Targeting Gb3 and apoptosis-related proteins to overcome cisplatin resistance

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Akademisk avhandling

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Avhandlingen kommer att förvaras på engelska.

Abstract

Background: Cisplatin is used for treatment of malignant pleural mesothelioma (MPM) and non-small cell lung cancer (NSCLC) but treatment with cisplatin often leads to acquired resistance to cisplatin, resulting in poor patient survival. Globotriaosylceramide (Gb3) and multidrug resistance protein 1 (MDR1) have been associated with cisplatin resistance. Gb3 serves as a receptor for verotoxin-1 (VT-1), which induces apoptosis, and has been shown to have a functional dependency to MDR1 and heat shock protein 70 (HSP70). The Bcl-2 family of proteins and inhibitors of apoptosis (IAPs) are key regulators of apoptosis. BH3-mimetics mimic pro-apoptotic BH3-only proteins, while Smac mimetics mimic the IAP-binding protein Smac/Diablo. These drugs have shown great promise in reversing cisplatin resistance. Exosomes are small bio-nanoparticles secreted and taken up by both cancer cells and normal cells. They have the ability to transfer properties between cells and normal cells. They have the ability to transfer resistance to cisplatin.

Methods: In this thesis, NSCLC cell line H1299 and MPM cell line P31 were studied using western blot, flow cytometry, proteome profilers, confocal microscopy and gene expression arrays to investigate changes in protein and gene expression after acquisition of cisplatin resistance (P31res and H1299res) or after incubation with exosomes or drugs that target these. The cytotoxic and apoptotic effects were studied using fluorometric cytotoxicity assay (FMCA) and terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay.

Results: This thesis confirms that Gb3 is a potential target for cisplatin resistance reversal. Incubation with glycosphingolipid production inhibitor DL-threo-1-phenyl-2-palmitoylamino-3-morpholino-1-propanol (PPMP) and VT-1 led to reduced Gb3 cell surface expression and increased cytotoxic effect of cisplatin in all cell lines. Gb3 and MDR1 was not co-localized in any studied cell line, but Gb3 and HSP70 were co-localized on the cell surface and PPMP and VT-1 led to a decrease of both Gb3 and HSP70. Both BH3-mimetic obatoclax and Smac mimetic AT-406 had an additive effect on cisplatin-induced cytotoxicity and apoptosis in P31 and a synergistic effect in P31res. Results indicate that exosomes from cisplatin-resistant cell lines can transfer HSP70 to the surface of cells.

Conclusion: Cell surface Gb3 and HSP70, the Bcl-2/IAP-family proteins and exosomal transfer of cisplatin resistance characteristics are potential targets in combatting cisplatin resistance that show therapeutic promise and warrant further research.

Keywords
Cisplatin resistance, exosomes, gb3, HSP70, the Bcl-2 family

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