Triple-negative human breast cancers (TNBC) lack three proteins commonly targeted by chemotherapy; estrogen and progesterone receptors, and her-2-neu. Most current protocols used to treat TNBC are largely ineffective and aggressive tumors frequently re-emerge, leading to metastasis and patient death. Thus, new therapies for TNBC are needed. Recent studies show that around 80% of TNBC express mutant p53 (mtp53), a functionally defective form of the p53 tumor suppressor protein. If mtp53 is converted into the active wild-type protein (wtp53), tumor suppressor functions are recovered. Most p53 mutations occur in the DNA-binding domain, causing normal regulation of p53 target genes involved in apoptosis, cell-cycle arrest, and angiogenesis to be blocked. This promotes metastasis and renders tumors resistant to chemotherapy. APR-246 is a small-molecule drug that re-activates mtp53 by covalent modification of the DNA-binding core domain of the mutant protein through alkylation of thiol groups and has been shown to reactivate mtp53 and restore p53 function. We examined whether APR-246 could inhibit TNBC growth, both *in vitro* and *in vivo*. Cell viability assays and FACS were used to measure *in vitro* TNBC cell growth and apoptosis respectively, in MDA-MB-231 and MDA-MB-468 cells, with MCF-7 cells (which express wtp53) as controls. Analysis of TNBC growth *in vivo* was assessed in a mouse model of MDA-MB-231 derived xenografts. Nuclear extracts of APR-246-treated TNBC cells exhibited significantly increased p53 DNA binding compared with untreated cells, indicating that APR-246 converts mtp53 to wtp53 in these cells. APR-246 significantly reduced TNBC cell viability *in vitro*, but had no effect on normal mammary cells or wtp53-expressing MCF-7 cells. Pro-apoptotic proteins, Bax, p21 and caspase-3 were elevated in APR-246 treated cells, while the cell survival protein Bcl-2 was suppressed. In the xenograft model, animals were given an intravenous (iv) tail vein injection of APR-246 alone (100 mg/kg/day) once tumors reached 100 mm^3. A second group received an intraperitoneal (ip) injection of 2aG4 antibody (100 μg/mouse/day), which targets phosphatidylserine and disrupts tumor blood vessel formation. A third group was given both APR-246 and 2aG4 using the same doses above. A control group received antibody C44 (100 μg/mouse/day, ip) and/or PBS (0.1 mL/day, iv). A total of 18 treatments were used. Administration of APR-246 alone or in combination with 2aG4, significantly reduced TNBC tumor growth, as well as two markers of angiogenesis (vascular endothelial growth factor expression and blood-vessel density). APR-246 in combination with 2aG4 completely eradicated almost 20% of the TNBC tumors. We conclude that TNBC is inhibited by APR-246 and 2aG4. Such treatment could represent an effective and innovative means of combating these particularly aggressive and deadly types of cancer.

Tumor Biology

HORMONE ACTIONS IN TUMOR BIOLOGY: FROM NEW MECHANISMS TO THERAPY

Inhibition of ALDH1A1 Activity in Cisplatin-Resistant Ovarian Cancer Cells Alters Their Cancer Stemness, Cell Cycle Profile and Mitochondrial Respiration Rate

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Introduction: Ovarian cancer is one of the leading cause of morbidity and death among women, with a five-year relative survival rate of only 30% in patients diagnosed with distant metastasis. The ovarian cancer cells initially respond to first-line platinum drug cisplatin [*cis*-diamminedichloroplatinum(II) (CDDP)] treatment. But, they subsequently develop resistance to CDDP and eventually exhibit chemoresistance. Aldehyde dehydrogenase 1 family member A1 (ALDH1A1) is one of the key functional markers of ovarian cancer stem cells (CSCs) that confers cancer stemness and therapeutic resistance, and is associated with poor prognosis and patient survival. In this study, we have assessed the anticancer effects of the ALDH1A1 inhibitor, A37, in CDDP-resistant ovarian cancer cells *in vitro*. **Experimental Methods:** SK-OV-3-CDDP, cisplatin-resistant ovarian cancer cells were treated with different concentrations of the small molecule inhibitor of ALDH1A1, A37. We determined the cell proliferation using water-soluble tetrazolium salt (WST-1) assay at 24 and 48 h. The distribution of cell division phases by cell cycle analysis and oxygen consumption rate (OCR) *via* seahorse extracellular flux analysis were assessed by flow cytometry and seahorse XFe24 analyzer, respectively. Furthermore, we examined the protein expression of key signaling molecules by western blot analysis and cancer stemness by tumorsphere formation assay. **Results:** Treatment of SK-OV-3-CDDP cells with A37 significantly reduced the ovarian cancer cell proliferation. Interestingly, A37 induced cell cycle arrest as observed by an increase in G_1 phase of the cell cycle. Additionally, A37 reduced the mitochondrial respiration of ovarian cancer cells as observed by the decrease in basal OCR. Moreover, A37 treatment markedly decreased the expression of WW domain containing transcription regulator 1 (WWTR1) protein [also called as transcriptional co-activator with PDZ-binding motif (TAZ)], which is a key downstream effector of mammalian Hippo signaling pathway that promotes cancer stemness, metastasis and chemoresistance. Importantly, A37 reduced the number and size of the tumorspheres. **Conclusions:** Our study suggests that inhibiting the ALDH1A1 activity using A37 reduced the cell proliferation and induced cell cycle arrest in CDPP-resistant ovarian cancer cells. The mechanism by which A37 elicits its anticancer effects on ovarian cancer cells include impairment in mitochondrial respiration that could alter cancer cell metabolism, and a decrease in WWTR1/TAZ expression and tumorsphere formation that could suppress cancer stemness. Our findings demonstrate that inhibition of ALDH1A1 could effectively eliminate the chemoresistant ovarian cancer cells, and therefore, new strategies targeting ALDH1A1 could lead to the development of novel therapeutics for aggressive chemoresistant ovarian cancer.