Exosome as Intercellular Shuttle of Chemotherapy Drugs
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Purpose
Exocytosis is a mechanism by which mammalian cells actively transport molecules via small membrane vesicles such as exosomes into extracellular space and to neighboring cells. Exosomes are between 30 and 100 nm in diameter and carry molecules (e.g., microRNAs, transcription factors, endosome-derived proteins, lipids) that participate in intercellular communications locally and distally (e.g., through systemic circulation). The present study evaluated the potential role of exosome in intercellular transfer of chemotherapeutic drugs. Paclitaxel (PTX) was used as the model drug.

Methods
Human breast (MCF7 and a lung metastatic subline of MDA-MB-231 cells, LM2) and ovarian (A2780 and OVCAR4) cancer cells were studied. Exosomes were isolated from cell growth medium after incubating cells with PTX (10-1000 nM) for 24-48 h, using ultra-centrifugation at 100,000g for 18 h, and analyzed for their acetylcholinesterase activity (Ellman’s assay), amounts of total proteins (BCA assay) and PTX (LC-MS/MS). The effects of exosomes derived from the PTX-treated cells (donor cells) on cell proliferation and migration were measured in recipient cells, using the sulforhodamine B and wound-healing assay, respectively. We further studied the effects of 24-h pretreatment with a proton pump inhibitor omeprazole or a ceramide synthesis inhibitor GW4869 on the exocytosis of PTX-containing exosomes and resulting biological effects. A quantitative pharmacological model was developed to characterize the intercellular transfer and pharmacodynamics of PTX-containing exosomes between donor and recipient cells.

Results
In the absence of PTX, cells in exponential growth phase excreted ~220 exosomes/cell. Addition of PTX stimulated exosome production from all four cancer cells in a dose- and time-dependent manner. This PTX effect was also cell-type specific, with greater sensitivity in ovarian cancer cells over breast cancer cells. Exosomes isolated from cells treated with 100 and 1000 nM PTX for 24 h contained 20 to 56 pmol PTX/10^6 cells, and inhibited cell viability and migration in vitro. Pretreatment with non-cytotoxic concentration of omeprazole (10 μM) or GW4869 (10 μg/mL) reduced the exosome production or secretion (by 19.5 to 36.6%) and lowered the total PTX content in exosomes (by 25.4 to 79.3 %); and the lowering of PTX-containing exosome release was accompanied by an increase of PTX cytotoxicity in parent cells.

Conclusion
Limited delivery of chemotherapy drugs to solid tumors and heterogeneous intratumoral distribution reduce their therapeutic efficacy. Our results indicate (a) exosome represents a mechanism of intercellular drug transfer, (b) PTX stimulates exosome production or secretion, and (c) exocytosis of PTX-containing exosomes is inhibited by a means of blocking exosome biogenesis and release.

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