Characterization of membrane microparticles derived from cancer cell lines

João Victor Vasconcelos de Sousa, Tandressa Souza Berguetti, Raquel Ciuvalschi Maia and Paloma Silva de Souza

Laboratório de Hemato-Oncologia Celular e Molecular, Programa de Hemato-Oncologia Molecular,
Instituto Nacional de Câncer (INCA), RJ, Brasil
e-mail: joaovv.sousa@gmail.com

ABSTRACT

Introduction and objective: Membrane microparticles (MP) are small membrane vesicles (0.1–2µm in diameter) derived from the ubiquitous cellular phenomenon of membrane budding. Numerous oncogenic molecules have been identified inside MP and its cargo can be transferred to neoplastic or non-malignant cells and consequently contribute to cancer dissemination. Studies have shown that P-glycoprotein (Pgp/ABCB1), a plasma membrane efflux transporter associated with multidrug resistance (MDR), can be carried by MP derived from resistant tumor cells and thus transferred to sensitive cells. In addition, our group demonstrated that sensitive cancer cells can acquire MDR phenotype through the intercellular transfer of MP derived from Pgp-positive cells. Based on this, the aim of this study was to identify and characterize MP derived from Pgp-positive and Pgp-negative cancer cells.

Material and Methods: The KB-3-1 (sensitive/ Pgp-negative) parental cell line and KB-C1 (resistant/Pgp-positive) cell line, both derived from cervical cancer. The KB-C1 cells were cultured with 1µg/ml colchicine. Pgp expression and efflux activity were analyzed by Western blotting and flow cytometry, respectively. Also, both cell lines were incubated with cisplatin, doxorubicin and colchicine, separately, for 24h, 48h and 72h and then cell viability was evaluated by MTT assay. For MP isolation, sensitive and resistant cell lines were seeded on the bottom of 6-well plate for 24h and then cultured for 24h in the presence or absence of 10% Fetal Bovine Serum (FBS), or in the presence of 10% dFBS (FBS filtered in 0,22µM pore filter membrane). Initially, supernatant was centrifuged at 2000g for 15 min twice for debris removal. After that, the supernatant was preserved and centrifuged at 16000g for 2:30h at 4°C. After centrifugation, supernatant was discarded and MP at pellet was washed with PBS at 16000g for 2:30h at 4ºC. The MP identification was performed trough annexin V labeling through by flow cytometry analysis. In addition, protein cargo from MP, and protein amount from donor cells were analyzed by Lowry protein assay protocol.

Results and conclusion: Our results showed that KB-3-1 was sensitive to doxorubicin, colchicine and cisplatin treatment. However, KB-C1 cell line showed resistance to doxorubicin and colchicine treatment, but sensitive to cisplatin. These data suggest that MDR phenotype of KB-C1 cells is mediated by Pgp expression. Regarding MP data, both cell lines were able to release MP spontaneously. The protein quantification data showed that MP are release in cell culture independently of Pgp expression. However, our data showed that absence of FBS in cell culture interfere on amount of MP. Together our results showed that Pgp expression contributes to MDR phenotype in KB-C1 cells, but does not interfere with MP release compered to KB-3-1 parental cells.

METHODS

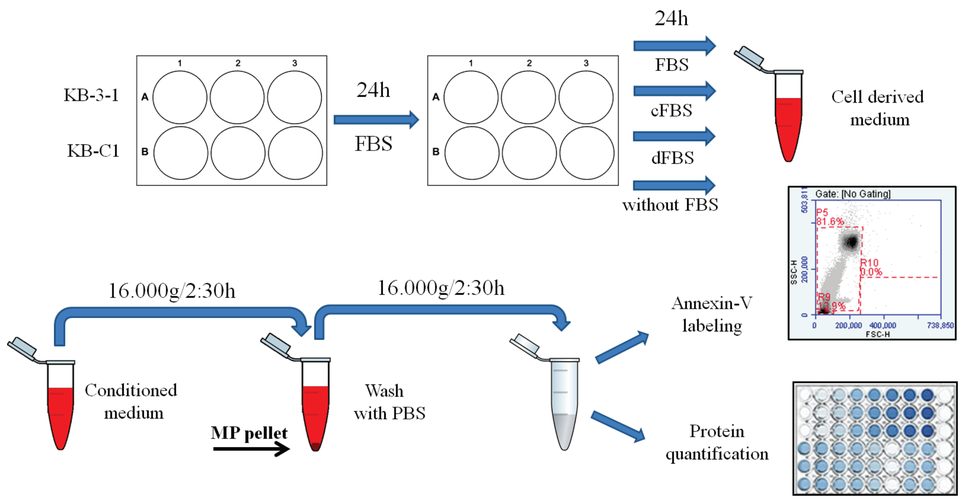

Figure 1: Representative scheme of membrane microparticles (MP) isolation. Cancer cell lines were seeded in 6-well plates and culture for 24 hours with fetal bovine serum (FBS) or centrifuged FSB (cFSB) or filtered FSB (dFSB) or without FSB(wFSB). The conditioned medium was preserved and centrifuged at 16000g for 2:30h at 4°C. After centrifugation, supernatant was discarded and MP at pellet was washed with PBS at 16000g for 2:30h at 4ºC. The MP derived from cancer cells were analyzed by flow cytometry and Lowry protein assay protocol.

RESULTS

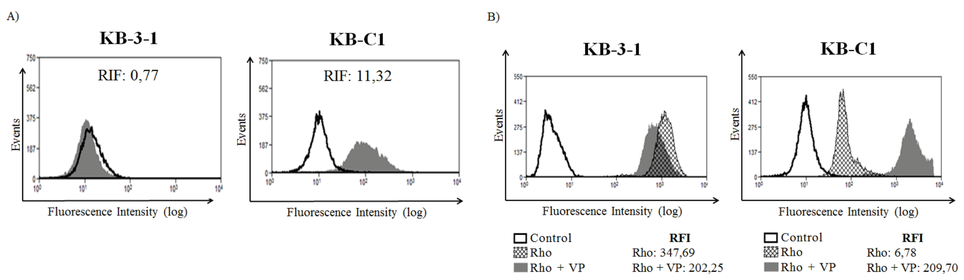

Figure 2: Expression and function of Pgp in KB-3-1 and KB-C1 cell lines. Pgp expression (A) and efflux activity (B) was evaluated in KB-3-1 and KB-C1 cells by flow cytometry. Empty histogram represents cell autofluorescence and gray histogram represents the labeling with conjugated anti-Pgp antibody (A). Empty histogram represents cell autofluorescence, hatched gray histogram represents fluorescence intensity after incubation with Rho and the gray histogram represents fluorescence intensity in presence of Rho and VP. Ratio Fluorescence Intensity (RFI).

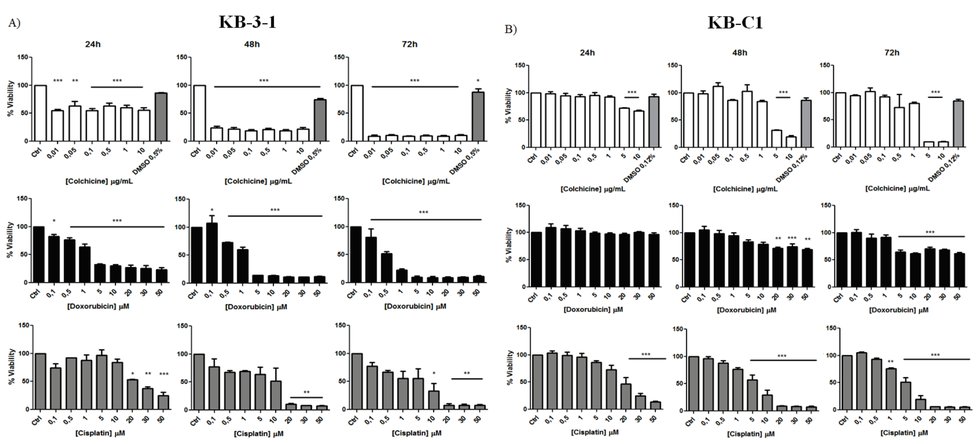

Figure 3: Viability of the KB-3-1 and KB-C-1 cell line after incubation with the chemotherapeutic agents. Cell viability of KB-3-1 (A) and KB-C1 (B) cells was assessed by the MTT assay in the presence of Doxorubicin, Cisplatin or Colchicine for 24h, 48h or 72h. The graphs represent the mean of three independent experiments and the error bars represent the standard deviation of three independent experiments.

Figure 4: KB-3-1 and KB-C1 tumor cells shed MP spontaneously. KB-3-1 (A) and KB-C1 (B) cell lines were cultivated in total FBS and spontaneous releasing of MP were identified through annexin V staining and analyzed by flow cytometry.

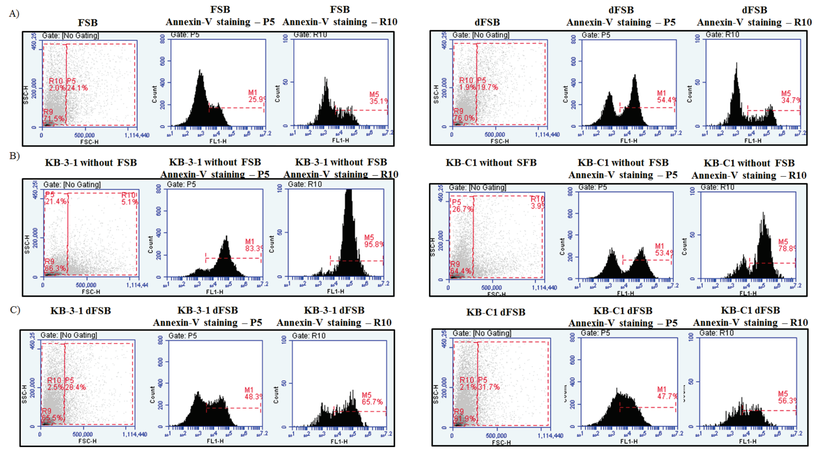

Figure 5: Analysis of MP derived from FBS and its effects in cancer cells MP releasing. MP derived from FBS (A- left panel) and filtered FBS (A – righr panel); MP derived from KB-3-1 cells cultured without FBS (B – left panel) and MP derived from KB-C1 cells cultured without FBS (B – right panel); MP derived from KB-3-1 cultured with filtered FBS (C- left panel) and MP derived from KB-C1 cultured with filtered FBS (C-right panel).

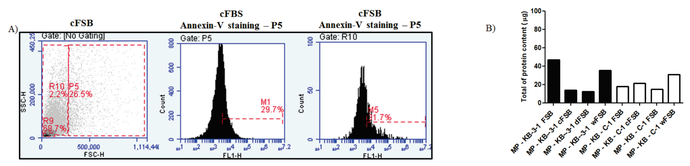

Figure 6: Analysis of MP derived from centrifuged FBS (cFBS) and its effects in protein content of MP derived from cancer cells. MP derived from cFBS (A). Protein content of MP derived from KB-3-1 and KB-C1 cells cultured with FBS, centrifuged FBS (cFSB), filtered FBS (dFBS) or without FBS (wFBS) (B).

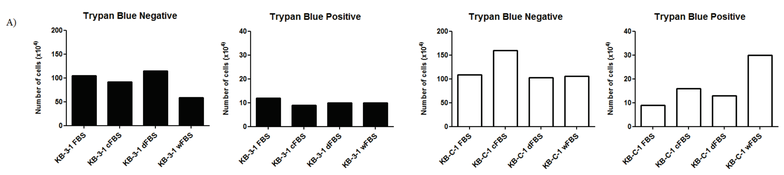

Figure 7: Analysis of different conditions of SFB in cancer cell lines viability. KB-3-1 (A) and KB-C1 (B) cell lines were cultured with FBS, cFBS, filtered FBS (dFBS) or without FBS (wFBS) and analyzed by trypan blue exclusion assay.

CONCLUSION

Characterization of membrane microparticles derived from cancer cell lines is influenced by different conditions of FBS and different condition of FBS interfere on cancer cell lines viability.

Financial Support: Programa de Oncobiologia, CNPq, FAPERJ and INCT

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