Microparticles confer multidrug resistance in breast cancer

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Rationale: Despite decades of using cytotoxic drugs, the incidence of cancer relapse is still prevalent. One mechanism contributing to this is multidrug resistance (MDR). MDR occurs when a tumour cell becomes resistant to a number of structurally and functionally unrelated cytotoxic drugs following exposure to a single agent, resulting in a tumour cell that no longer responds to therapy even if drugs of different classes are used. One mechanism contributing to the emergence of MDR is the over-expression of efflux transporters, in particular, P-glycoprotein (P-gp). This transporter maintains a sublethal intracellular drug concentration, effectively rendering cancer cells treatment unresponsive.

Microparticles (MP) are plasma membrane-derived vesicles 0.1-1μm in diameter released by blebbing from various cell types. As such, MP are made up of fragments of the parent cell's plasma membrane and contain its cell surface proteins and cytoplasmic material (Mack *et al.*, 2000). We have previously shown in human acute lymphoblastic leukaemia cells that MP are shed spontaneously from drug-resistant cells, are capable of binding to drug-sensitive cells, and in doing so, transfer functional P-gp to drug-sensitive cells, conferring MDR (Bebawy *et al.*, 2009). Here we demonstrate that this also occurs in solid tumour cells such as the MCF-7 breast cancer cell line. Specifically, we demonstrate that the multidrug resistant MCF-7/Dx cell line spontaneously sheds MP and that these MP carry the multidrug transporter. Furthermore, we demonstrate that MP derived from these cells also transfer transporter transcript and regulatory nucleic acids (microRNAs).

Methods: To determine if MP are shed from drug-resistant cells, the isolated MP population was labelled with FITC-annexin V and analysed by flow cytometry. To determine if MP transferred P-gp to drug-sensitive cells, a co-cultured population of drug-sensitive cells with MP were labelled with FITC-anti-P-gp and analysed by flow cytometry. Transferred P-gp was deemed functional by the daunorubicin dye exclusion assay. Quantitative real time PCR was used to determine the levels of expression of transcripts and microRNAs in donor cells, MP and recipient cells following MP coculture.

Results: Our results show that the multidrug resistant MCF-7/Dx cell line spontaneously sheds MP and that these MP are capable of carrying functional P-gp to the drug-sensitive cell line, rendering recipient cells MDR. We have also determined that MP are capable of incorporating and transferring transporter transcripts and microRNAs, leading to changes in the recipient cells which are reflective of the donor cell phenotype.

Conclusion: The significance of this study is in the elucidation of a non-genetic pathway for the acquisition of P-gp mediated MDR in cancer. This has the potential for translation into clinical outcomes as it provides another avenue by which resistance to chemotherapy can be addressed.

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