Role of TNF-α and P glycoprotein in multifactorial cancer resistance

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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 is 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. Studies have shown that Y-box protein 1 (YB-1) and NFB may regulate Pgp expression, acting as regulators of *MDR1/ABCB1* gene. 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. KB-3-1 and KB-C1 cell lines were treated with recombinant TNF-α (rTNF-α) for 30min or 24h and apoptosis index was measured by Annexin-V/PI staining using flow cytometry. Pgp expression, function and subcellular localization were analyzed by Western blot, flow cytometry and immunofluorescence respectively. TNF-α, YB-1 and NFκB expression and subcellular localization were also investigated. Transcription of *TNFA* and *ABCB1* genes were analyzed by qRT-PCR.

Results and conclusion: Our data showed that resistance present by KB-C1 is probably related to a functional overexpression of Pgp. Then, we observed that KB-3-1 cells showed higher expression of YB-1 and NFκB/p65 subunit than KB-C1, but lower expression of NFκB/p105 subunit. We observed a perinuclear, nuclear and cytoplasmatic subcellular distribution of NFκB in both cell lines. Also, YB-1 was detected in cytoplasm and nuclear foci in both cell lines, but apparently wide larger in KB-C1 than KB-3-1. KB-C1 cells exhibited cytoplasmatic and nuclear TNF-α distribution, while KB-3-1 cells showed mostly nuclear localization. Further, we observed low apoptosis rate following rTNF-α treatment in both cell lines. We observed that mRNA levels of *ABCB1* decrease in KB-C1 cells, while increase in KB-3-1 cells. Besides that, Pgp expression was increased after treatment with rTNF-α in KB-C1 and no effect in KB-3-1. Also, KB-C1 cells showed strong Pgp staining in membrane. In summary, our results suggest that rTNF-α did not alters cell viability significantly, independently of Pgp expression. Also, Pgp expression in KB-C1 cell line might be regulated by YB-1 pathway. In addition, rTNF-α treatment may induce an epigenetic regulation in *ABCB1* 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

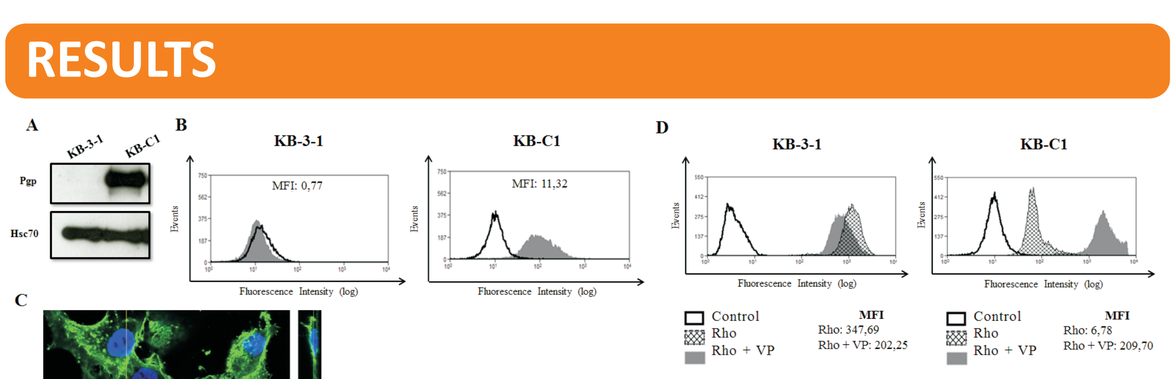

Figure 1: Comparison of P-glicoprotein (Pgp) expression, localization and efflux activity in KB-3-1 and KB-C1 cell lines. Total Pgp expression was analyzed by *Western* blot (A), and Pgp expression on cell surface was performed by immunolabeling and fluorescence intensity was recorded by FACs analysis (B) or confocal analysis (C). Pgp efflux activity was assessed by flow cytometry after incubation with rhodamine 123 (Rho) in the presence or absence of verapamil (VP) (D). Hsc70 was used as loading control for *Western* blot (A). Empty histograms represent cell autofluorescence and solid gray histograms represent Pgp immunolabeling (B). DAPI was used for nuclear staining (blue), and anti-Pgp staining (green); images captured at 60x magnification (C). Empty histogram represents cell autofluorescence, hatched black histogram represents cells with Rho, and solid gray histogram represents cells with Rho and VP (D). Fluorescence intensity (MFI).

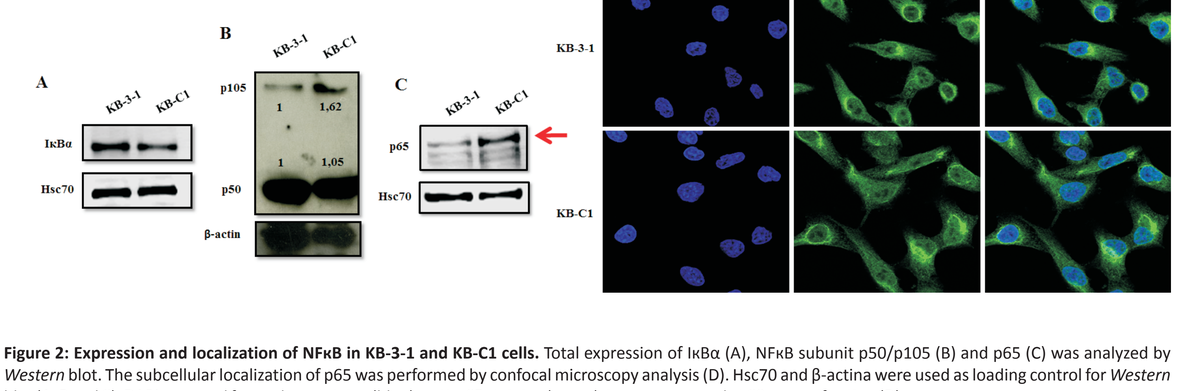

Figure 2: Expression and localization of NFκB in KB-3-1 and KB-C1 cells. Total expression of IκBα (A), NFκB subunit p50/p105 (B) and p65 (C) was analyzed by *Western* blot. The subcellular localization of p65 was performed by confocal microscopy analysis (D). Hsc70 and β-actina were used as loading control for *Western* blot (A, B and C). DAPI was used for nuclear staining (blue), anti-p65 staining (green); images captured at 60x magnification (D).

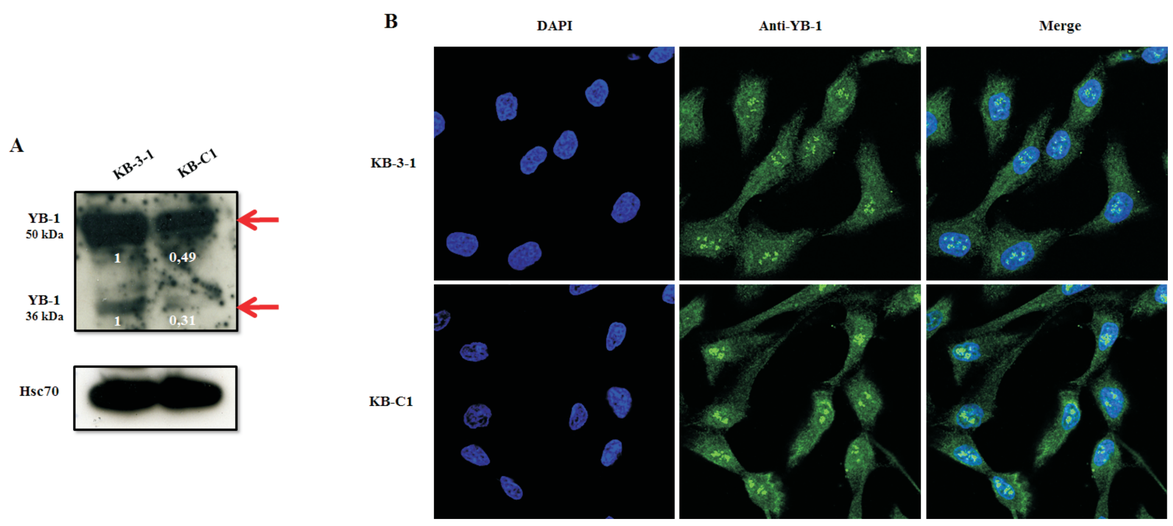

Figure 3: Expression and localization of YB-1 in KB-3-1 and KB-C1 cell lines. Total YB-1 protein level was analyzed by *Western* blot (A). The subcellular localization of YB-1 was performed by confocal microscopy analysis (B). Hsc70 was used as loading control for *Western* blot (A). DAPI was used for nuclear staining (blue) and anti-YB-1 staining (green); images captured at 60x magnification (B).

Financial support FAPERJ CNPq inct Programa de Oncobiologia

Figure 4: 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).

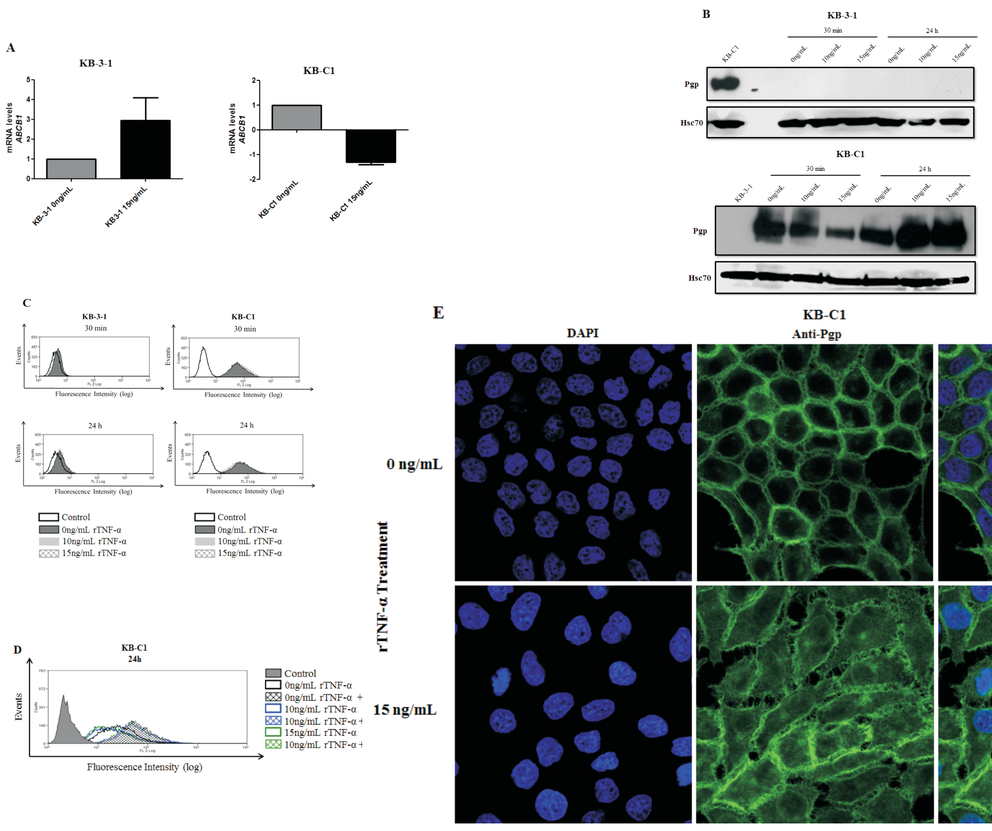

Figure 5: Effect of rTNF-α on expression and localization of Pgp in KB-3-1 and KB-C1 cells. The mRNA levels of *MDR1* were analyzed using RT-qPCR in KB-3-1 and KB-C1 cell lines after 24h treatment with rTNF-α (A). Total and cell surface protein levels of Pgp were analyzed by *Western* blot and FACs, respectively, in KB-3-1 and KB-C1 cells (B and C) after 30min and 24h treatment with rTNF-α. Total 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 10 M 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 10 M 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 10 M of CsA treatment (D). DAPI was used for nuclear staining (blue) and anti-Pgp staining (green); images captured at 60x magnification (E).

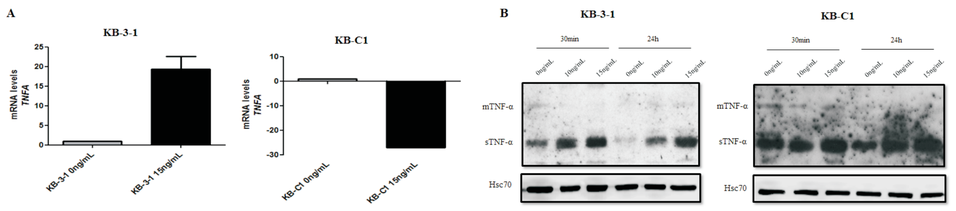

Figure 6: Effect of rTNF-α in expression of endogenous TNF-α in KB-3-1 and KB-C1 cell lines. The mRNA levels of *TNFA* were analyzed using RT-qPCR after 24h treatment with rTNF-α (A) and protein levels of TNF-α was analyzed by *Western* blot in KB-3-1 and KB-C1 cells after 30min or 24h treatment with rTNF-α (B). Hsc70 was used as loading control for *Western* blot (A). GAPDH gene was used as endogenous control for RT-qPCR (B).

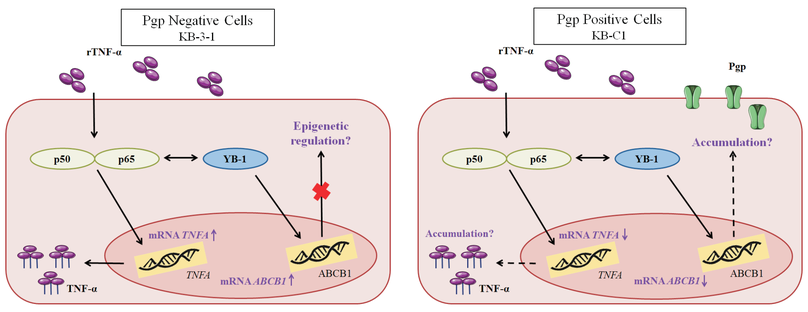

Figure 7: Representative scheme of effect of rTNF-α in KB-3-1 and KB-C1 cell lines. The rTNF-α induced an increase of *TNFA* and *MDR1* mRNA levels in Pgp negative cells, while decrease these mRNA levels in Pgp positive cells. Both cell lines presented TNF-α protein accumulation. rTNF-α did not induce Pgp expression in KB-3-1 cell line, suggesting an epigenetic regulation. Although , rTNF-α induced higher Pgp total expression in KB-C1 cells, suggesting an accumulation.

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