MEMBRANE MICROPARTICLES DERIVED FROM MULTIDRUG RESISTANT CELLS INDUCE A PROLIFERATIVE PROFILE IN NON-TUMOR CELLS

JOÃO VICTOR VASCONCELOS DE SOUSA; TANDRESSA BERGUETTI; LUCAS DE SIQUEIRA PENNA QUINTAES; RAQUEL CIUVALSCHI MAIA; PALOMA SILVA DE SOUZA

Programa de Hemato-Oncologia Molecular, INSTITUTO NACIONAL DE CÂNCER (INCA), RIO DE JANEIRO - RJ - BRAZIL

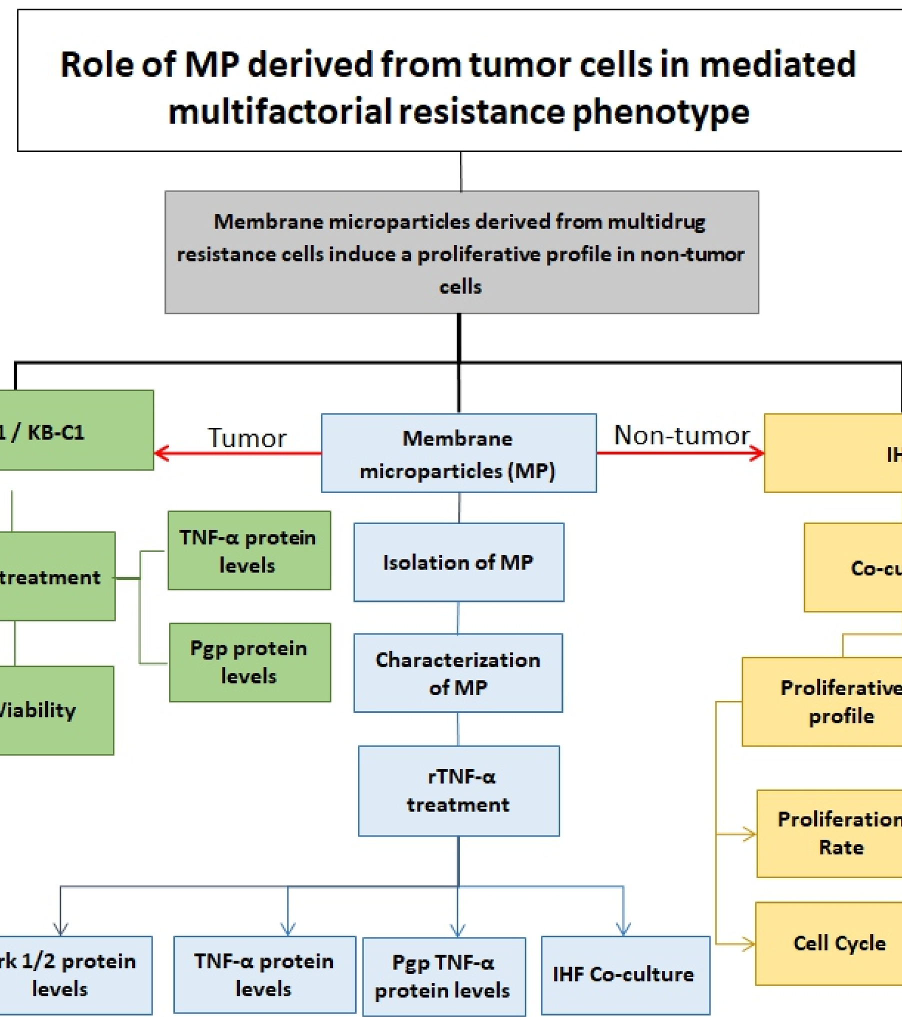
ABSTRACT

Introduction: The multidrug resistance (MDR) is characterized by overexpression of P-glycoprotein (Pgp / ABCB1), and is one of the major causes of cancer treatment failure with chemotherapy. Factors associated with deregulation of the cell death process also support MDR, contributing to a multifactorial resistance phenotype. MDR molecules that inhibit apoptosis have already been identified in membrane microparticles (MP) secreted by tumor cells. MP are small enclosed fragments (0.1 to 1 μM in diameter) that derive from plasma membrane budding and may carry different cellular molecules. In the context of cancer, Tumor Necrosis Factor-alpha (TNF-α) is an important cytokine that can induce cell death through activation of death receptors and consequent activation of caspases, or tumor growth. In addition, data from our group demonstrated a correlation between Pgp expression and TNF superfamily proteins. Thus, the aim of our study was to evaluate the role of MP derived from tumor cells in mediated multifactorial resistance phenotype (**Figure 1**).

Methods and Results: Using two cervical cancer cell lines KB-3-1 (Pgp-negative) and KB-C1 resistant (Pgp-positive), and the Immortalized Human Fibroblast cell line (IHF), we evaluated the effect of recombinant TNF-α (rTNF-α) in the induction of cell death and proliferation (**Figure 2, Figure 3, Figure 4**). We analyzed the protein levels of endogenous Pgp and TNF-α in tumor cells and in MP derived from these cells (**Figure 5**). The results showed that rTNF-α did not induce changes in the tumor cells viability but did contribute to a decreasing of their proliferation rate. It was observed that tumor cells spontaneously release MP *in vitro* (**Figure 6**), and that MP carry TNF-α and Pgp as content (**Figure 7, Figure 8**). Also, MP derived from rTNF-α treated cells had higher levels of endogenous TNF-α (**Figure 9**). The results obtained by co-culturing non-tumor cells and MP suggest a higher proliferation rate of IHF cells in comparison to cells with no co-culture condition (**Figure 10, Figure 11**).

Conclusion: Although our data suggest that rTNF-α did not induce changes in tumor cells viability independent of Pgp expression, it may contribute to a decreasing in the proliferative rate of these cells. However, MP derived from tumor cells treated with rTNF-α carry high amount of endogenous TNF-α and may promote a proliferative profile in IHF cells. Altogether, our data suggest that endogenous TNF-α carried by MP derived from drug resistant cells contribute to a proliferative phenotype of non-tumor cells.

Keyword 1: Membrane microparticles; **Keyword 2:** Multidrug resistance; **Keyword 3:** TNF-α



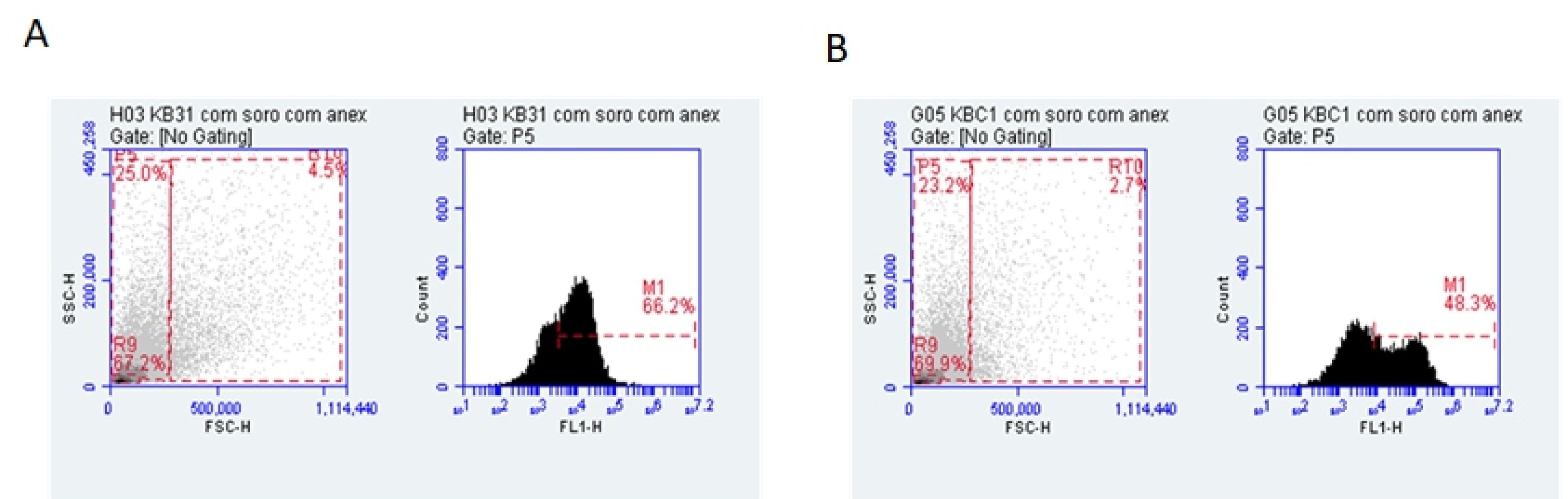


Figure 6: Cell lines release spontaneously microparticles (MP). MP isolated from KB-3-1 (A) and KB-C1 (B) cells were identified by flow cytometry. 66.2% of the events concerning the MPs derived from the KB-3-1 (A) cells and 48.3% of the events concerning the MPs derived from the KB-C1 cells were positive for FITC-annexin V.

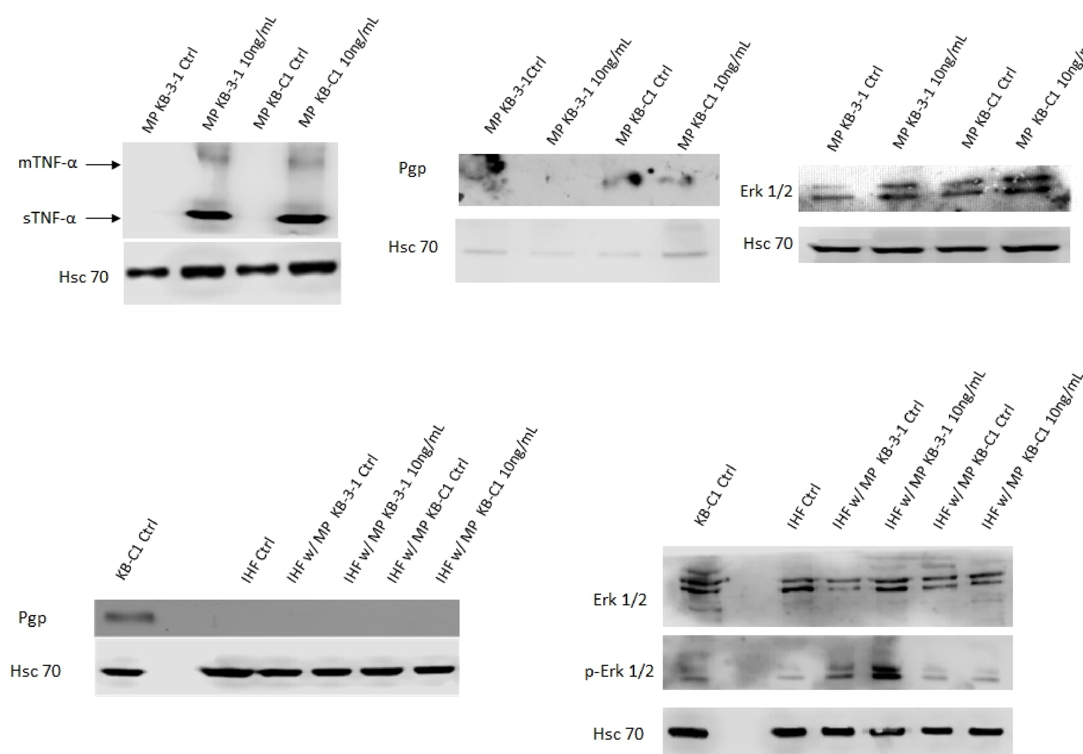


Figure 7: Analysis of protein content in microparticles (MP) derived from tumor cell lines. The protein content of of endogenousTNF-α, Pgp and ERK 1/2 was assessed by Western blot in released MP from KB-3-1 cells and KB-C1 cells after treatment with rTNF-α. Hsc70 was used as an endogenous control. Image representative of three independent experiments.

Figure 8: Expression of P glycoprotein (Pgp), ERK 1/2 and pErk 1/2 in non-tumor cells after co-culture with Microparticles (MP) derived from tumor cell lines. Protein content of Pgp, Erk 1/2 and p-Erk 1/2 was evaluated by Western blot ,after co-culture with MP derived from KB-3-1 and KB-C1 in the presence or absence of 10 ng/mL of rTNF-α. Hsc70 was used as an endogenous control. Image representative of two independent experiments.

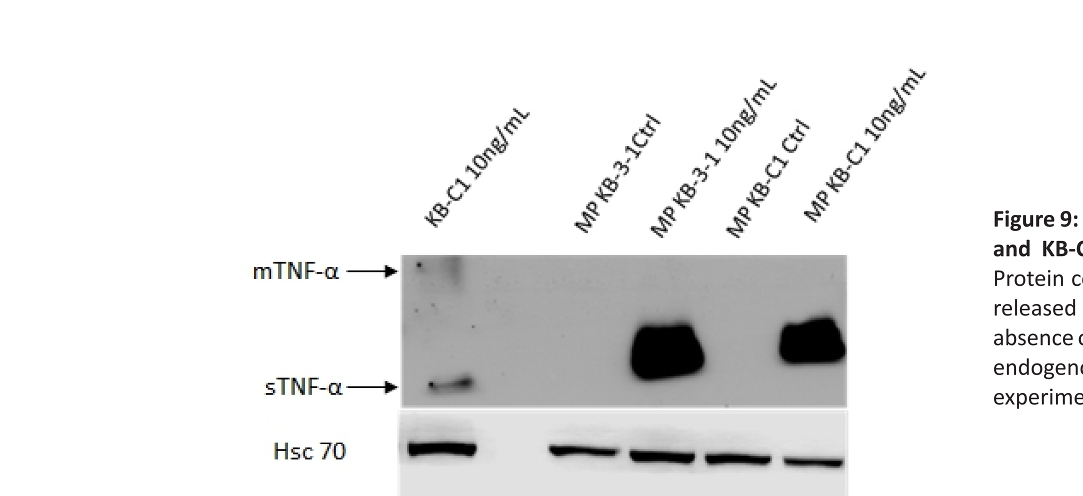


Figure 9: Analysis of endogenous TNF-α protein levels in KB-3-1 and KB-C1 cell lines and their released microparticles (MP). Protein content of TNF-α was evaluated in tumor cells and their released MP by Western blot after treatment in the presence or absence of 10 ng/mL of recombinant TNF-α. Hsc70 was used as an endogenous control. Image representative of three independent experiments.

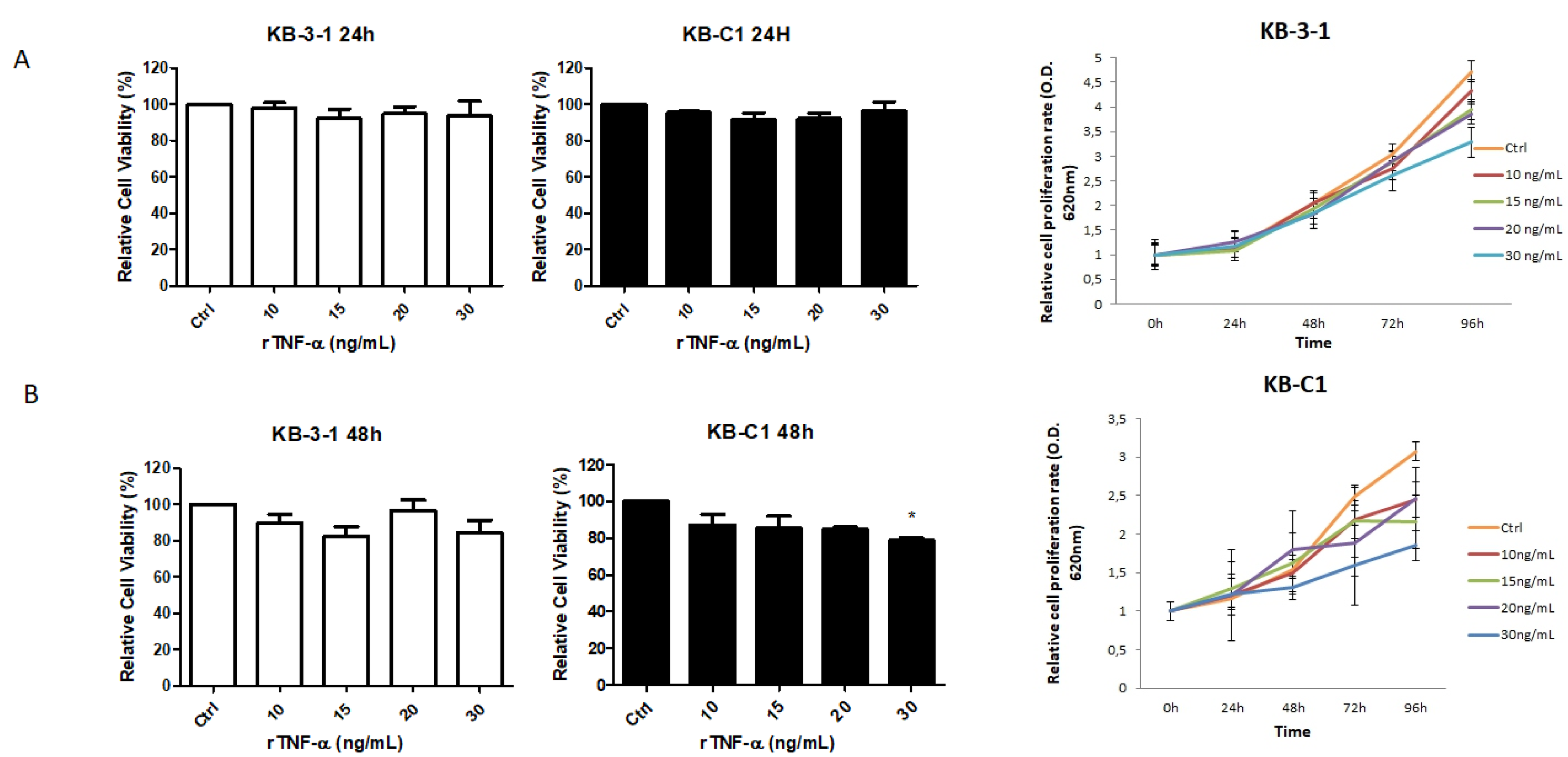


Figure 2: Viability of KB-3-1 and KB-C1 cell lines after incubation with recombinantTNF-α (rTNF). The cell viability of KB-3-1 and KB-C1 cells was assessed by the MTT assay in the presence or absence of 10, 15, 20 and 30 ng/mL of rTNF-α at 24 h (A) or 48 h (B). The graphs are representative of at least three independent experiments. The error bars represent the standard deviation of at least three independent experiments; * p <0,05.*cter pylori* positive; INT- = Intestinal Gastric Cancer *Helicobacter pylori* negative.

Figure 3: Proliferation kinetic analyses by crystal violet staining. KB-3-1 and KB-C1 were analyzed for proliferation daily by incorporation of crystal violet in the presence or absence of 10, 15, 20 and 30 ng/mL of recombinant TNF-α. The graphs represent the mean of at least three independent experiments. The error bars represent the standard deviation of at least three independent experiments.

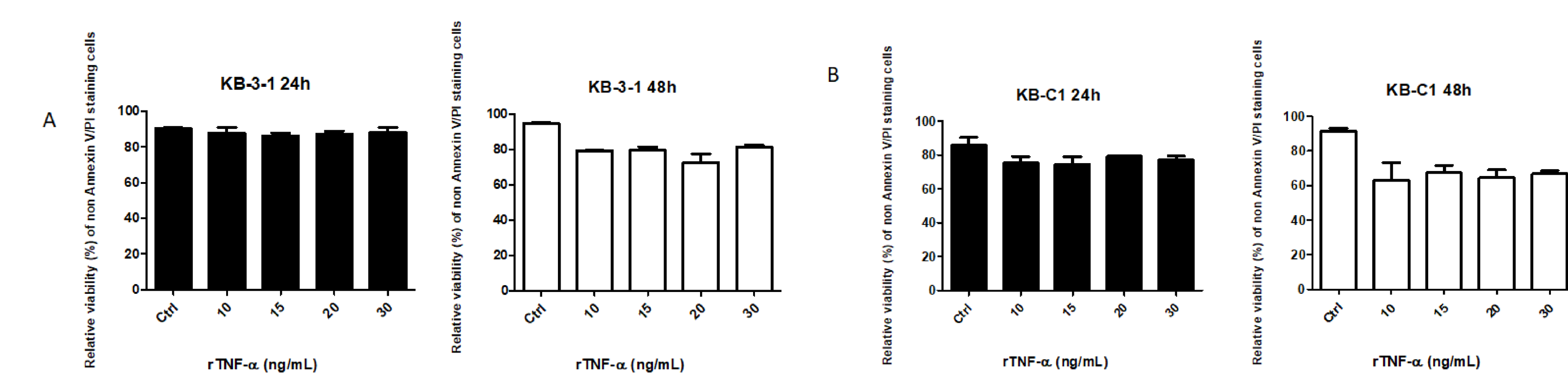


Figure 4: Annexin V / PI labeling in KB-3-1 and KB-C1 lines by recombinantTNF-α (rTNF)treatment. The KB-3-1 (A) and KB-C1 (B) lines were incubated in the presence or absence of 10, 15, 20 and 30 ng/mL of rTNF-α for 24 h or 48 h,for evaluation of cell death by Annexin-V labeling and Propidium iodide (PI) incorporation. The graphs represent the mean of two independent experiments and the error bars represent the standard deviation of these experiments

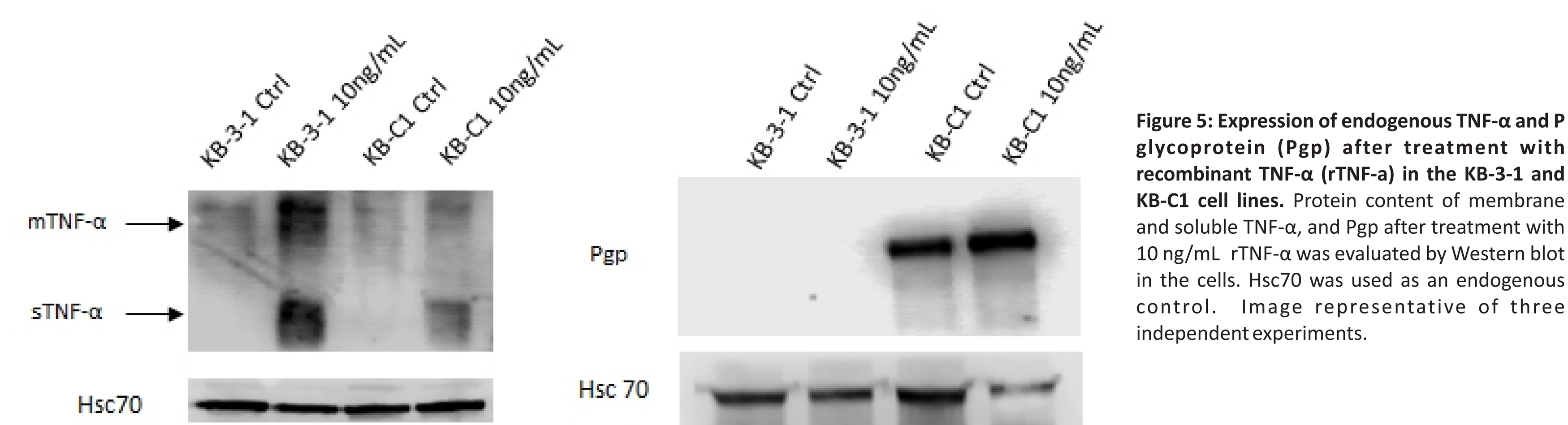


Figure 5: Expression of endogenous TNF-α and P glycoprotein (Pgp) after treatment with recombinant TNF-α (rTNF-a) in the KB-3-1 and KB-C1 cell lines. Protein content of membrane and soluble TNF-α, and Pgp after treatment with 10 ng/mL rTNF-α was evaluated by Western blot in the cells. Hsc70 was used as an endogenous control. Image representative of three independent experiments.

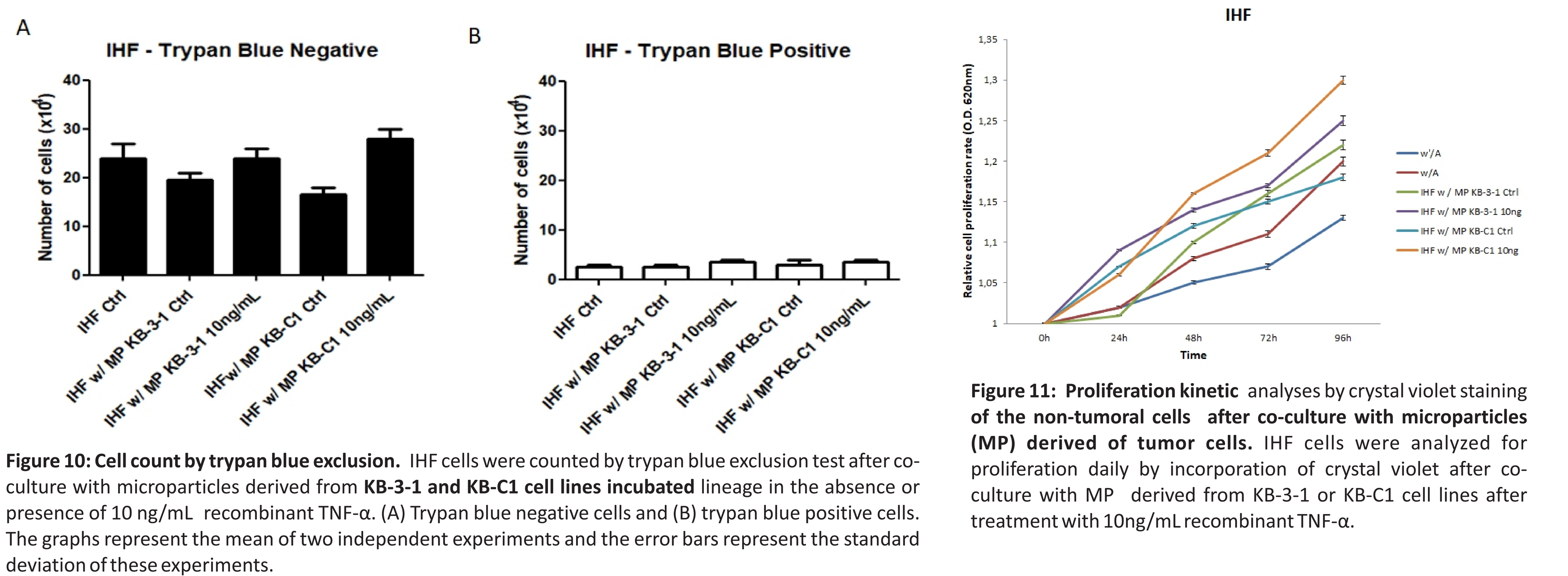


Figure 10: Cell count by trypan blue exclusion. IHF cells were counted by trypan blue exclusion test after co-culture with microparticles derived from **KB-3-1 and KB-C1 cell lines incubated** lineage in the absence or presence of 10 ng/mL recombinant TNF-α. (A) Trypan blue negative cells and (B) trypan blue positive cells. The graphs represent the mean of two independent experiments and the error bars represent the standard deviation of these experiments.

Figure 11: Proliferation kinetic analyses by crystal violet staining **of the non-tumoral cells after co-culture with microparticles (MP) derived of tumor cells.** IHF cells were analyzed for proliferation daily by incorporation of crystal violet after co-culture with MP derived from KB-3-1 or KB-C1 cell lines after treatment with 10ng/mL recombinant TNF-α.

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