INVESTIGATION OF THE EFFECTS OF TOB2 MODULATION IN TUMOR AND NON-TUMOR CELL LINES AND THE ASSOCIATION BETWEEN MYC AND TOB2

THAIS HANCIO[1], MARCELA ROBAINA[1], RAFAELA FAGUNDES[2], LEONARDO KARAM TEIXEIRA[2], FERNANDA COSTAS CASAL DE FARIA[1], RAQUEL CIUVALSCHI MAIA[1]

[1]Laboratório de Hemato-Oncologia Celular e Molecular – Instituto Nacional de Câncer

[2]Laboratório de Biologia Celular – Instituto Nacional de Câncer

INTRODUCTION AND AIMS

Tob2 (*transducer of ERBB2, 2*) is a protein from BTG family mainly involved in mRNA deadenylation mediated by CCR4-Not Complex. The Tob2 binding to CCR4-Not complex can lead to the decrease expression of targets involved in cell cycle progression, for this reason Tob2 is considered as an antiproliferative protein and a tumor suppressor, although its role in tumor cells and tumor transformation is almost unknown. The aim of this study is to understand the effect of Tob2 modulation in tumor and non-tumor cells and to investigate the mechanism by which Tob2 is modulated in these cell lines.

MATERIALS AND 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 (Wb) were applied to evaluate mRNA and protein levels respectively. Human Tob2 was cloned to a plasmidial vector in order to evaluate the effect of transient overexpression in the cell lines. Tob2 overexpression was evaluated in tumor cell lines MDA-MB-231 (breast cancer), U251 (glioblastoma), H460 (lung cancer) and in non-tumor cell line HEK293T. The siRNA assay was performed to inhibit Tob2 in non-tumor cell line HEK293T. The proliferative effects of Tob2 overexpression or Tob2 inhibition were evaluated by crystal violet assay, clonogenic assay and Trypan blue assay. The association between Myc and Tob2 expression was investigated in tumor cells (MDA-MB-231 and U251) and in non-tumor cell line HEK293T after Tob2 overexpression and was also evaluated in the B-cell line P493-6 that present Myc promotor responsive to tetracycline.

RESULTS AND CONCLUSIONS

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. Wb evaluation demonstrated that U937 cell line exhibit high levels of Tob2 protein expression when compared to the others AML cell lines. Tob2 overexpression for 24h in MDA-MB-231 cells led to an increase of Trypan blue stained cells and a decrease of colonies formation in clonogenic assay. The opposite effect was observed in 72h Tob2 overexpression, demonstrating an increase on cell proliferation. Otherwise, Tob2 overexpression for 24h in H460 cell line led to increased cell proliferation and colonies formation. Tob2 inhibition for 24h in non-tumor cell line HEK293T increased colonies formation and proliferation. Tob2 overexpression in MDA-MB-231 and HEK293T showed a decrease of Myc protein levels in these cell lines, effect that was not observed in U251. The cell line P493-6 treated with tetracycline showed lower levels of Myc, concomitantly with increased levels of Tob2 protein. Taken together, our results demonstrate that Tob2 may have different effects in tumor and non-tumor cell lines. Furthermore, our results indicate that may exist a regulatory modulation feedback between Myc and Tob2.

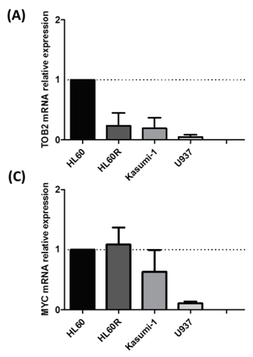

Fig 1. Expression of TOB2 and MYC in AML cell lines. TOB2 and MYC mRNA levels and protein levels were evaluated by Real time PCR and Western Bloting respectively in HL60, HL60R, Kasumi-1 and U937 AML cell lines. (A) Mean of TOB2 mRNA expression in AML cell lines in two independents expeiments. (B) TOB2 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.

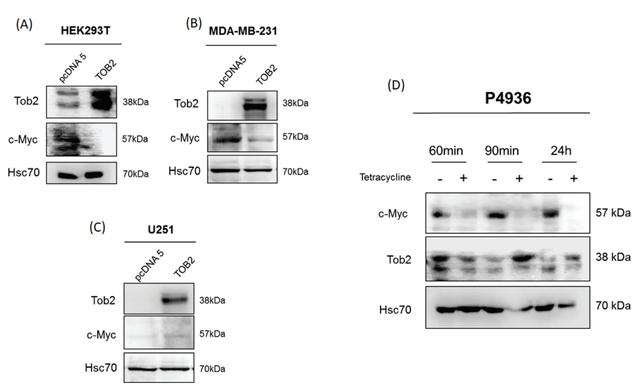

Fig 2. Regulation feedback between MYC and TOB2. Tob2 overexpression in non-tumor cell line (A) HEK-293T and in breast cancer cell line (B) MDA-MB-231 lead to a decrease in MYC protein expression. The Tob2-Myc reletionship is not observed in glioblastoma cell line (C) U251. The cell line (D) P493-6 treated with tetracycline showed lower levels of Myc, concomitantly with increased levels of Tob2 protein.

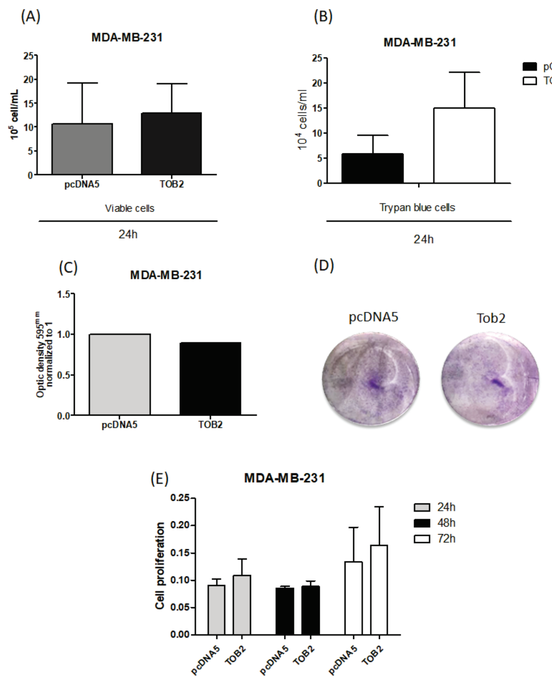

Fig 3. Tob2 overexpression in breast cancer cell line. (A and B) Tob2 overexpression in MDA-MB-231 cell line increase Trypan blue stained cells but did not change the number of viable cells. (C and D) The clonogenic potencial was reduced after Tob2 overexpression. (E) Cell proliferation increase was observed after 24, 48 and 72h after Tob2 overexpression.

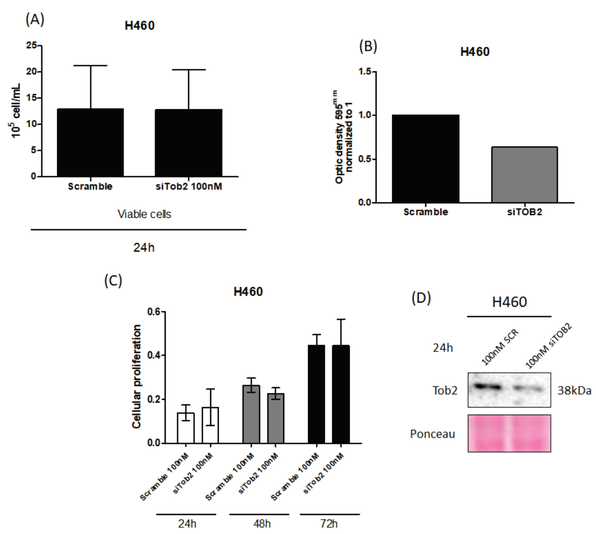

Fig 6. Tob2 inhibition in H460 cell line. Tob2 inhibition by siRNA do not change the number of viable cells (A), decrease the clonogenic potencial (B) and decrease cell proliferation until 48h evaluated (C and D).

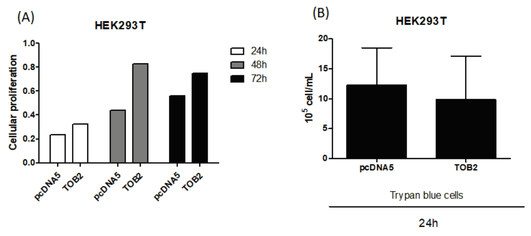

Fig 7. Tob2 overexpression in non-tumor cell line. Tob2 overexpression in HEK293T cells induce proliferation increase in 24, 48 and 72h (A) and reduce the trypan blue cells stained after 24h (B).

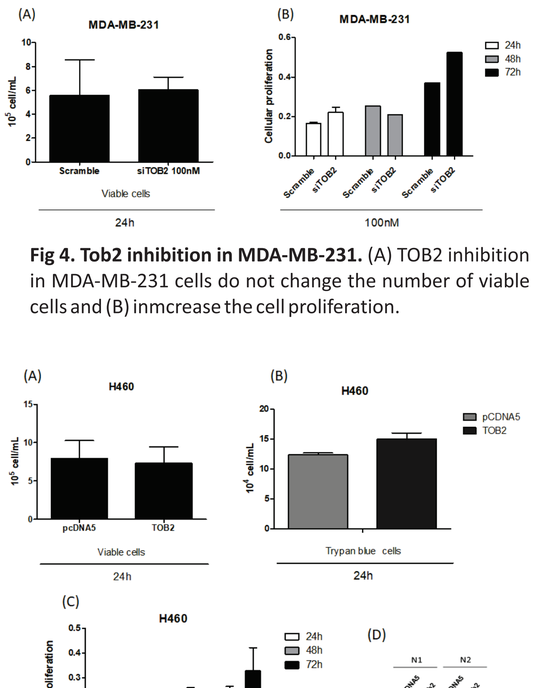

Fig 4. Tob2 inhibition in MDA-MB-231. (A) TOB2 inhibition in MDA-MB-231 cells do not change the number of viable cells and (B) inmcrease the cell proliferation.

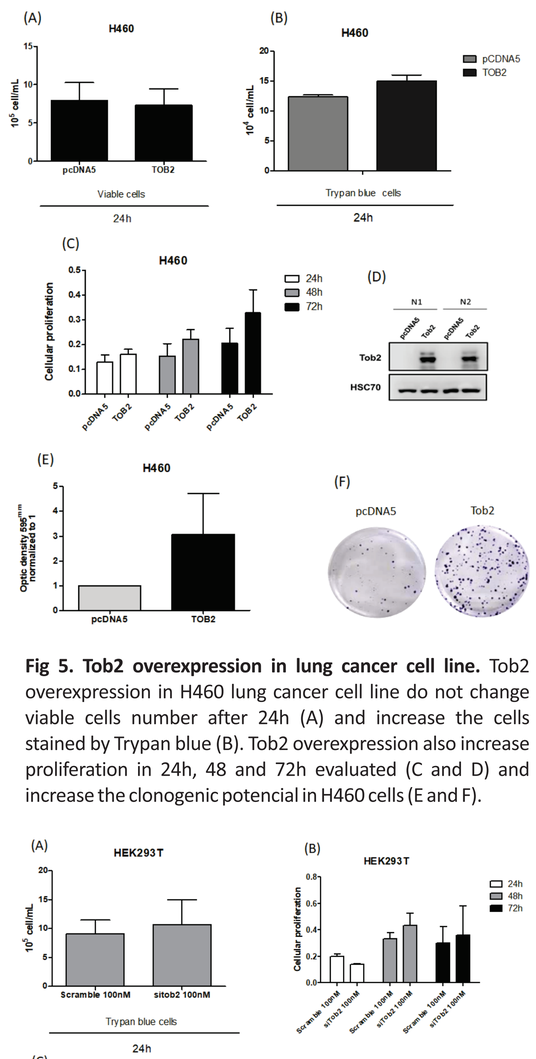

Fig 5. Tob2 overexpression in lung cancer cell line. Tob2 overexpression in H460 lung cancer cell line do not change viable cells number after 24h (A) and increase the cells stained by Trypan blue (B). Tob2 overexpression also increase proliferation in 24h, 48 and 72h evaluated (C and D) and increase the clonogenic potencial in H460 cells (E and F).

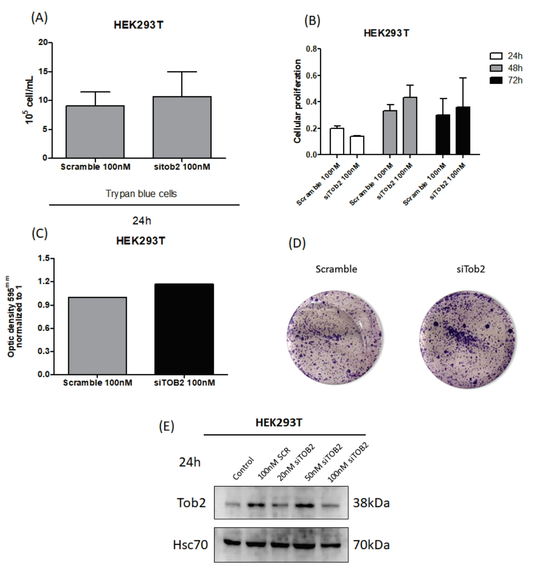

Fig 8. Tob2 inhibition in HEK293T cells. Tob2 inhibition by siRNA do not change viable cells number after 24h (A and E), increase proliferation for 24,48 and 72h (B) and increase clonogenic potencial in HEK293T cells (C and D).

FINANCIAL SUPPORT: CAPES, FAPERJ, Programa de Oncobiologia (UFRJ/Fundação do Câncer), CNPQ, Ministério da Saúde, INCA.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA