ROLE OF TNF-α AND TRANSCRIPTIONAL FACTORS YB-1 AND NFκB IN P-GLYCOPROTEIN EXPRESSING 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 chemotherapy cancer treatment failure. However, MDR is considered a multifactorial phenotype associated with molecular pathways deregulation, such as changes in apoptosis-associated proteins or transcriptional regulators expression. Y-box protein 1 (YB-1) and NFκB may regulate Pgp expression, acting as regulators of MDR1/ABCB1 gene. Studies have shown that Pgp and apoptosis-associated proteins expression may also contribute to multifactorial cancer resistance. Therefore, the aim of this study was to investigate the role of Pgp expression, the proapoptotic protein TNF-α and YB-1 and NFκB transcriptional factors in the contribution of multifactorial resistance phenotype in cancer cells.

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 doxorubicin, cisplatin and colchicine for 24, 48 or 72h and cell viability was analyzed by MTT assay. Pgp expression was analyzed by Western blot and flow cytometry and efflux activity was analyzed by flow cytometry. TNF-α, YB-1 and NFκB expression were analyzed by Western blot. Subcellular location of Pgp, TNF-α, YB-1 and NFκB were analyzed by immunofluorescence using confocal microscopy. KB-3-1 and KB-C1 cell lines were also treated with 10 or 15 ng/mL of recombinant TNF-α (rTNF-α) for 30min or 24h and apoptosis index was measured by Annexin-V/PI staining using flow cytometry and caspase-3 and -8 by Western blot.

Results and conclusion: Our data showed that KB-C1 cells were resistant to doxorubicin and colchicine and it is probably related to a functional overexpression of Pgp. However, KB-3-1 cells were sensitive to drugs treatment and did not show Pgp expression. Also, both cell lines showed sensitivity to high doses of cisplatin, a non-Pgp substrate. Then, we observed that YB-1 and NFκB/p50 subunit presented similar expression levels in both cell lines. However, KB-C1 cells showed higher expression of NFκB/p105 subunit than KB-3-1 cells. Moreover, we observed a perinuclear, nuclear and cytoplasmatic subcellular distribution of NFκB/p65 and NFκB/p105 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. After that, we analyzed KB-3-1 and KB-C1cellular viability treated with rTNF-α. We observed no apoptosis following rTNF-α treatment in both cells. In summary, our results suggest that Pgp expression in KB-C1 cell line may be regulated by NFκB/p105 and YB-1 pathways, supporting its resistance phenotype. However, more data is required to understand the role of TNF-α protein in MDR phenotype.

RESULTS

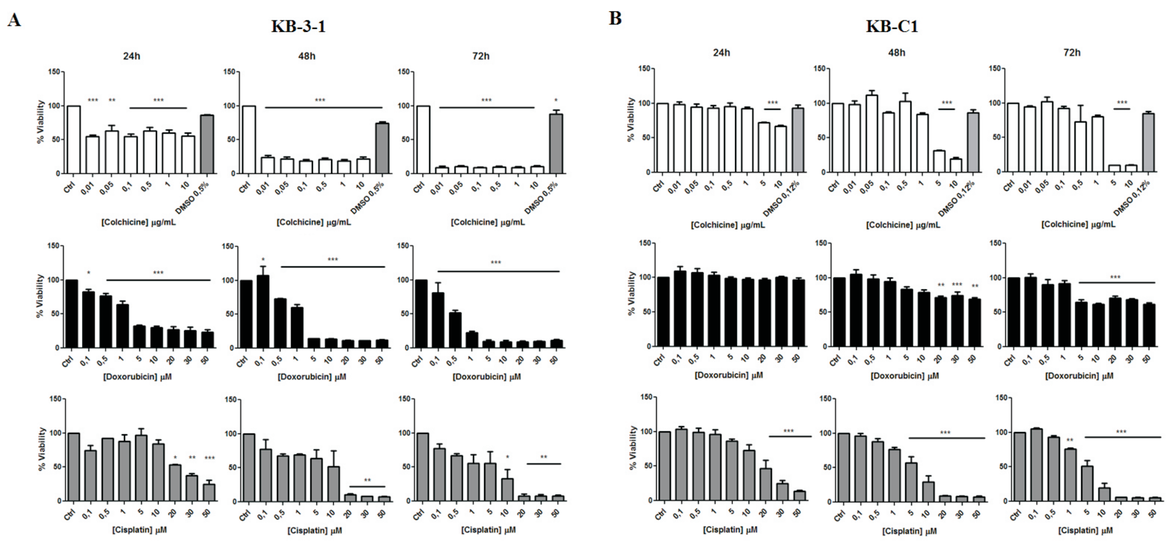

Figure 1: Effect of colchicine, doxorubicin and cisplatin on KB-3-1 and KB-C1 cell lines viability. Cellular viability of KB-3-1 (A) and KB-C1 (B) cell lines was analyzed by MTT assay after treatment with colchicine, doxorubicin and cisplatin, chemotherapeutic drugs for 24, 48 or 72h. Graphs represent the mean +/- standard error of three independent experiments. *p<0.05, **p<0.01, ***p<0.001.

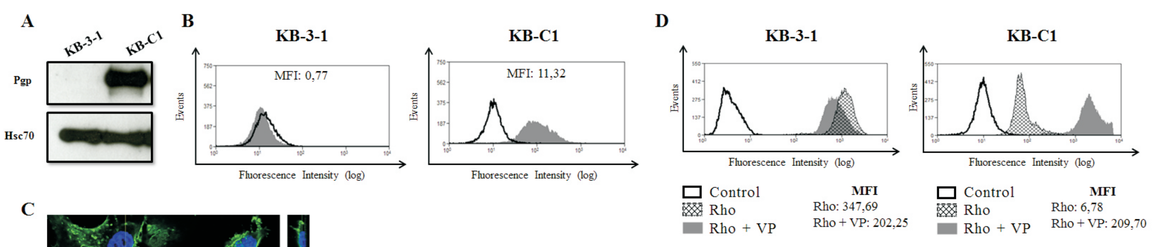

Figure 2: Comparison of P-glicoprotein (Pgp) expression, localization and efflux activity in KB-3-1 and KB-C1 cell lines. Total Pgp expression was analyzed by *Western* blotting (A), and Pgp expression on cell surface was performed by immunolabeling and fluorescence intensity was recorded by FACs analysis (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).

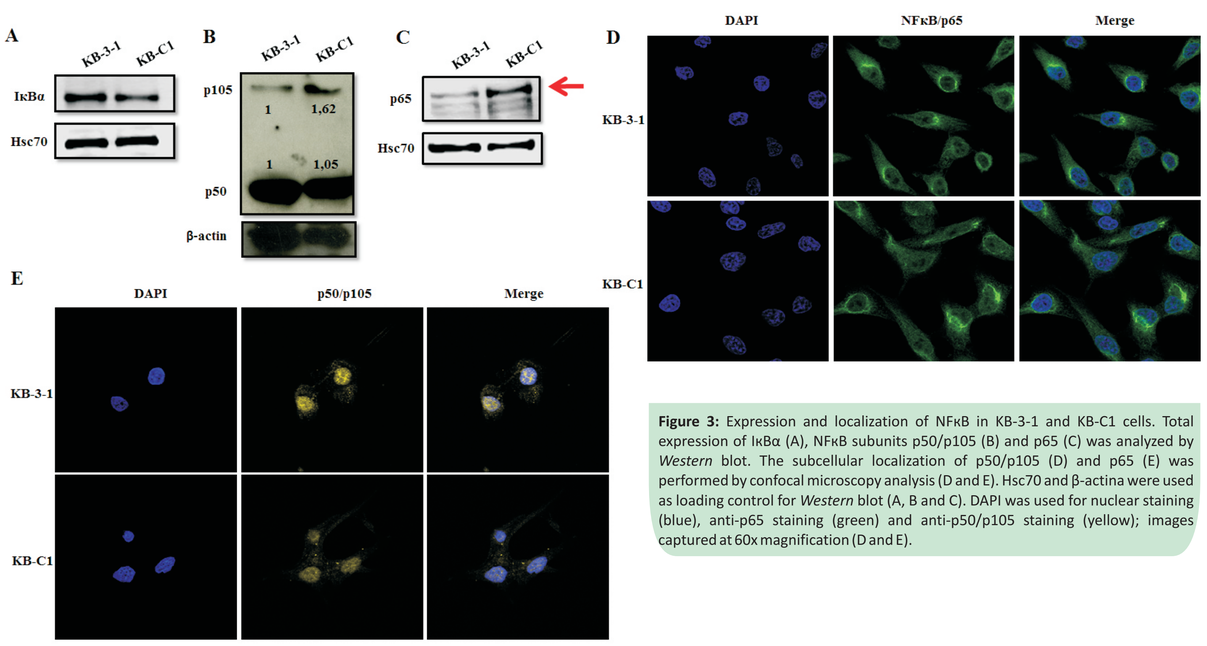

Figure 3: Expression and localization of NFκB in KB-3-1 and KB-C1 cells. Total expression of IκBα (A), NFκB subunits p50/p105 (B) and p65 (C) was analyzed by *Western* blot. The subcellular localization of p50/p105 (D) and p65 (E) was performed by confocal microscopy analysis (D and E). Hsc70 and β-actina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

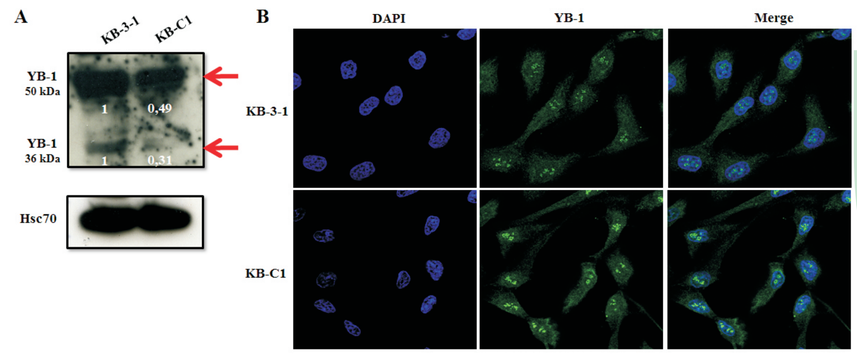

Figure 4: Expression and localization of YB-1 in KB-3-1 and KB-C1 cell lines. Total YB-1 expression was analyzed by *Western* blot (A). The subcellular localization of YB-1 was performed by confocal microscopy analysis (B). Hsc70 was used as loading control for *Western* blot (A). DAPI was used for nuclear staining (blue) and anti-YB-1 staining (green); images captured at 60x magnification (B).

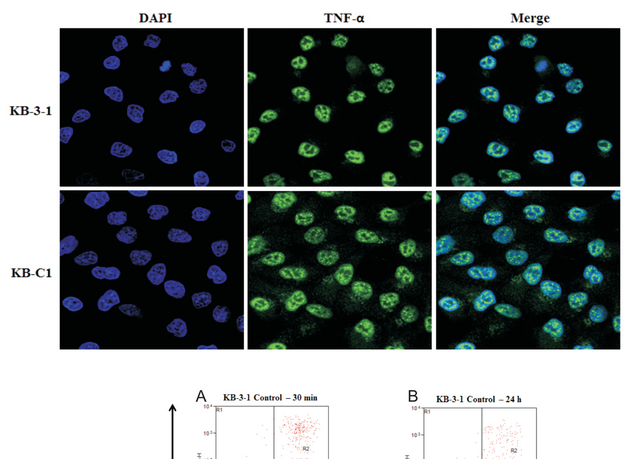

Figure 5: Localization of TNF-α in KB-3-1 and KB-C1 cell lines. The subcellular localization of TNF-α was performed by confocal microscopy analysis. DAPI was used for nuclear staining (blue) and anti-TNF-α staining (green); images captured at 60x magnification.

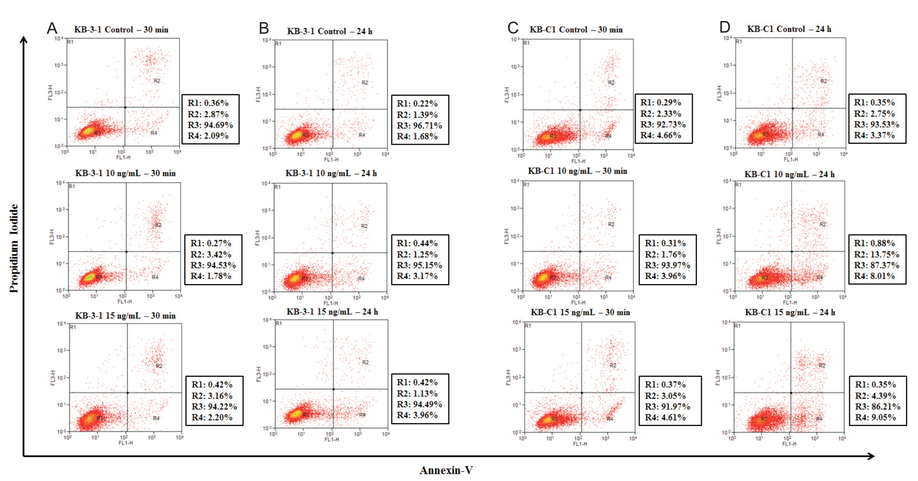

Figure 7: Effect of recombinant TNF-α (rTNF-α) on inducing apoptosis in KB-3-1 and KB-C1 cell lines. The apoptosis index of KB-3-1 (A and B) and KB-C1 (C and D) cell lines was measured by Annexin-V/Propidium Iodide staining after treatment with rTNF-α for 30min or 24h.

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