Osteopontin-c mediates drug resistance in ovarian carcinoma cells

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Summary

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. This work aimed to evaluate OPNc expression patterns and its associations with resistance to cisplatin (CDDP) in ovarian tumor cell lines. We used one ovarian cancer cell line resistant to CDDP (ACRP), as well as their corresponding parental control cell line A2780.

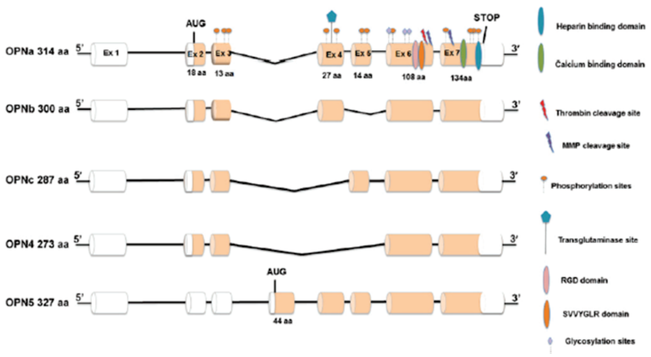
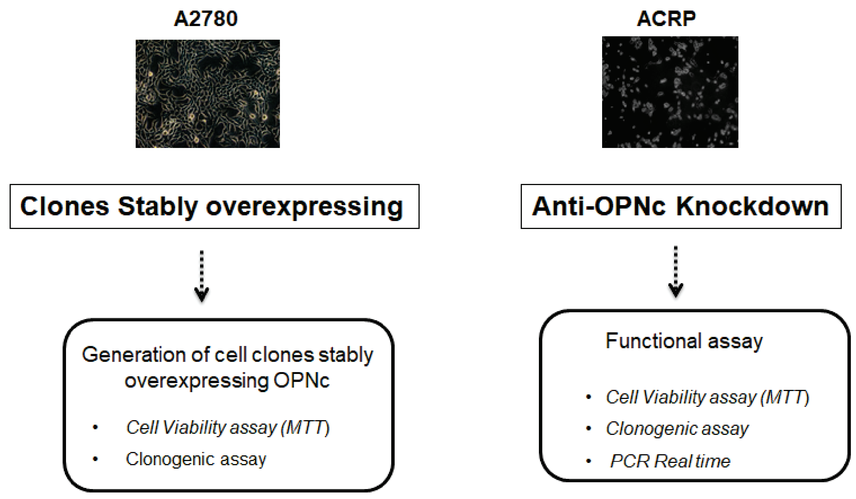


Figure 1: Structure of OPN isoforms. Gimba, E. R. P; Brum, M.C.M; Nestal de Moraes, G. 2018.

Keywords: Osteopontin and resistance

Methodology



Results

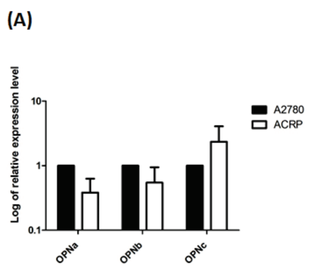


Figure 3: OPN isoforms and P-gp expression levels in ACRP resistant cell line and their corresponding parental cell line. OPNc isoform (A) and P-gp (B) is expressed in higher levels in resistant cell lines in relation to parental cells line. Besides, OPNc is overexpressed in relation to OPNa and OPNb isoforms.

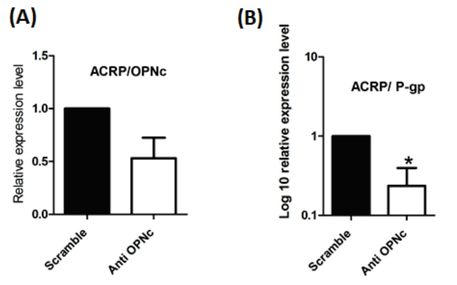


Figure 4: OPNc knockdown using anti-OPNc oligomers inhibits OPNc and P-glycoprotein (P-gp) expression in ACRP resistant cell line. ACRP resistant cell lines were transfected with the anti-OPNc oligomers and 24 hs after transfection OPNc (A) and P-gp (B) transcriptional expression levels have been measured. Transfection with the anti-OPNc oligomers reduced OPNc (A) and P-gp (B) expression ACRP resistant cell line. *p<0,05.

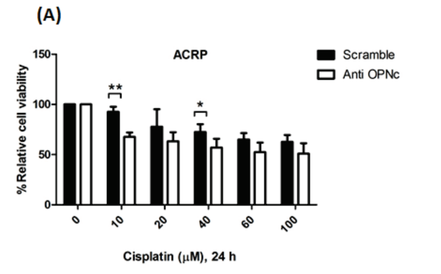


Figure 5: OPN-c knockdown sensitizes drug resistant ACRP cells to cisplatin treatment. ACRP cells were treated with increasing concentrations of cisplatin (CDDP) (0μM- 100μM) for 24 (A) and 96 (B) hs after drug treatment. Cell viability has been measured using MTT assays. Bar graphs represent relative viability of ACRP cells in response to CDDP treatment. OPNc knockdown using anti-OPNc oligomers decreases ACRP cell viability, indicating OPNc role on mediating cell survival.

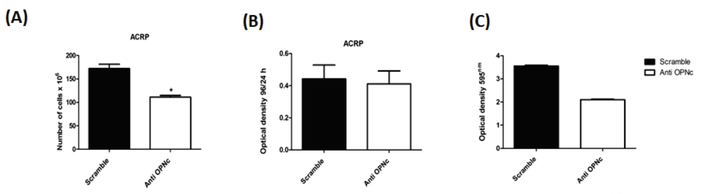


Figure 8: OPN-c knockdown reduces cell growth in drug resistant ACRP cells. ACRP cells display a decreased cell number (A) and a reduced cell growth (B), in response to OPNc knockdown, as evidenced by the clonogenic assay using crystal violet staining (C). * p< 0,05.

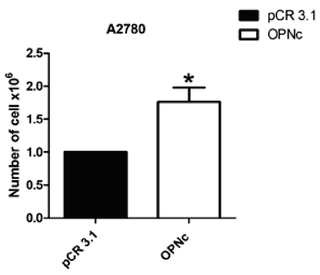


Figure 10: OPNc overexpression in A2780 cell induces cell growth. Cell growth was analyzed by trypan blue exclusion assay in A2780 cells that were transfected with the plasmid pCR3.1 in relation to A2780 OPNc overexpressing cells (A). Statistical analyzes were performed by Student´s t test, with the significance level set at p<0.05.

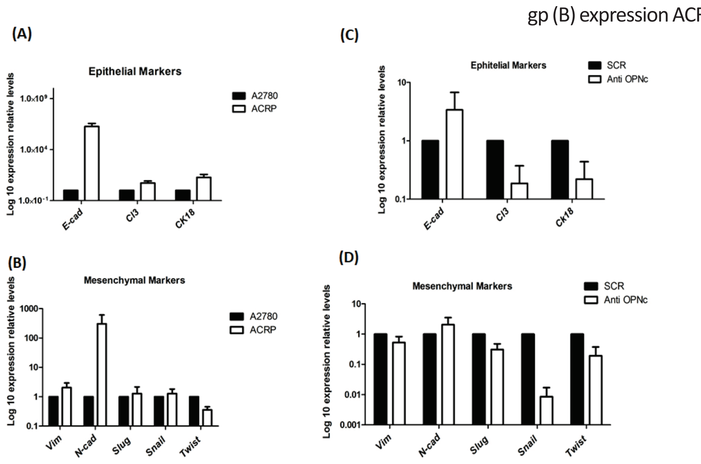


Figure 6: OPNc knockdown partially reverses the drug resistant ACRP epithelial-mesenchymal transition (EMT)-like transcriptional phenotype. The transcriptional pattern of epithelial (E-CADHERIN (E-cad), CLAUDIN-3 (Clau-3) and CITOKERATIN 18 (CK18) and mesenchymal (VIMENTIN (Vim), N-CADHERIN (N-cad), SLUG (Slug), SNAIL (Snail) and TWIST (Twist) markers has been measured by quantitative real time PCR. Bar graphs on the left panel (A) represent the relative transcriptional levels of epithelial and mesenchymal markers in ACRP in relation to A2780 parental cell line. ACRP exhibit a EMT-like phenotype in relation to A2780; Bar graphs on the right panel represent the expression of epithelial and mesenchymal markers when transfecting ACRP cells with the anti-OPNc oligomers in relation to cells transfected with the scramble control oligomers. In response to OPNc knockdown, the ACRP EMT-like phenotype has been partially reversed.

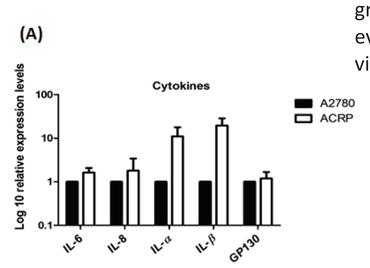


Figure 7: ACRP drug resistant cell line display upregulated expression of epithelial-mesenchymal transition (EMT)-related cytokines. The transcriptional expression of EMT-related cytokines (Interleukin-6 (IL-6), Interlekin-8 (IL-8), Interleukin-α (IL-α), Interleukin-β (IL-β) and GP130 (GP130) has been measured by real time PCR. The expression of EMT-related cytokines is overexpressed in ACRP cells in relation to A2780 parental cell lines, respectively.

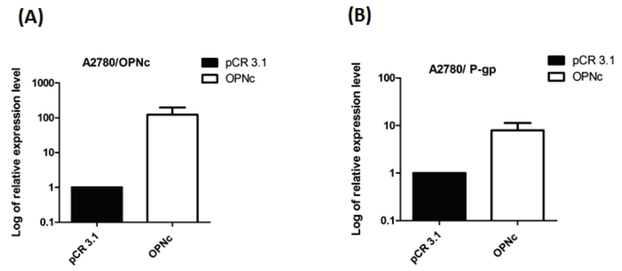


Figure 9: A2780 cells ectopically overexpressing OPNc and P-gp. A2780 cells were transfected with the plasmid pCR3.1, in which OPNc complete cDNA has been cloned. Cell clones stably overexpressing OPNc have been selected using geneticin (G418) at 300 μg/ml in the culture media. These results have been analyzed using a pool of stably overexpressing cell clones. The transcriptional expression of OPNc (A) and P-gp (B) has been evaluated in A2780 cell clones stably expressing OPNc and in those cells transfected with the empty vector control clone.

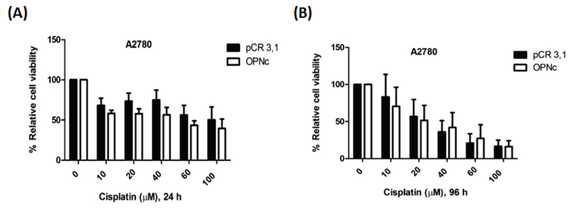


Figure 11: Osteopontin-c (OPNc) overexpression sensitizes A2780 to CDDP. in parental cells (A2780). A2780 cells were transfected with the plasmid pCR3.1and A2780 cell overexpressing OPNc cells were treated with increasing concentrations of cisplatin (0 μM - 100μM) for 24 (A) and 96 (B) hs after CDDP treatment. Cell viability was measured using MTT assays. Bar graphs represent relative viability of A2780 cells in response to CDDP treatment. OPNc overexpression decrease ACRP cell viability, indicating OPNc role on mediating the resistant phenotype.

Conclusion

OPNC-overexpressing cells induce cell growth, while sensitizes ACPR cell to CDDP treatment. Our data further reinforce evidence that OPNc could represent a potential additional molecular target to therapeutic strategies. The specific OPNc isoform knockdown, in combination to CDDP treatment approach has the potential to overcome resistance to chemotherapeutic drugs

Finantial support: CNPq, CAPES, FAPERJ, Programa de Oncobiologia/UFRJ and Fundação do Câncer, INCT/INBEB, Ministério da Sáude, Proppi-UFF.

Projeto Gráfico: Área de Edição e Produção de Materiais Técnico-Científicos / INCA