ROLE OF TNF-α AND TRANSCRIPTIONAL FACTORS YB-1 AND NFκB IN P-GLYCOPROTEIN EXPRESSING CANCER CELLS

Tandressa Souza Berguetti[1], **Paloma Silva de Souza**[1] **and Raquel Ciuvalschi Maia**[1]
1Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular,
Instituto Nacional de Câncer (INCA), RJ, Brasil
e-mail: tata.berguetti@hotmail.com

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 NFB 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. Tumor Necrosis Factor-alpha (TNF-α) is an important cytokine that present ambiguous function on cancer development, since may act as death signaling or tumor growth factor. Also, TNF-α is main activator of NFB pathway. Therefore, the aim of this study was to investigate the role of Pgp expression, the proapoptotic protein TNF-α, and YB-1 and NFB 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. 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.
Results and conclusion: Our data showed that KB-3-1 cells were sensitive to drugs treatment while KB-C1 cells were resistant to doxorubicin and colchicine drugs, and it is probably related to a functional overexpression of Pgp. However, both cell lines showed sensitivity to high doses of cisplatin, a non-Pgp substrate. 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 peri-nuclear, 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-3-1 and KB-C1 cells exhibited cytoplasmatic and nuclear TNF-α distribution. Further, we observed low apoptosis rate following rTNF-α treatment in both cell lines. Besides that, Pgp expression was increased and showed strong membrane staining in KB-C1 cells treated with rTNF-α. Also, KB-3-1 and KB-C1 showed higher expression of TNF-α and cytoplasmatic localization after treatment with rTNF-α. In summary, our results suggest that Pgp expression in KB-C1 cell line might be regulated by YB-1 pathway. In addition, rTNF-α treatment changed Pgp expression in resistant cells, suggesting a possible role of TNF-α in supporting resistance phenotype. More data are required to understand the role of TNF-α protein in MDR phenotype.

RESULTS

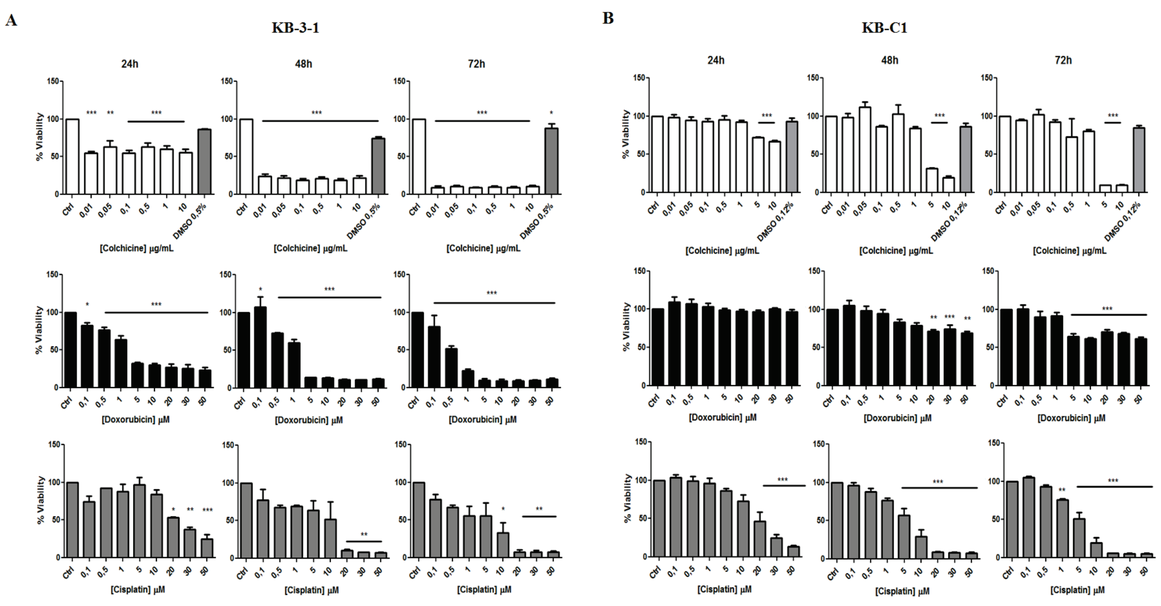

Figure 1: Effect of colchicine, doxorubicin and cisplatin on KB-3-1 and KB-C1 cell lines viability. Cellular viability of KB-3-1 (A) and KB-C1 (B) cell lines was analyzed by MTT assay after treatment with colchicine, doxorubicin and cisplatin, chemotherapeutic drugs for 24, 48 or 72h. Graphs represent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

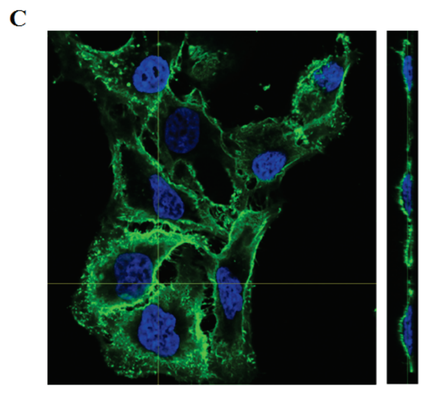

Figure 2: Comparison of P-glicoprotein (Pgp) expression, localization and efflux activity in KB-3-1 and KB-C1 cell lines. Total Pgp expression was analyzed by *Western* blotting (A), and Pgp expression on cell surface was performed by immunolabeling and fluorescence intensity was recorded by FACs analysis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

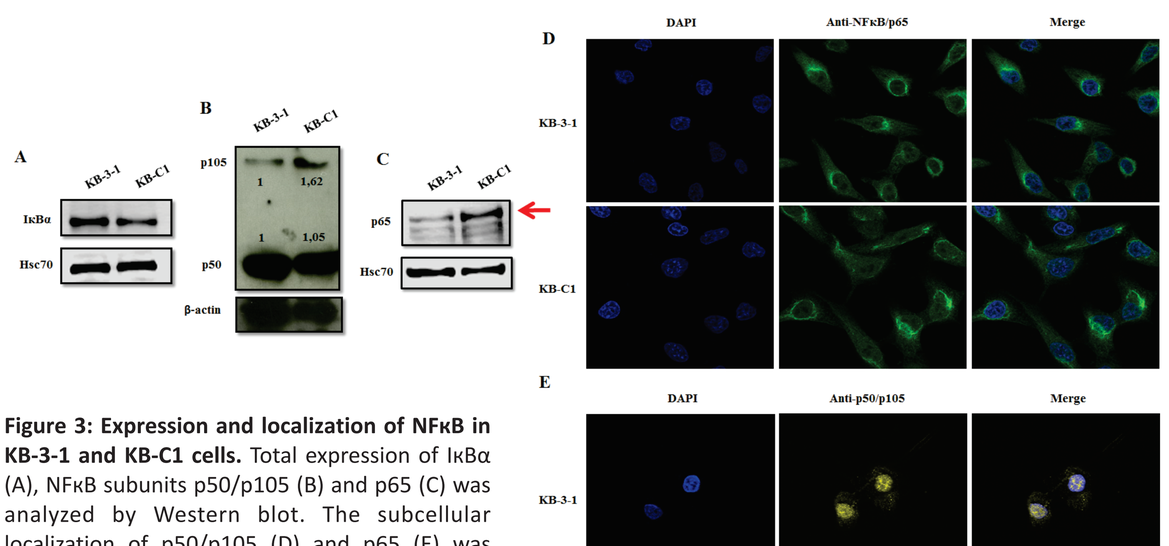

Figure 3: Expression and localization of NFκB in KB-3-1 and KB-C1 cells. Total expression of IκBα (A), NFκB subunits p50/p105 (B) and p65 (C) was analyzed by Western blot. The subcellular localization of p50/p105 (D) and p65 (E) was performed by confocal microscopy analysis (D and E). Hsc70 and β-actina 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).

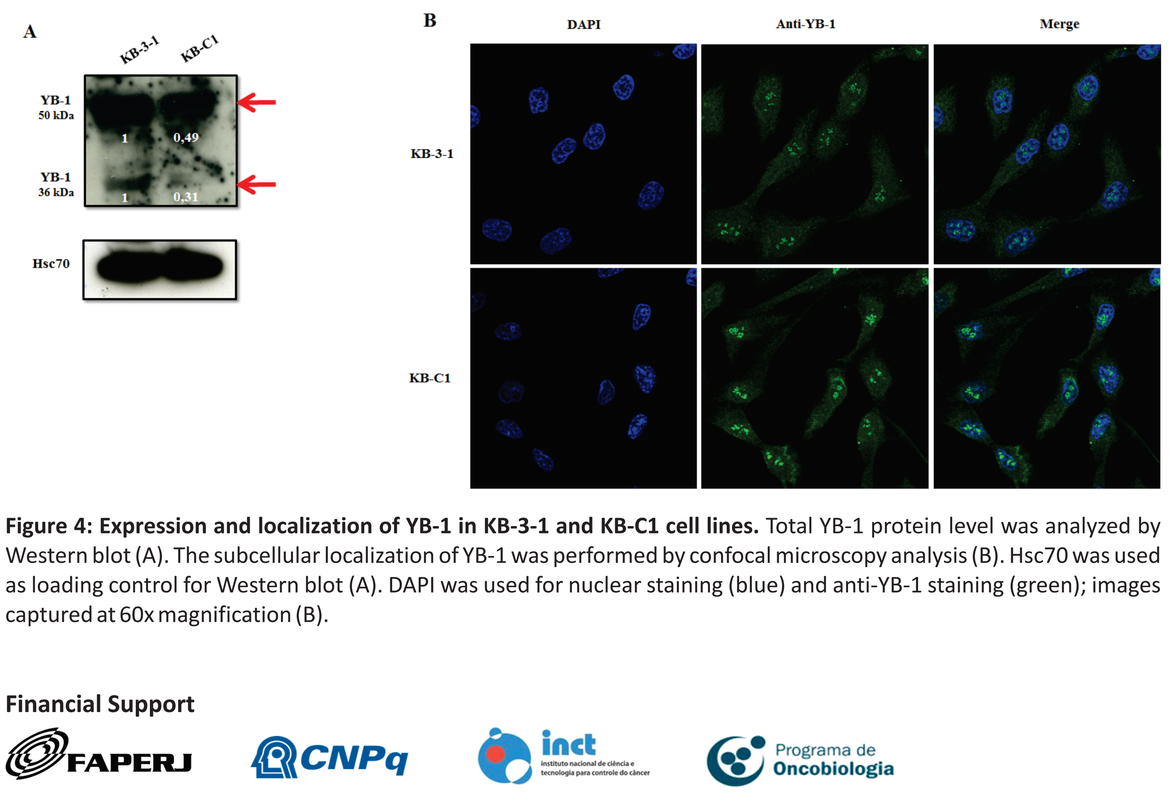

Figure 4: Expression and localization of YB-1 in KB-3-1 and KB-C1 cell lines. Total YB-1 protein level was analyzed by Western blot (A). The subcellular localization of YB-1 was performed by confocal microscopy analysis (B). Hsc70 was used as loading control for Western blot (A). DAPI was used for nuclear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

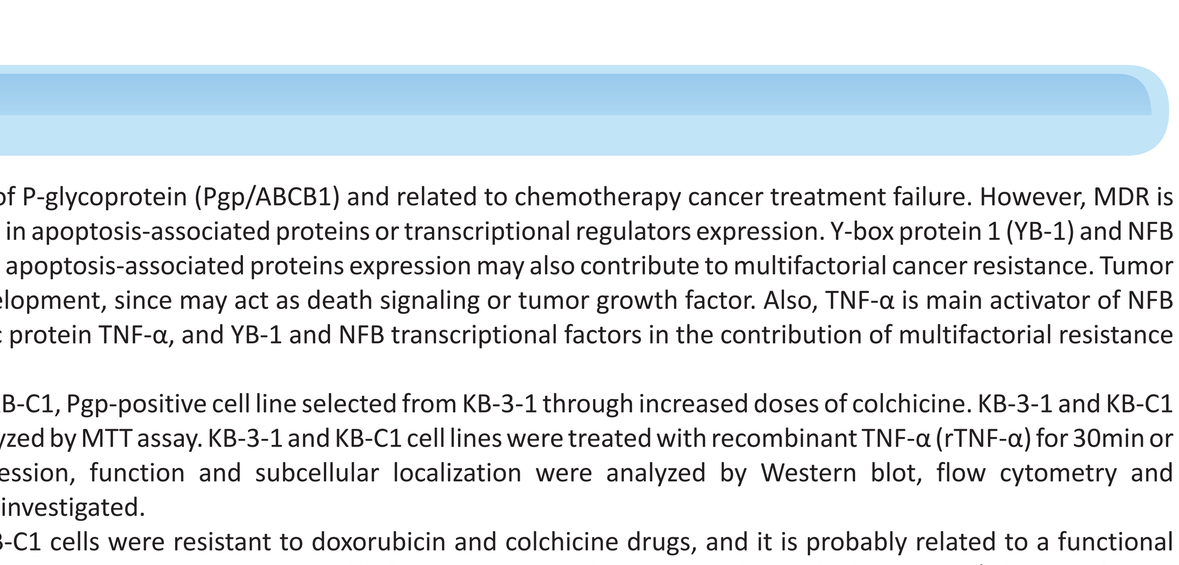

Figure 5: Effect of recombinant TNF-α (rTNF-α) on inducing apoptosis in KB-3-1 and KB-C1 cell lines. The apoptosis index of KB-3-1 (A and B) and KB-C1 (C and D) cell lines was measured by Annexin-V/Propidium Iodide staining and pro-caspase-3 was analyzed by Western blot (E and F) after treatment with rTNF-α for 30min or 24h. Hsc70 was used as loading control for Western blot (E and F).

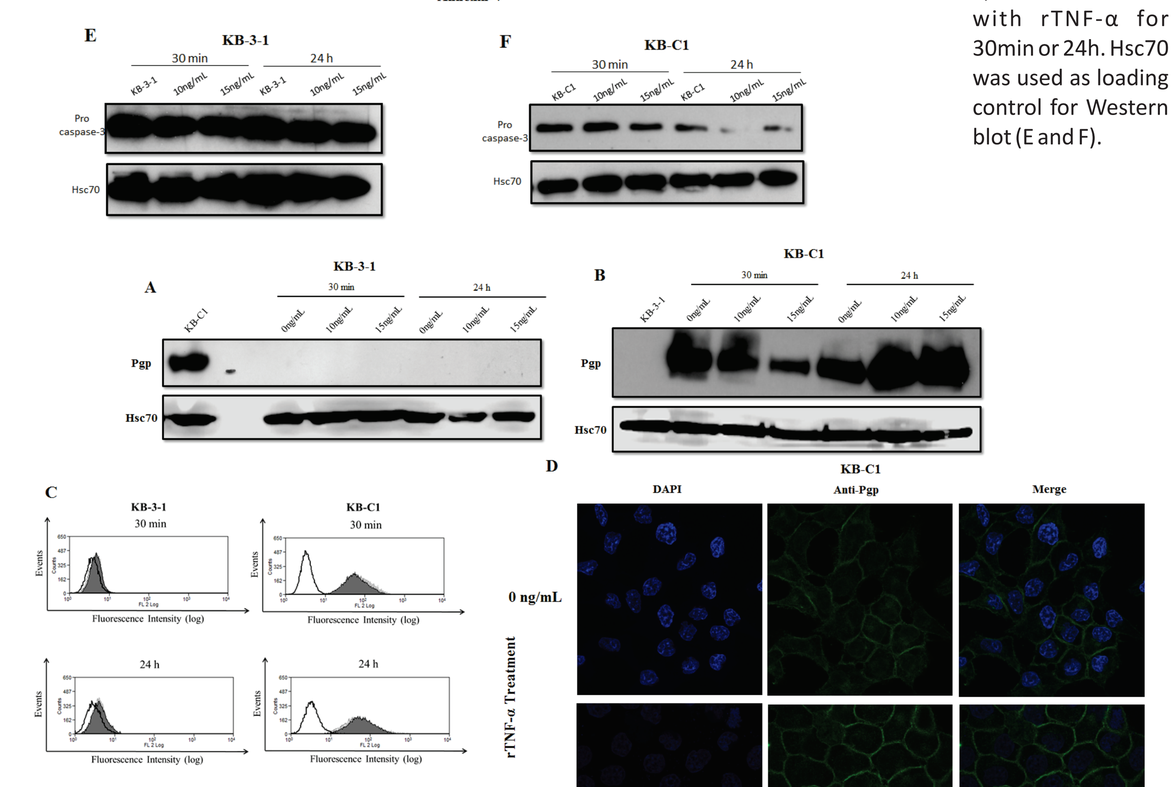

Figure 6: Effect of rTNF-α on expression and localization of Pgp in KB-3-1 and KB-C1 cells. Total and cell surface protein levels of Pgp were analyzed by Western blot and FACs, respectively, in KB-3-1 (A-C) and KB-C1 cells (B-C) after 30min and 24h treatment with rTNF-α. The subcellular localization of Pgp in KB-C1 after 24h treatment with rTNF-α cells was performed by confocal microscopy analysis (D). Hsc70 was used as loading control for Western blot (A and 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 DAPI was used for nuclear staining (blue) and anti-Pgp staining (green); images captured at 60x magnification (D).

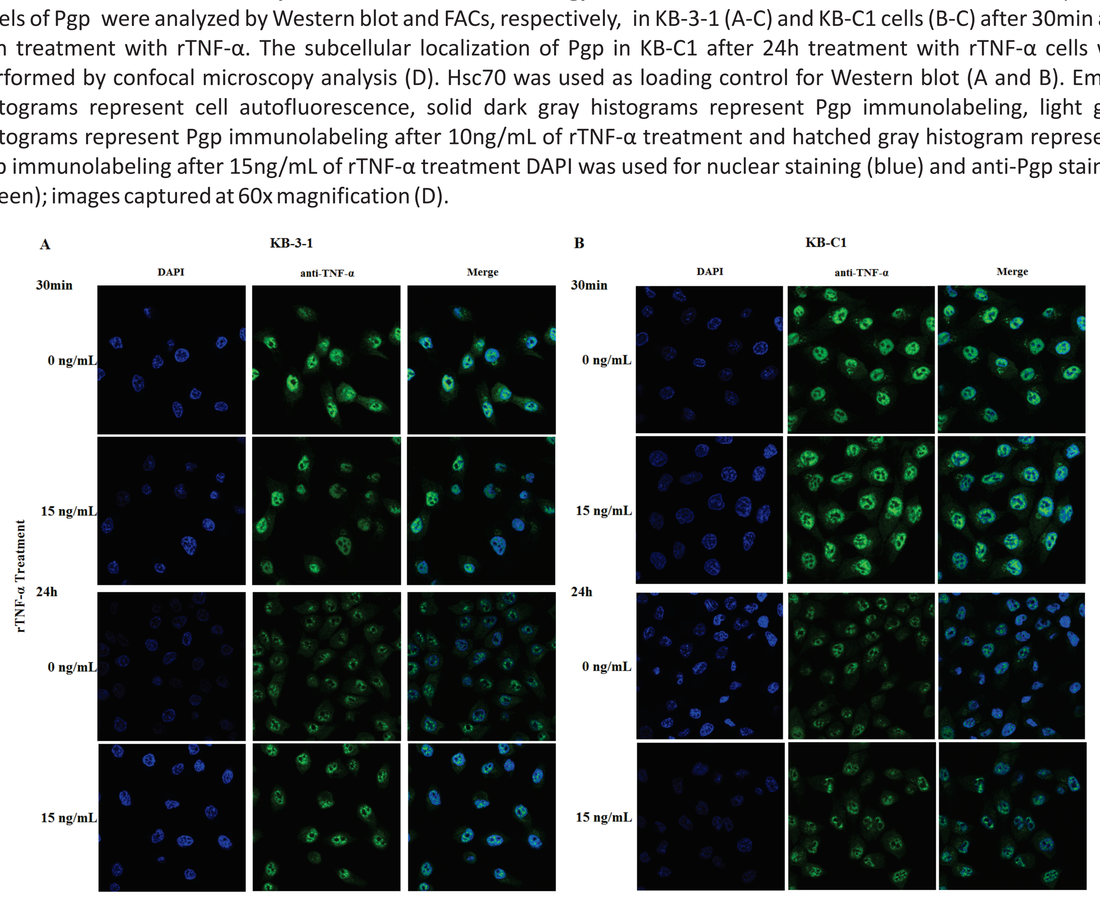

Figure 7: Effect of rTNF-α on localization of endogenous TNF-α in KB-3-1 and KB-C1 cell lines. The subcellular localization of TNF-α was performed by confocal microscopy analysis after 30 min or 24h treatment with rTNF-α in KB-3-1 cells (A) or KB-C1 cells (B). DAPI was used for nuclear staining (blue) and anti-TNF-α staining (green); images captured at 60x magnification.

Financial Support
FAPERJ CNPq inct Instituto nacional de ciência e tecnologia para controle do câncer Programa de Oncobiologia

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

INCA SUS MINISTÉRIO DA SAÚDE BRASIL GOVERNO FEDERAL