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

Tandressa Souza Berguetti, Paloma Silva de Souza, Marcela Cristina Robaina and Raquel Ciuvalschi Maia
Laboratório 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

Multidrug resistance phenotype (MDR) is characterized by P-glycoprotein (Pgp/ABCB1) overexpression and related to chemotherapy failure. However, MDR may be associated with apoptotic pathways deregulation. The Tumor Necrosis Factor-alpha (TNF-α) is an important cytokine that may trigger signal death or tumor growth. In addition, our group previously demonstrated a correlation between Pgp and TNF superfamily proteins. Therefore, this study aimed to investigate the TNF-α role in multifactorial cancer resistance phenotype associated with Pgp expression. We used two cervical cancer cells: KB-3-1, parental and KB-C1, Pgp-positive. The TNF superfamily members were analyzed by TaqMan Array Human Apoptosis 96-well. Cells were treated with recombinant TNF-α (rTNF-α) and apoptosis were measure through annexin-V/PI staining and pro-caspase-3 levels. Pgp/ABCB1 and endogenous TNF-α/TNFA were analyzed by Western blot, immunofluorescence and qRT-PCR. Pgp efflux activity was analyzed by flow cytometry. The TNFA mRNA was up regulated in KB-C1 cells compared to the parental cells. We also observed low apoptosis rate following rTNF-α treatment in both cell lines by annexin-V/PI staining, however KB-C1 cells showed pro-caspase-3 drop down protein levels. While rTNF-α induced an increase of ABCB1 mRNA levels in KB-3-1 cells, no changes were observed in protein levels. Instead, rTNF-α did not alter ABCB1 mRNA expression levels in KB-C1 cells, but induced downregulation in protein levels with no efflux activity changes. However, TNF-α mRNA and protein showed upregulated in both cell lines after rTNF-α treatment. In summary, our results suggest that rTNF-α does not significantly alter cell viability, independent of Pgp expression. However, the downregulation of pro-caspase-3 may be associated with Pgp expression. In addition, rTNF-α induces a differential regulation of Pgp/ABCB1, which suggests an epigenetic regulation of Pgp expression in KB-3-1 cells. The maintenance of Pgp activity even after downregulation of Pgp expression levels, suggest 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.

KB-C1				
Gene	**Protein**	**Up regulated**	**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.

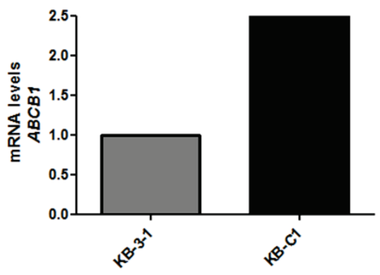

Figure 1: TNFA mRNA expression levels in KB-3-1 and KB-C1 cell lines. The mRNA levels of *TNFA* were analyzed using RT-qPCR in KB-3-1 and KB-C1 cells. GAPDH gene was used as endogenous control for RT-qPCR.

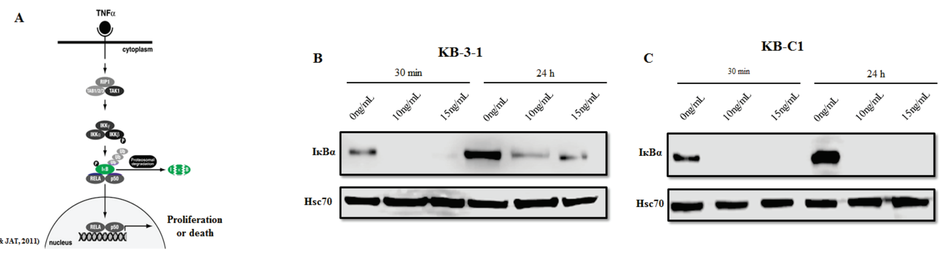

Figure 2: Expression of IκBα in KB-3-1 and KB-C1 cells. Representative scheme of NκFB pathway (A). Total expression of IκBα after treatment with rTNF-α for 30min or 24h was analyzed by *Western blot* in KB-3-1 (B) and KB-C1 (C) cells. Hsc70 was used as loading control.

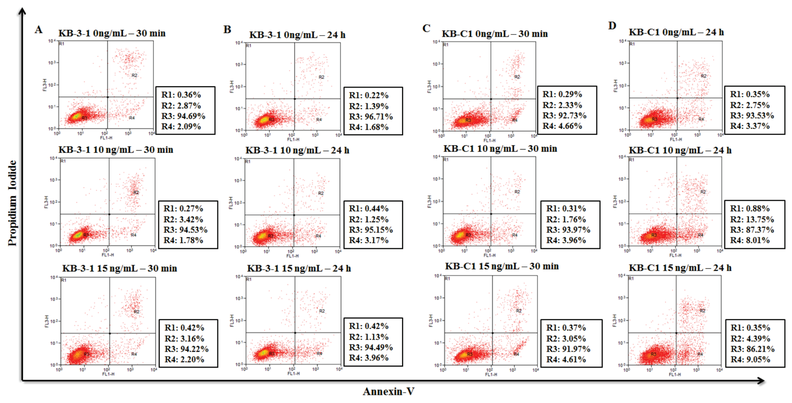

Figure 3: Effect of recombinant TNF-α (rTNF-α) on inducing apoptosis in KB-3-1 and KB-C1 cell lines. The apoptosis index of KB-3-1 (A and B) and KB-C1 (C and D) cell lines was measured by annexin-V/propidium iodide staining. Pro-caspase-3 (E and F) and caspase-8 (G and H) was analyzed by *Western* blot after treatment with rTNF-α for 30min or 24h. Hsc70 or Na+/K+ ATPase was used as loading control for *Western* blot (E to H).

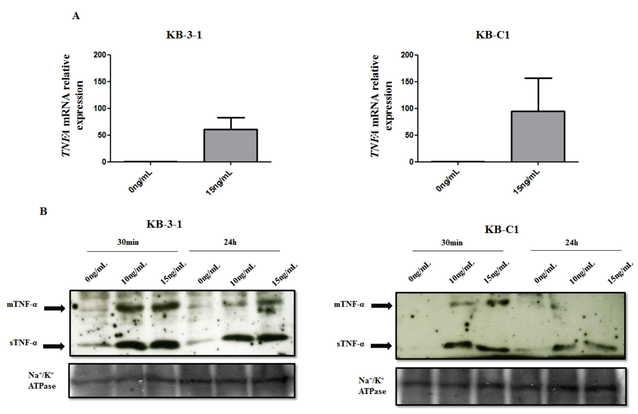

Figure 4: Effect of recombinant TNF-α (rTNF-α) in expression of endogenous TNF-α in KB-3-1 and KB-C1 cell lines. The mRNA levels of TNFA were analyzed using RT-qPCR after 24h treatment with rTNF-α (A) and protein levels of TNF-α was analyzed by *Western* blot in KB-3-1 and KB-C1 cells after 30min or 24h treatment with rTNF-α (B). Na+/K+ ATPase was used as loading control for *Western* blot (A). GAPDH gene was used as endogenous control for RT-qPCR (B).

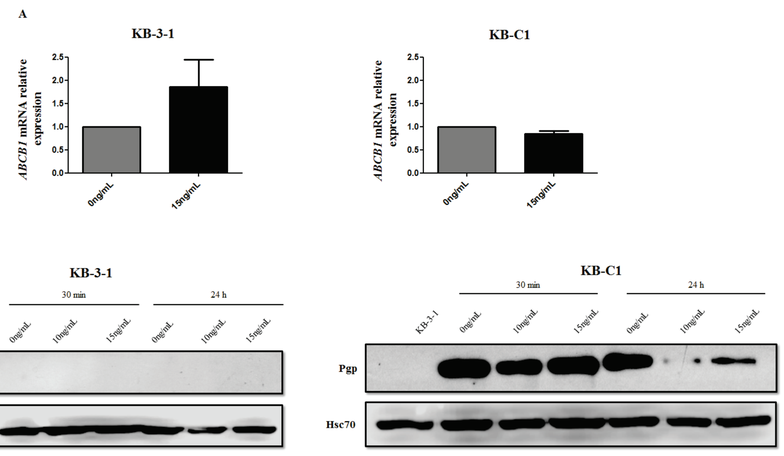

Figure 5: Effect of recombinant TNF-α (rTNF-α) on Pgp expression in KB-3-1 and KB-C1 cells. The mRNA levels of ABCB1 were analyzed using RT-qPCR in KB-3-1 and KB-C1 cell lines after 24h treatment with rTNF-α (A). Protein levels of Pgp were analyzed by Western blot in KB-3-1 and KB-C1 cells after 30min and 24h treatment with rTNF-α (B). GAPDH gene was used as endogenous control for RT-qPCR (A) and Hsc70 was used as loading control for *Western* blot (B).

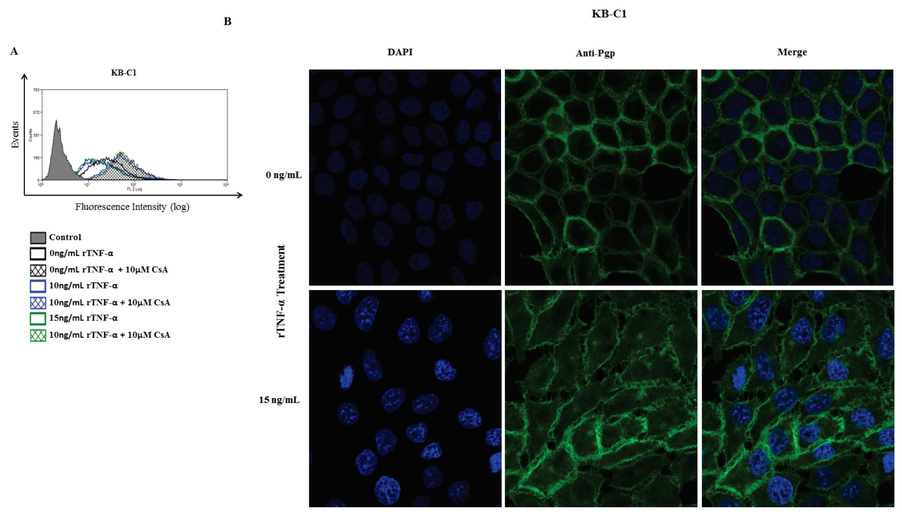

Figure 6: Effect of recombinant TNF-α (rTNF-α) on localization of Pgp in KB-C1 cells. The cell surface protein levels of Pgp were analyzed by shift assay in KB-C1 cells after 24h treatment with rTNF-α using FACs analysis (A). The subcellular localization of Pgp in KB-C1 after 24h treatment with rTNF-α cells was performed by confocal microscopy analysis (B). DAPI was used for nuclear staining (blue) and anti-Pgp staining (green); images captured at 60x magnification (B).

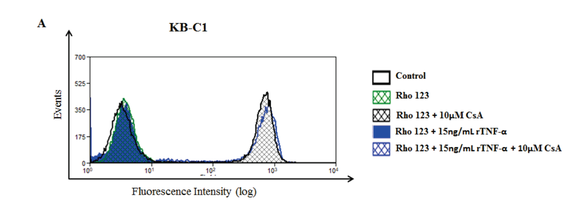

Figure 7: Effect of Pgp efflux function on *TNFA* and *ABCB1* expression levels. The efflux activity of Pgp was analyzed by Rhodamine 123 (Rho 123) assay, using FACs analysis (A) and mRNA levels of ABCB1 (B) and TNFA (C) were analyzed using RT-qPCR in KB-C1 cell lines after 24h treatment with 15ng/mL of rTNF-α plus 10ng/mL of CsA. Empty histogram represent cell autofluorescence, hatched green histogram represent Rho 123 accumulation, hatched black histogram represent Rho 123 accumulation after 10ng/mL of ciclosporine (CsA) treatment, blue histogram represents Rho 123 accumulation after 15ng/mL of rTNF-α treatment and hatched blue histogram represents Rho 123 accumulation after 15ng/mL of rTNF-α plus 10ng/mL of CsA (A). GAPDH gene was used as endogenous control for RT-qPCR (B and C).

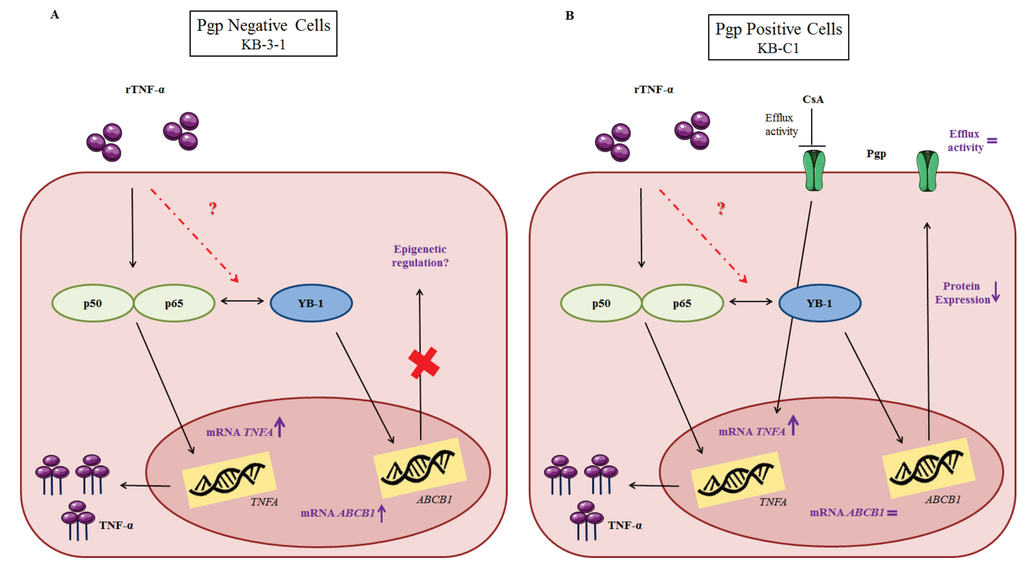

Figure 8: Representative scheme of recombinant TNF-α (rTNF-α) effect in KB-3-1 and KB-C1 cell lines. The rTNF-α in Pgp negative cells induces an increase of mRNA and protein levels of endogenous TNF-α and ABCB1 mRNA levels, with no change in Pgp protein expression (A). In Pgp positive cells, rTNF-α induced a decrease in Pgp protein expression with no change in *ABCB1* mRNA expression levels. Also, rTNF-α induced no changes in Pgp efflux activity. However, rTNF-α induced an increase in endogenous TNF –α mRNA and protein. Besides, the efflux activity of Pgp may interfer on rTNF-α effect in endogenous TNF-α.

Financial support: FAPERJ CNPq inct Programa de Oncobiologia

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

INCA SUS MINISTÉRIO DA SAÚDE BRASIL GOVERNO FEDERAL