INVESTIGATION OF THE ASSOCIATION BETWEEN MYC AND TOB2 IN IN AML CELL LINES

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INTRODUCTION

Acute myeloid leukemia (AML) comprehends 80% of adult leukemias. 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 of the mechanisms have been described, resistance to treatment has not yet been fully elucidated, mainly because of its multifactorial resistance characteristics. Previous data demonstrate that MYC overexpression could 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 cell cycle inhibition. The aim of the study is Investigate the role of MYC transcription factor and the antiproliferative protein TOB2 in AML cell lines and in response to treatment with Cytarabine.

METHODS

AML cell lines HL60, HL60R, Kasumi-1 and U937 were used to evaluate the basal levels of MYC and TOB2. Real time PCR and Western blotting were applied to evaluate mRNA and protein levels respectively. siRNA MYC were used to silence MYC in HL60R cell line for 72 hours and cell death was accessed by G0 analysis and Annexin V/PI assays. Trypan blue exclusion was used to investigate the proliferation after MYC silencing and cell cycle distribution was analyzed by flow cytometry. In order to develope a plasmid with TOB2 wild-type gene, a TOB2 gene from a non-tumoral cell line was used for cloning in an *E. coli* strain.

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 others AML cell lines evaluated. Western blotting evaluation demonstrated that U937 cell line exhibit high levels of TOB2 protein expression when compared to the others 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 bacterial plasmid with the transgene TOB2 wild-type. 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.

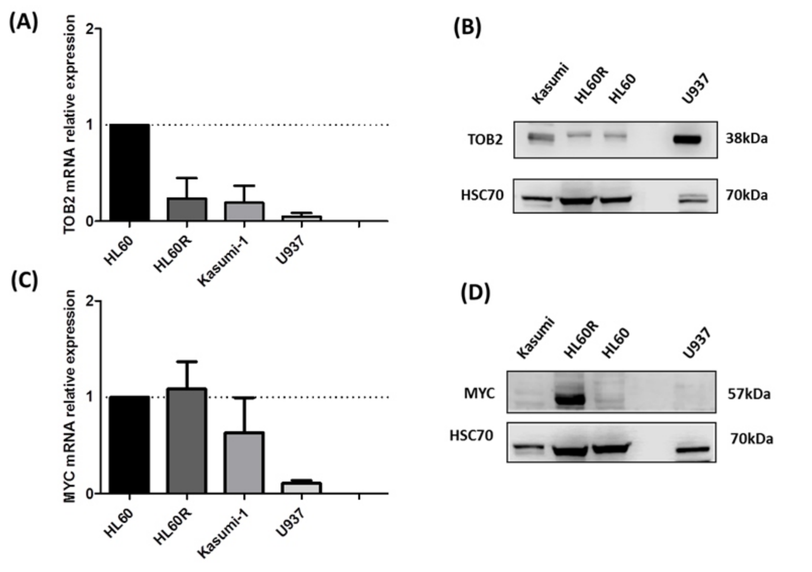

Figure 1 : Expression of TOB2 and MYC in AML cell lines wih different responses to Cytarabine. TOB2 and MYC mRNA levels and protein levels were evaluated by Real time PCR and Western Bloting respectively in HL60, HL60R, Kasumi-1 and U937 AML cell lines. (A) Mean of TOB2 mRNA expression in AML cell lines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

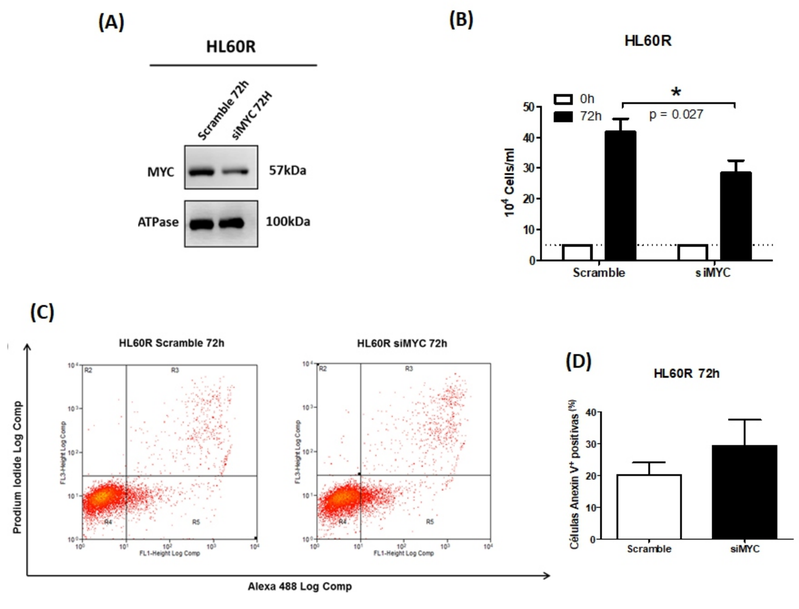

Figure 2: Evaluation of MYC silencing in HL60R cell line death. After 72 hours MYC protein was silencing (A) by siRNA MYC and compared with Scramble control. The silencing of MYC protein suggests a decrease of cells number (B) and an increase of Anexin V positive cells and Anexin V and Propidium iodide (PI) (C and D). Analyzes of double staining of Annexin V and Annexin / PI were considered the average of three independent experiments ± SD.

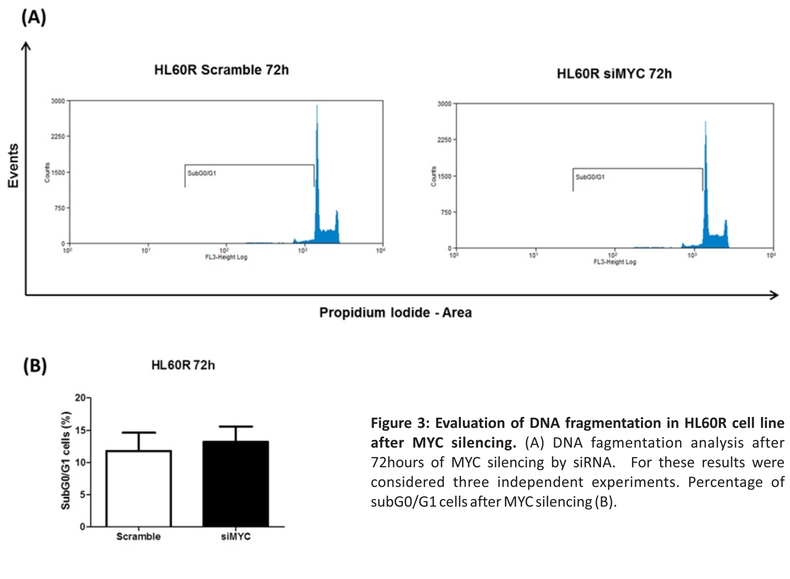

Figure 3: Evaluation of DNA fragmentation in HL60R cell line after MYC silencing. (A) DNA fagmentation analysis after 72hours of MYC silencing by siRNA. For these results were considered three independent experiments. Percentage of subG0/G1 cells after MYC silencing (B).

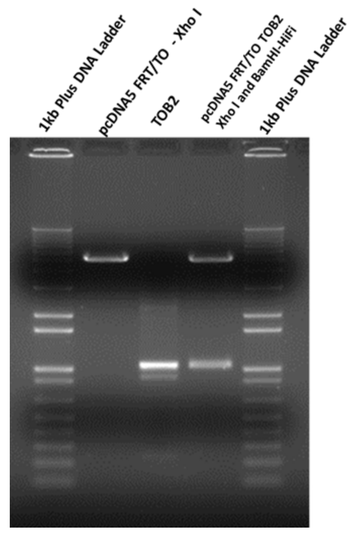

Figure 5: Plasmid encoding the human TOB2 gene. pcDNA5FRT/TO was used as a backbone to insert the TOB2 human gene and the plasmid contains Ampicilin resistance gene and restriction sites to Xho I and BamHI-FI restriction enzymes. TOB2 human gene was amplified by specific primes and purified to be inserted in the bacterial plasmid. After the cloning process the plasmid and TOB2 human gene were sequenced by Sanger sequencing to avoid possible mutations generated in the process.

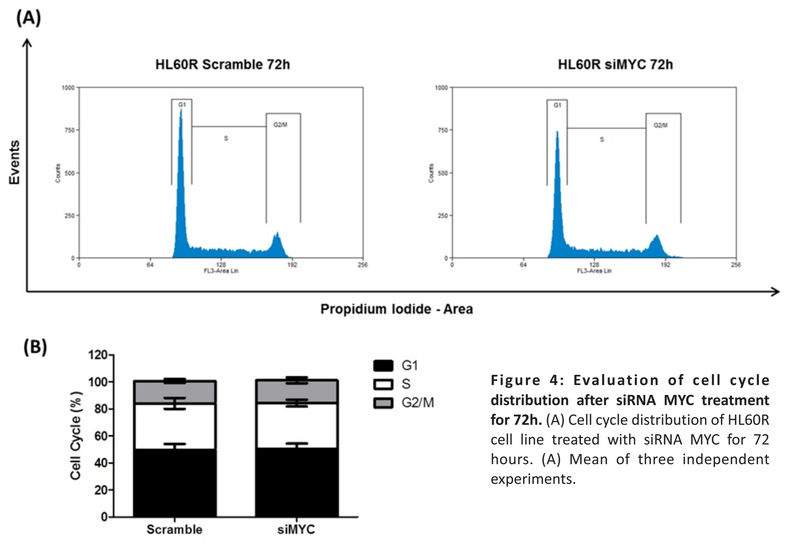

Figure 4: Evaluation of cell cycle distribution after siRNA MYC treatment for 72h. (A) Cell cycle distribution of HL60R cell line treated with siRNA MYC for 72 hours. (A) Mean of three independent experiments.

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