOB2 and MYC mRNA and protein levels were evaluated by Real time PCR and Western Bloting respectively in HL60, HL60R, Kasumi-1 and U937 AML cell lines. (A) Mean of TOB2 mRNA expression in AML cell lines in two independents expeiments. (B) TOB2 protein levels evaluation in AML cell lines. (C) Mean of three independent experiments of MYC mRNA expression and (D) MYC protein levels in AML cell lines.

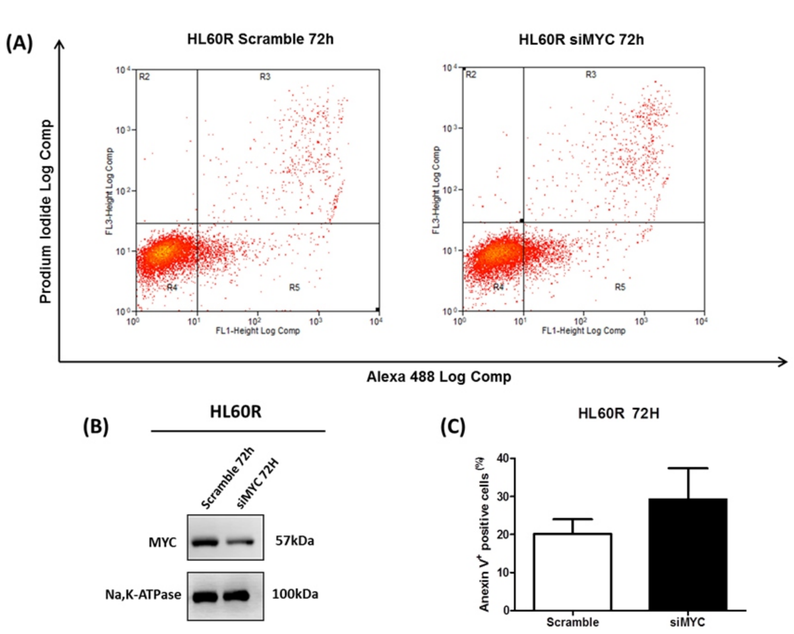

Figure 2: Evaluation of cell death after MYC silencing in HL60R cell line. Analysis of Anexin V positive cells and Anexin V and Propidium iodide (PI), in comparison to Scramble control (A and C), after 72 hours MYC protein was silencing (B) by siRNA. Analysis of double staining of Annexin V and Annexin / PI were considered the average of three independent experiments ± SD (C).

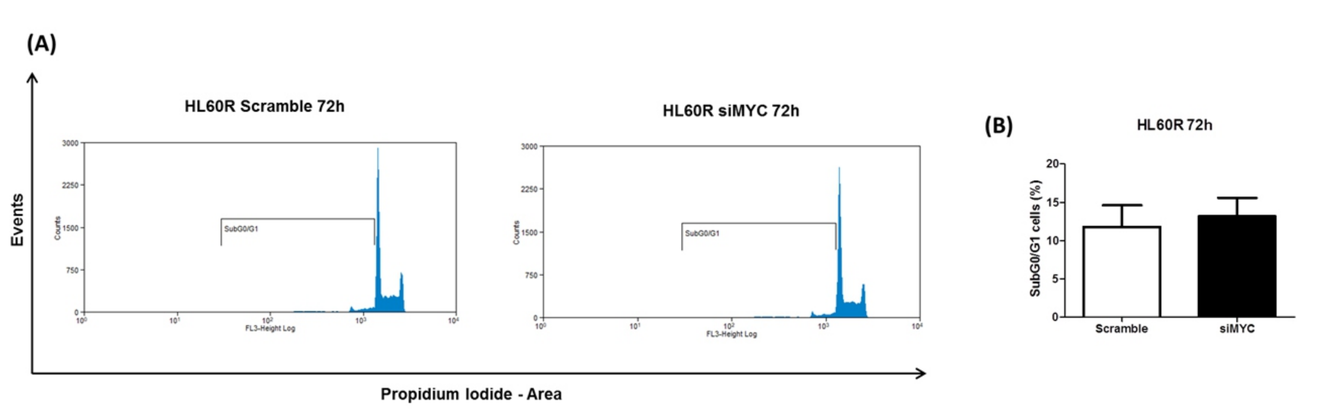

Figure 3: Evaluation of DNA fragmentation in HL60R cell line after MYC silencing. (A) DNA fagmentation analysis after 72hours of MYC silencing by siRNA. For these results were considered three independent experiments. Percentage of subG0/G1 cells after MYC silencing (B).

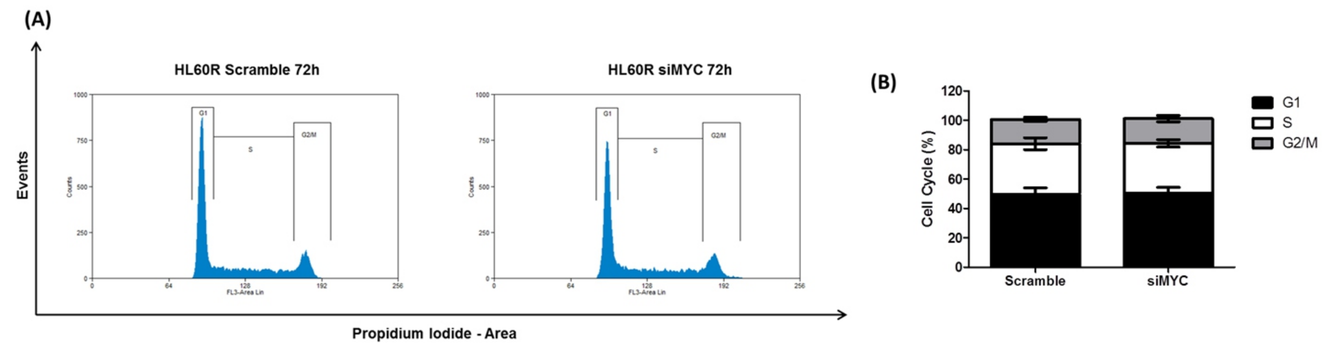

Figure 4: Evaluation of cell cycle distribution after siRNA MYC treatment for 72h. (A) Cell cycle distribution of HL60R cell line after 72 hour of siRNA MYC exposure (B) Mean of three independent experiments.

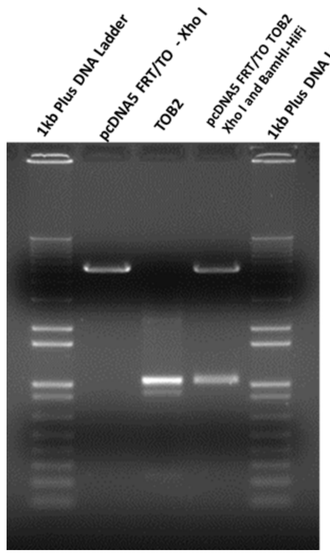

Figure 5: Plasmid encoding the human TOB2 gene. pcDNA5FRT/TO was used as a backbone to insert the TOB2 human gene and the plasmid contains Ampicilin resistance gene and restriction sites to Xho I and BamHI-FI restriction enzymes. Amplification of TOB2 human gene by specific primes followed by eletrophoresis indicating the empty vector in linear plasmid (pcDNA5FRT/TO – Xho I); TOB2 plasmid (TOB2); and TOB2 plasmid after incubation with restriction enzymes (pcDNA5FRT/TO TOB2– Xho I and BamHI-FI.

CONCLUSION

Together our data indicate that MYC silencing is not enough to induce cell death in resistant cell line HL60R, but it modulates HL60R cell line proliferation rate. Furthermore our results indicate that a regulation between MYC and TOB2 may exist in AML models.

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