Is P-glycoprotein relevant for the release of microvesicles by tumor cells?

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Aim: In this study, we aimed to verify if MDR cells without expression of P-gp also produced more microvesicles and less exosomes than their DS counterpart cells.

Introduction: Cancer multidrug resistance (MDR) is a major cause of chemotherapy failure and is highly associated with overexpression of drug-efflux pumps such as P-glycoprotein (P-gp). The identification of mechanisms specific of P-gp overexpressing cells may contribute to the identification of biomarkers of MDR.

It was recently discovered that a drug-resistant phenotype may be horizontally transferred from drug-resistant (DR) to drug-sensitive (DS) cells, mediated by the cargo of extracellular vesicles (EVs) released by DR cells and captured by DS cells. These EVs include smaller exosomes and larger microvesicles. Our previous work showed that MDR cells with overexpression of P-gp released more microvesicles than exosomes, unlike their DS counterpart cells. However, it is not known if this phenomenon is restricted to MDR cells with overexpression of P-gp or if it is extensive to all DR cells (with other mechanism of drug resistance).

Methods: Drug-response curves of MDR and DS counterpart cells were obtained, using resazurin and trypan blue assays, to confirm the resistant or sensitive phenotype of the cell lines. Confirmation of their P-gp status was possible by Western-Blot. EVs released by both DS and MDR cells were isolated by ultracentrifugation and characterized by transmission electron microscopy, dynamic light scattering, nanoparticle tracking analysis and Western blot analysis.

Results: We confirmed that MDR cells without expression of P-gp release EVs with similar sizes to the ones released by their DS counterparts.

Conclusion: So, P-gp may be associated with the release of larger EVs by MDR cells. These results will be further confirmed by characterizing the EVs released by P-gp overexpressing MDR cell lines following downregulation of P-gp expression and the EVs released by DS cell lines following transfection of P-gp.

References

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Uterine protein oxidative modifications may condition trophoblast function

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Aim: Evaluate whether protein carbonylation resulting from uterine altered redox imbalance interferes with extravillous trophoblast viability.

Introduction: Local redox homeostasis is believed to have a pivotal role in uterine transformation necessary for blastocyst implantation and placenta development. By contrast, redox status imbalance plays a role in deficient placenta and the development of pregnancy-related complications (e.g. preeclampsia or gestational diabetes) with increased incidence in older women. Thus, it was hypothesized that at an older reproductive age, loss of redox homeostasis is a contributor to disruption of foetal/placental interactions and the development of such complications.

Methods: Uterine human samples were collected at delivery by elective caesarean section. The protocol was approved by the ethical committee of “Centro Materno-Infantil do Porto”, volunteers gave written consent to be included in the study. Total protein carbonylation was detected by oxyblot and protein expression was quantified by western blotting. Specific protein carbonylation was verified by immunoprecipitation. Albumin was carbonylated using hydrogen peroxide (H2O2), followed by dialysis, and western blotting to confirm carbonylated albumin. HST-8SV neo extravillous trophoblasts were treated with carbonylated/non-carbonylated albumin, followed by cell viability assay. A P value less than 0.05 was assumed to denote significant difference.

Results: At the placental site, carbonylated albumin normalized to total albumin expression showed a positive and significant association with maternal age. (r = 0.6909, P = 0.0021) In vitro, carbonylated albumin displayed a cytotoxic effect, at concentrations ranging from 10 to 100 μg/ml. Lower concentrations did not affect trophoblast viability.

Conclusion: Uterine aging is accompanied by selective albumin oxidative modifications, which appears to interfere with trophoblast ability to invade and transform the maternal placental site.

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