DIFFERENTIAL ROLE OF TNF-α IN MULTIFACTORIAL RESISTANCE IN CANCER CELLS

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ABSTRACT

Introduction and objective: Multidrug resistance phenotype (MDR) is characterized by overexpression of P-glycoprotein (Pgp/ABCB1) and related to cancer chemotherapy treatment failure. However, MDR may be considered a multifactorial phenotype associated with deregulation of apoptotic pathways or changes in transcriptional regulators. The Tumor Necrosis Factor-alpha (TNF-α) is an important cytokine that present ambiguous function on cancer development, since it may act as death signaling or tumor growth factor. In addition, our group previously demonstrated a correlation between Pgp and proteins of TNF superfamily. Therefore, the aim of this study was to investigate the role of TNF-α in the contribution of multifactorial cancer resistance phenotype associated with Pgp expression.

Material and Methods: In this study we used two cervical cancer cell lines: KB-3-1, parental cell line; and KB-C1, Pgp-positive cell line selected from KB-3-1 through increased doses of colchicine. mRNA levels of different members of TNF superfamily were analyzed by PCR array in both cell lines. KB-3-1 and KB-C1 cells were treated with recombinant TNF-α (rTNF-α) and apoptosis index was measured by Annexin-V/PI staining and pro-caspase-3 expression levels. Pgp and endogenous TNF-α (TNF-α) expression levels, and subcellular localization were analyzed by Western blot and immunofluorescence, respectively. Pgp efflux activity was analyzed by flow cytometry mRNA levels of *TNFA* and *ABCB1* genes were analyzed by qRT-PCR.

Results and conclusion: Our data showed that *TNFA* mRNA was up regulated in KB-C1 cells compared to parental cell line. Further, the *TNFA* mRNA expression level was confirmed by qRT-PCR. We also observed low apoptosis rate following rTNF-α treatment in both cell lines by Annexin-V/PI staining, however KB-C1 cells showed a reduction of pro-caspase-3 after 24h. The rTNF-α treatment promoted inversely results at transcription and translation levels of Pgp and TNF-α in both cell lines. While rTNF-α induced an increase of *TNFA* and *MDR1* mRNA levels in KB-3-1 cells, the KB-C1 cells showed a decrease of these mRNA levels. TNF-α protein was increased in both cell lines after rTNF-α treatment. However, rTNF-α did not induce Pgp expression in KB-3-1 cell line. We also observed that rTNF-α induced higher Pgp total expression in KB-C1 cells, although reduced superficial protein. Futhermore, rTNF-α did not alters Pgp efflux activity. In summary, our results suggest that rTNF-α did not alters cell viability significantly, independently of Pgp expression. In addition, rTNF-α treatment may induce an epigenetic regulation in *MDR1* gene in KB-3-1 cells and Pgp protein accumulation in KB-C1 cells, suggesting a possible role of TNF-α in supporting resistance phenotype.

RESULTS

Table 1. Analysis of superfamily TNF genes expression based at Pgp expression in KB-C1.

Gene	Protein	KB-C1 Up regulated	KB-C1 Down regulated	Similar
TNFA	TNF-α	1,92	-	-
TNFRSF10A	DR4	-	-	1,22
TNFRSF10B	DR5	-	1,67	-
TNFRSF1A	TNFR1	-	-	1,02
TNFRSF1B	TNFR2	1,71	-	-
TNFRSF21	DR6	-	-	1,33
TNFRSF25	DR3	-	8,89	-
TNFSF10	TRAIL	-	2,02	-

Parental KB-3-1 cells were used as reference cell line. The fold-change cutoff were ±1,5.
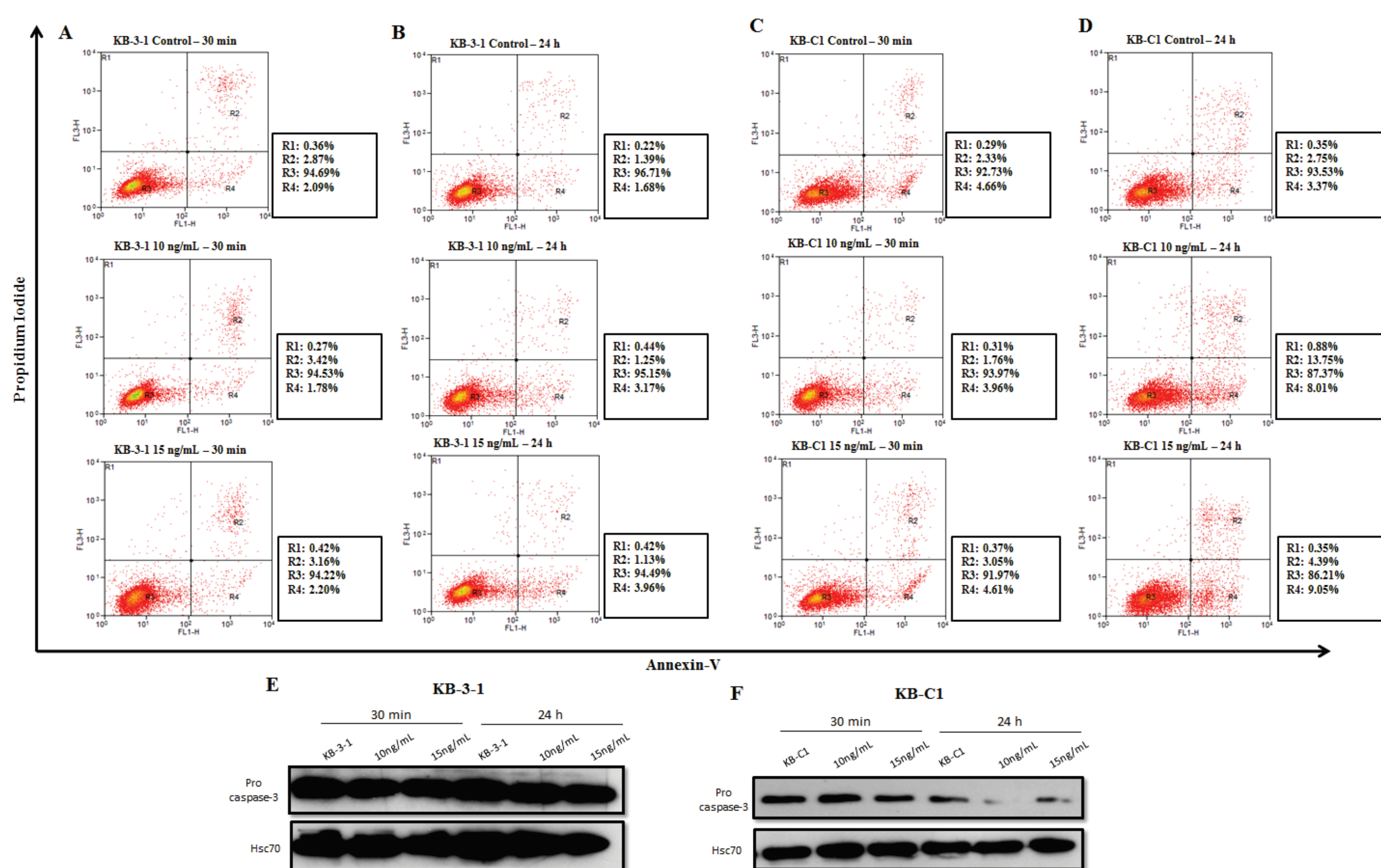

Figure 2: Effect of recombinant TNF-α (rTNF-α) on inducing apoptosis in KB-3-1 and KB-C1 cell lines. The apoptosis index of KB-3-1 (A and B) and KB-C1 (C and D) cell lines was measured by Annexin-V/Propidium Iodide staining and pro-caspase-3 was analyzed by *Western blot* (E and F) after treatment with rTNF-α for 30min or 24h. Hsc70 was used as loading control for *Western blot* (E and F).
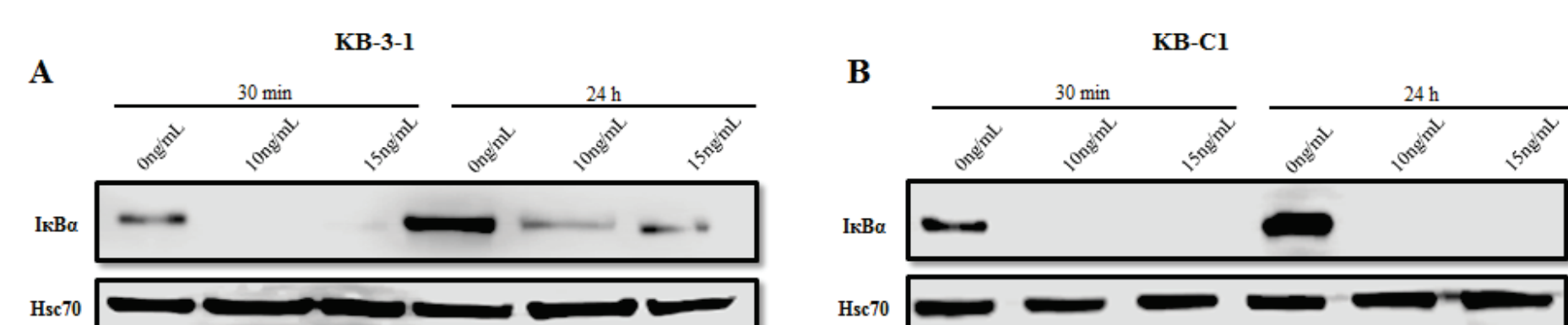

Figure 3: Expression of IκBα in KB-3-1 and KB-C1 cells. Total expression of IκBα after treatment with rTNF-α for 30min or 24h was analyzed by *Western blot* in KB-3-1 (A) and KB-C1 (B) cells. Hsc70 was used as loading control.
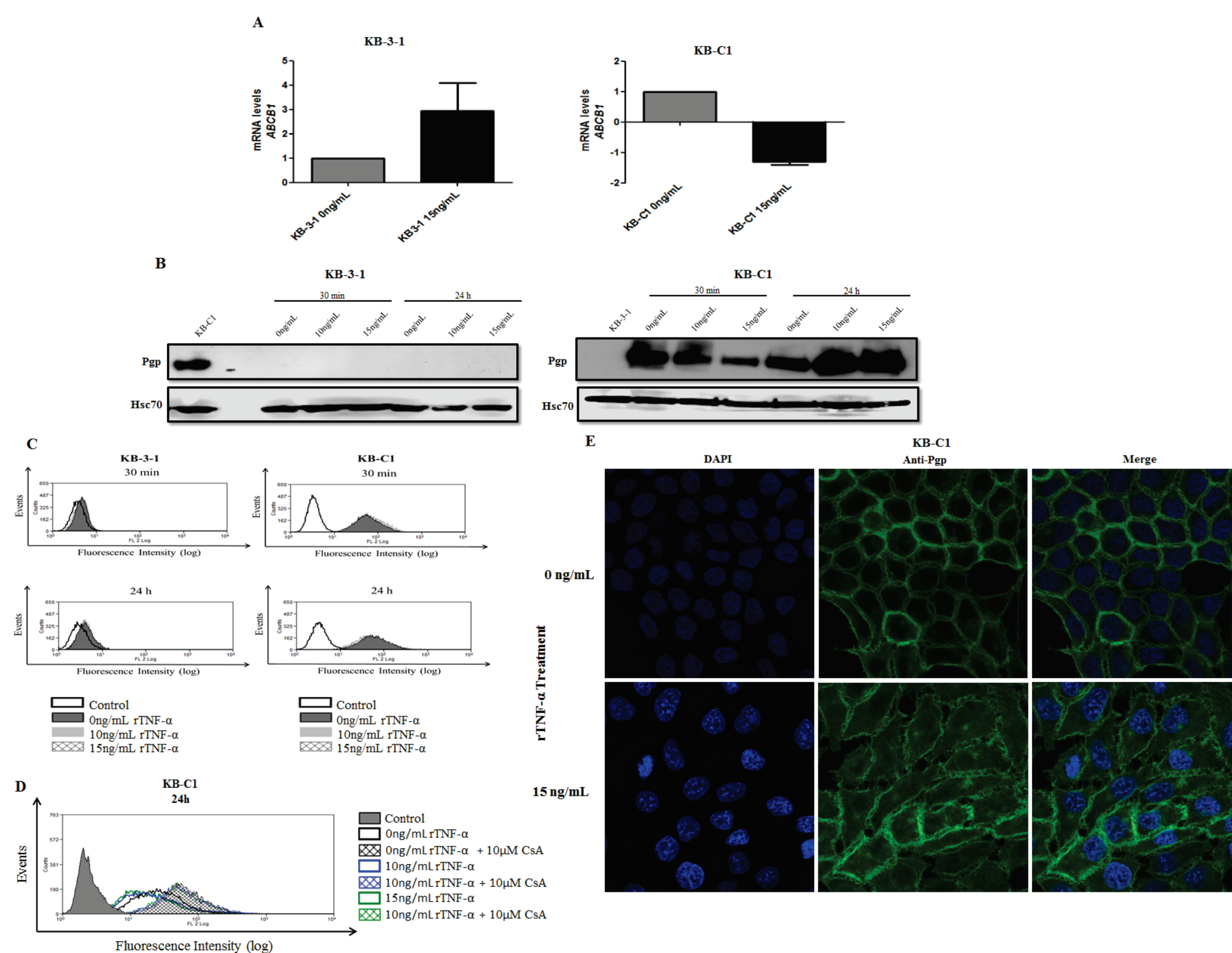

Figure 4: Effect of recombinant TNF-α (rTNF-α) on expression and localization of Pgp in KB-3-1 and KB-C1 cells. The mRNA levels of *MDR1* were analyzed using RT-qPCR in KB-3-1 and KB-C1 cell lines after 24h treatment with rTNF-α (A). Total and cell surface protein levels of Pgp were analyzed by *Western blot* and FACs, respectively, in KB-3-1 and KB-C1 cells (B and C) after 30min and 24h treatment with rTNF-α. Superficial Pgp expression was analyzed by shift assay using FACs analysis (D). The subcellular localization of Pgp in KB-C1 after 24h treatment with rTNF-α cells was performed by confocal microscopy analysis (E). GAPDH gene was used as endogenous control for RT-qPCR (A). Hsc70 was used as loading control for *Western blot* (B). Empty histograms represent cell autofluorescence, solid dark gray histograms represent Pgp immunolabeling, light gray histograms represent Pgp immunolabeling after 10ng/mL of rTNF-α treatment and hatched gray histogram represents Pgp immunolabeling after 15ng/mL of rTNF-α treatment (C). Gray histogram represents cell autofluorescence, empty black histogram represents Pgp immunolabeling, hatched black histogram represents Pgp immunolabeling after 10μM of Ciplosporine (CsA) treatment, empty blue histogram represents Pgp immunolabeling after 10ng/mL of rTNF-α treatment, hatched blue histogram represents Pgp immunolabeling after 10ng/mL of rTNF-α plus 10μM of CsA treatment, empty geen histogram represents Pgp immunolabeling after 15ng/mL of rTNF-α treatment, hatched green histogram represents Pgp immunolabeling after 15ng/mL of rTNF-α plus 10μM of CsA treatment (D). DAPI was used for nuclear staining (blue) and anti-Pgp staining (green); images captured at 60x magnification (E).
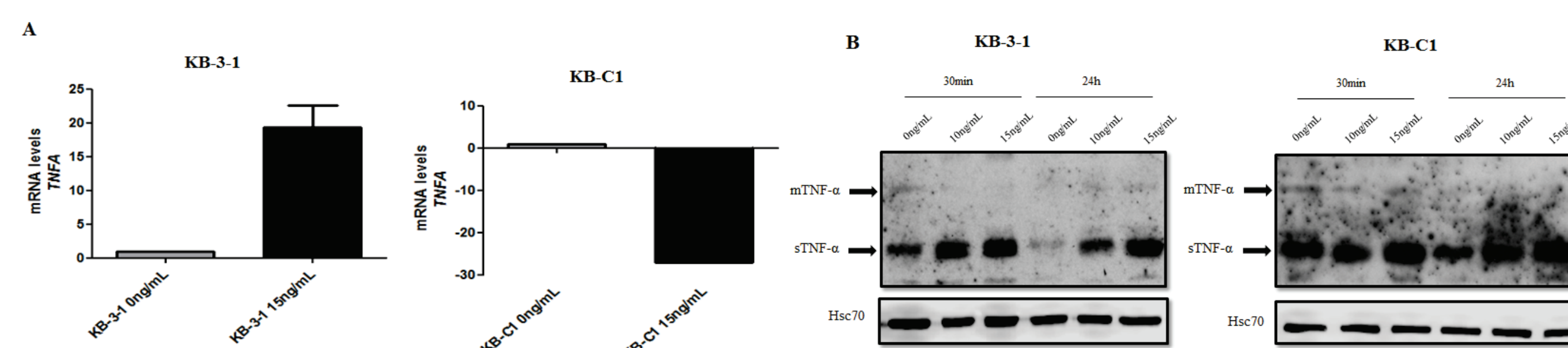

Figure 5: Effect of recombinant TNF-α (rTNF-α) in expression of endogenous TNF-α in KB-3-1 and KB-C1 cell lines. The mRNA levels of *TNFA* were analyzed using RT-qPCR after 24h treatment with rTNF-α (A) and protein levels of TNF-α was analyzed by *Western blot* in KB-3-1 and KB-C1 cells after 30min or 24h treatment with rTNF-α (B). Hsc70 was used as loading control for Western blot (A). GAPDH gene was used as endogenous control for RT-qPCR (B).
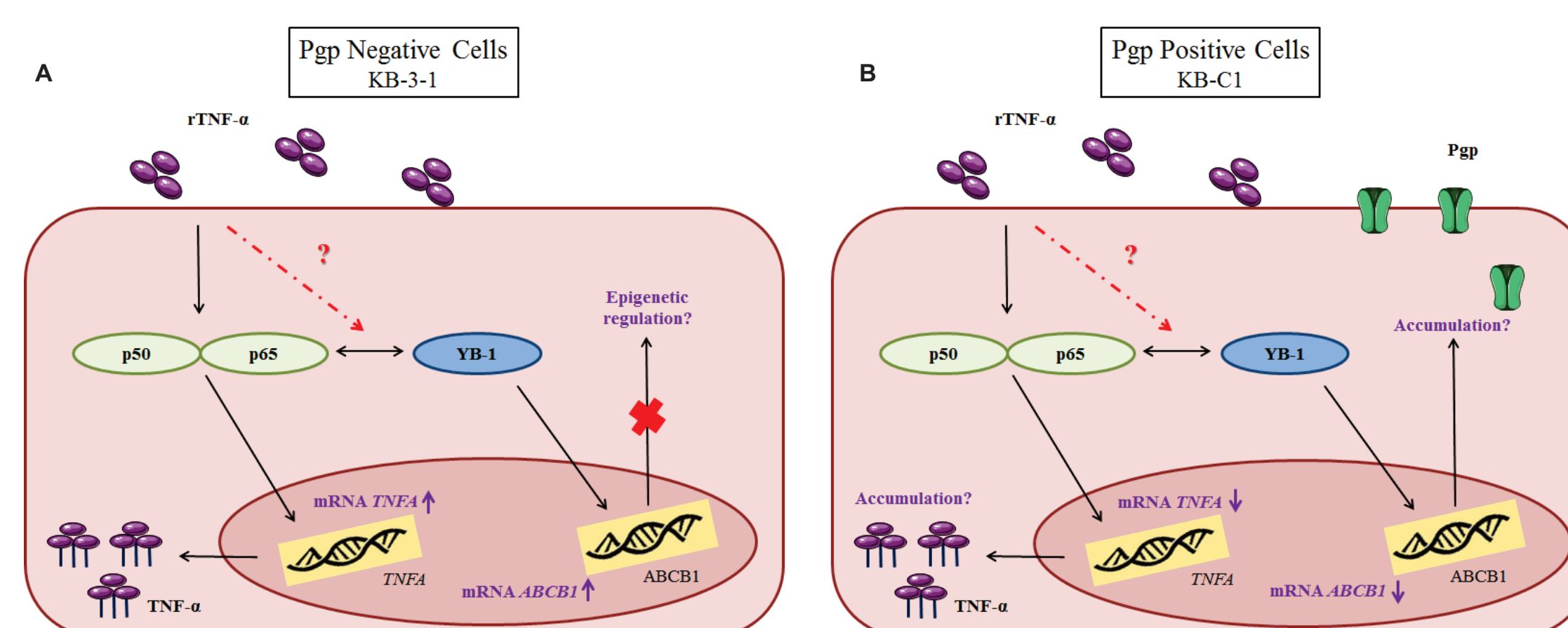

Figure 6: Representative scheme of recombinant TNF-α (rTNF-α) effect in KB-3-1 and KB-C1 cell lines. The rTNF-α in Pgp negative cells induces an increase of mRNA and protein levels of endogenous TNF-α and ABCB1 mRNA levels, with no change in Pgp protein expression (A). In Pgp positive cells, rTNF-α induces a decrease of TNFA and ABCB1 mRNA levels, with an increase of Pgp protein expression (B).

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

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