Osteopontin-c mediates drug resistance in ovarian carcinoma cells

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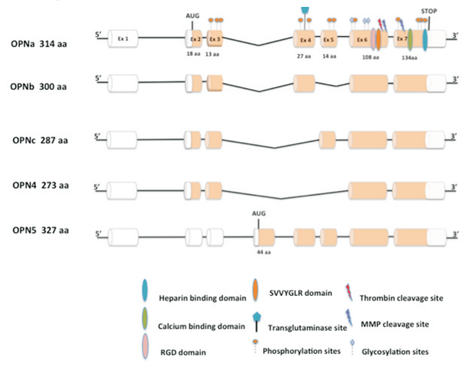
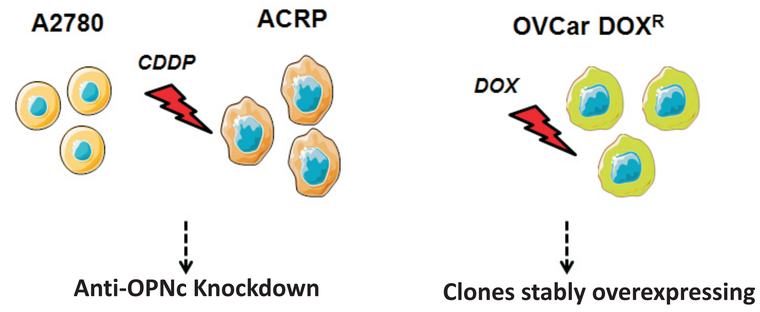
INTRODUCTION

Osteopontin (OPN) has been described as an important gene product mediating resistance to chemotherapeutic drugs. However, the specific roles of each OPN splice variant on mediating chemoresistance should be further investigated. In this context, this work aimed to evaluate OPNc expression patterns and its correlations with resistance to cisplatin (CIS) in ovarian tumor cell lines.



Gimba et al.,2018 Submetido

METHODOLOGY



- PCR Real time
- Functional Assays

Cell viabillity assay (MTT)
Clonogenic assay

Generation of cells clones
Stably overexpressing OPNc

RESULTS

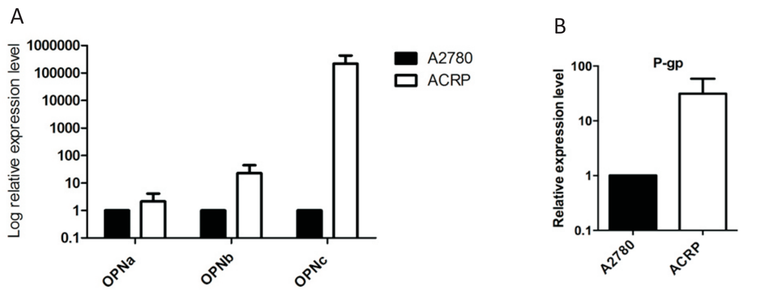


Fig. 1. OPNc isoform and P-gp are overexpressed in ACRP cisplatin-resistant ovarian cancer cells (A) ACRP cells were harvested for qRT-PCR analysis of OPNa, OPNb and OPNc isoforms expression levels. OPN-SI mRNA levels were compared to their parental counterparts A2780 cells (B) ACRP cells cells were collected for P-glycoprotein (P-gp) mRNA expression levels by qRT-PCR analysis.

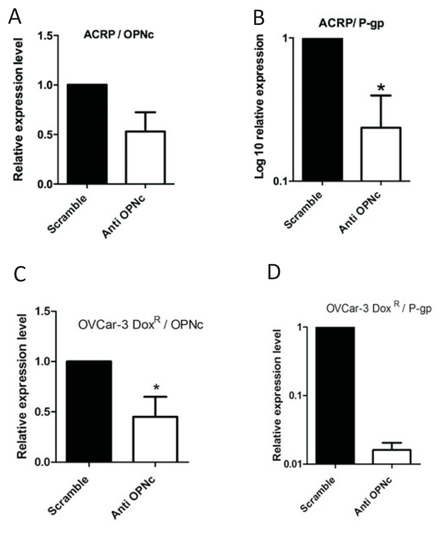


Fig. 2. Knockdown of OPNc isoform downregulates P-glycoprotein mRNA expression levels. ACRP cisplatin-resistant ovarian cancer cells were seeded for 24 h, after which they were transfected with 100 nM scramble and anti-OPNc oligomers using the Lipofectamine 2000 reagent. Following 24 h of transfection, cells were harvested for qRT-PCR analysis of OPNc (A and C) and P-glycoprotein (B and D) mRNA expression

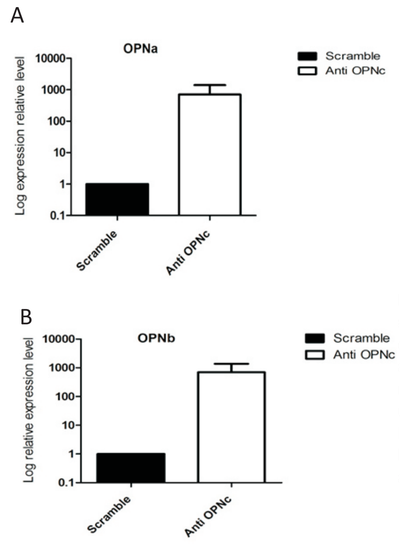


Fig. 3. OPNc knockdown modulates OPNa and OPNb levels. ACRP cisplatin-resistant ovarian cancer cells were seeded for 24 h, after which they were transfected with 100 nM scramble and anti-OPNc oligomers using the Lipofectamine 2000 reagent. Following 24 h of transfection, cells were harvested for qRT-PCR analysis of OPNa (A) and OPNb (B) relative expression levels.

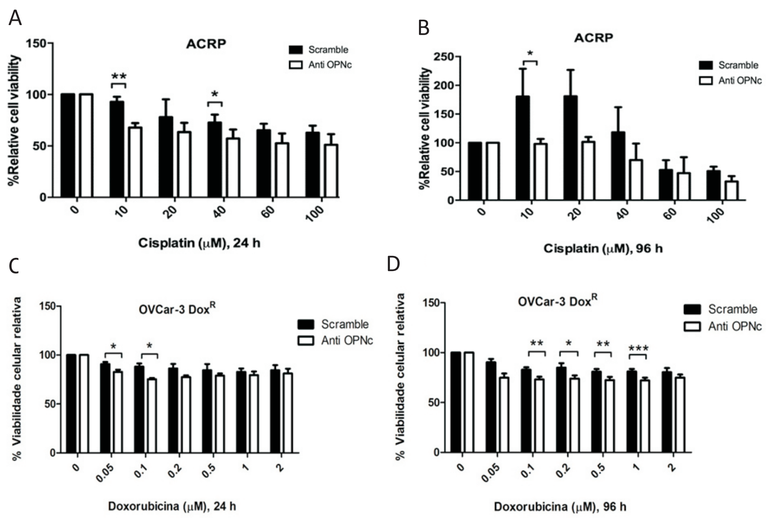


Fig. 4. OPNc knockdown sensitizes ACRP cisplatin-resistant ovarian cancer cells to drug treatment and impairs cell growth and colony formation. ACRP cisplatin-resistant ovarian cancer cells were seeded for 24 h, after which they were transfected with 100 nM scramble or anti-OPNc oligomers. Following 24 h of transfection, cells were reseeded in 96-well plates and treated with increasing concentrations of CDDP for 24 h (A and C) and 96 h (B and D). ACRP cell viability was measured by the MTT assay and compared between scramble or anti-OPNc oligomers-transfected cells.

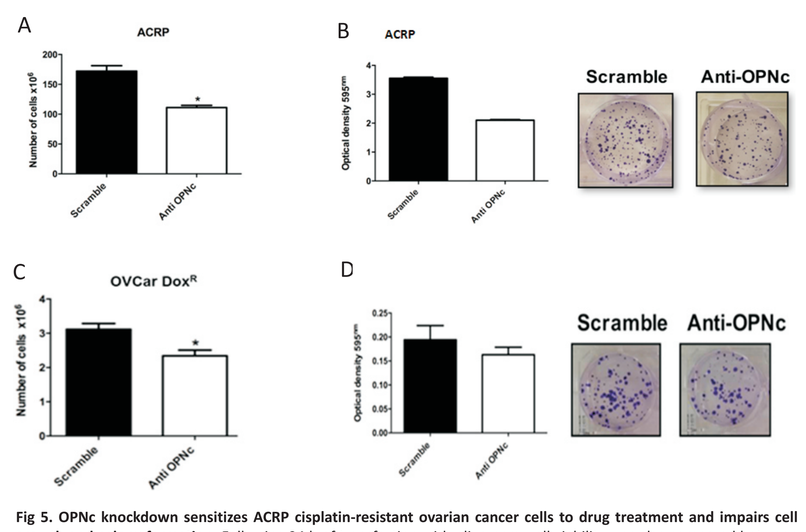


Fig 5. OPNc knockdown sensitizes ACRP cisplatin-resistant ovarian cancer cells to drug treatment and impairs cell growth and colony formation. Following 24 h of transfection with oligomers, cell viability was also compared between scramble and anti-OPNc transfected cells through trypan blue exclusion analysis (A and C) ACRP and OVCar-DOX^R cells were also assessed for clonogenicity, in which they were grown in fresh media until colony formation (around 14 days) and stained with crystal violet (B and D). Optical density was obtained at 595 nm following crystal dissolution in 33 % acetic acid.

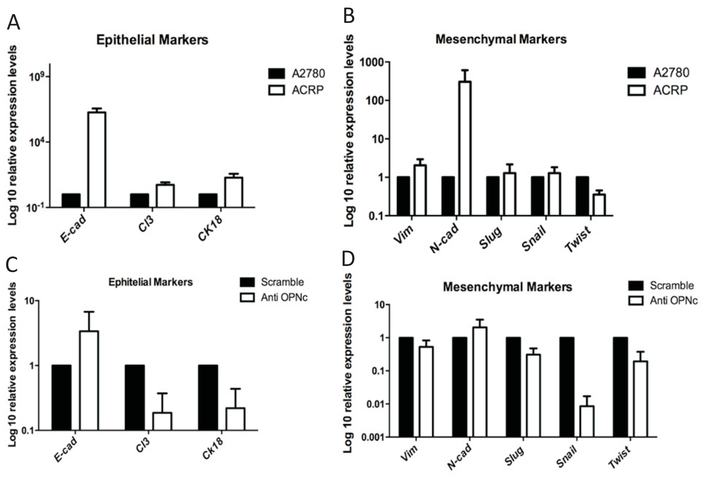


Fig. 6. OPNc modulates the epithelial-mesenchymal plasticity phenotype in ACRP cisplatin-resistant ovarian cancer cells. A2780 and ACRP were harvested and had epithelial (A) and mesenchymal markers (B) analyzed through qRT-PCR analysis. ACRP cells were seeded for 24 h, after which they were transfected with 100 nM scramble or anti-OPNc oligomers. Following 24 h of transfection, cells were harvested for qRT-PCR analysis of epithelial (C) and mesenchymal markers (D).

CONCLUSION

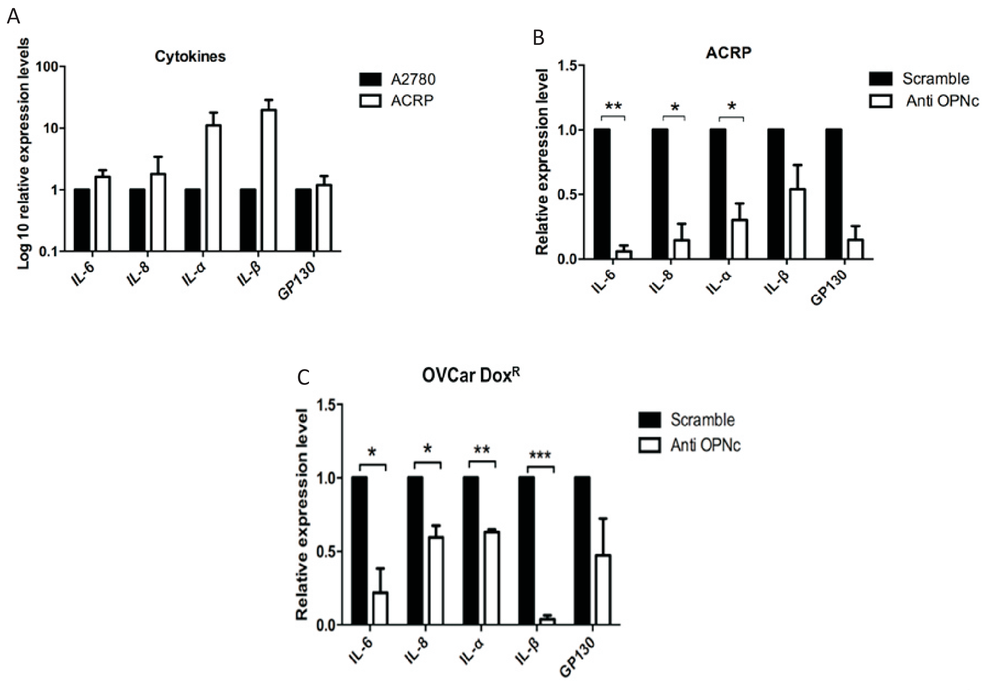


Fig. 7. OPNc regulates the expression of epithelial-mesenchymal-transition-related cytokines in drug-resistant cancer cells. (A) A2780, ACRP and OVCar DOX^R ovarian-derived cancer cells were harvested and analysed by qRT-PCR for the mRNA expression of IL6, IL8, IL1α, IL1β cytokines and the GP130 receptor. ACRP and OVCar DOX^R drug-resistant cell lines (B and C) were seeded for 24 h, after which they were transfected with 100 nM scramble or anti-OPNc oligomers. Following 24 h of transfection, cells were harvested for analysis of IL6, IL8, IL1α, IL1β cytokines and the GP130 receptor by qRT-PCR.

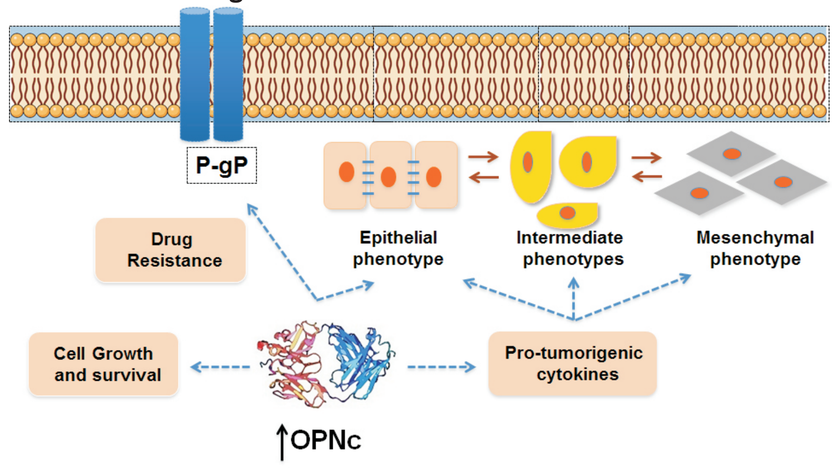


Fig 8. Schematic model for OPNc isoform in mediating chemoresistance in tumour cells. OPNc splicing isoform is overexpressed in ACRP cisplatin-resistant ovarian cancer cells, compared to their sensitive counterparts. Based on our data resulting from OPNc specific knockdown we hypothesized that this splice variant may modulate cell growth, sensitizing chemoresistant cells to cisplatin treatment, in association with OPNc action on controlling P-glycoprotein (P-gp) expression. We also postulate that OPNc controls the expression of epithelial-mesenchymal transition (EMT) markers in response to the expression of EMT-related cytokines. Altogether, these findings point to a role of OPNc isoform as a promising target for future therapeutic interventions aiming to revert drug resistance.

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

MINISTÉRIO DA SAÚDE