Review of: "Cross-resistance of cisplatin selected cells to anti-microtubule agents: Role of general survival mechanisms"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

As the chemotherapeutic treatment of epithelial ovarian cancer usually comprises a combination of cisplatin with a microtubule active compound, such as paclitaxel. As a taxane-cisplatin combination is a pilaster of treatment of this type of cancer. As the literature about the induction of cross-resistance by cisplatin are conflicting, the authors want to make their contribution by the development of a cisplatin-resistant cell line model for the investigation of the effect of cisplatin-resistance towards a group of compounds normally combined with cisplatin. The results could have a strong impact on the current therapy approaches for ovarian cancer. In addition, a better understanding of cross-resistance at the functional, molecular, and drug-drug interaction (DDI) levels of these compounds can make a meaningful contribution to the improvement of the treatment of cancer.

The authors devoted considerable effort exploring to what extent the exposure of cancer cells (ovarian in this case) to DNA targeting agents like cisplatin could induce drug resistance towards anti-microtubule agents and to explore the molecular basis for such cross-resistance. For a better understanding of whether there is a relationship between resistance against cisplatin and compounds targeting microtubule, such as paclitaxel, vincristine or colchicine, the authors created a resistance model by exposing an ovarian cancer cell line to increasing concertation of cisplatin. After several months this resulted in two daughter lines, one resistant to 1µM and another to 5µM cisplatin (OVCAR8-CP5). The resistance was confirmed by a reduced cell cycle arrest induced by cisplatin itself. The more resistant cells showed a change of cell shape in comparison to the parental cells. In addition, cross-resistance was shown by a lower response to paclitaxel, vincristine or colchicine. This became clearly evident at an IC_{50} of each compound, respectively. Moreover, all the compounds induced a lower apoptosis rate in the resistant cells as compared to the parental cell line. Although RNA-seq data identified increased levels of ABCB1 and ABCG2 in highly resistant OVCAR8-CP5 subpopulation, the intense bioinformatics elaboration of these data on the resistant subpopulations pointed to the activation of Tumor Necrosis Factor alpha (TNFα) as well as Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling, this was confirmed by immune-blotting.

The authors concluded that there is cross-resistance in OVCAR8-CP5 ovarian cancer cells resistant to high dose cisplatin due to an up-regulation of TNFα and NF-κB signaling. These two signaling pathways are related to each other, with NF-κB signaling down-stream to that of TNFα.

Platinum (Pt) compounds, including cisplatin are a group of chemotherapeutic agents, which exert an anti-proliferative effect via the formation of Pt/DNA lesions. Cellular platinum accumulations resulted in binding to DNA and consequently to intra-strand crosslinking. This process initiates multiple cellular responses leading to cell death [1]. Acquired cisplatin resistance comprises several mechanisms, multiple pathways, and complex processes, that range from enhanced DNA repair, over-expression of anti-apoptotic proteins, reduced drug influx/efflux transporters, to over-expression of detoxifying proteins [2]. TNF/NF-κB is suggested to be a key player of the underlining mechanism of the cross-resistance. NF-κB is a transcription factor and major regulator of genes that promote tumor cell proliferation, survival, metastasis, inflammation, invasion, and angiogenesis. Constitutive or aberrant activation of NF-κB is frequently encountered in many human tumors and is associated with a resistant phenotype and poor prognosis [3] . The role of NF-κB for the induction of resistance has been controversially discussed. Lui and colleagues indicated the association of NF-κB with drug resistance in liver cancer cells. Using RT-qPCR they reported an increased levels of NF-κB and MDR1 mRNA and enhanced protein expression of both NF-κB and MDR1 at resistant HepG-2/ADM compared to sensitive HepG-2 cells [4]. Annunziata et al showed that in advanced ovarian cancers NF-κB signaling had a role to play in increasing the tumor-initiating cells (TIC) populations by regulating the cancer stem-like associated enzyme aldehyde dehydrogenase (ALDH) via the NF-κB transcription factor [5]. Yu et al. found that cisplatin resistant A549 cells not only exhibited an epithelial-mesenchymal transition (EMT) phenotype, but also exhibited an increased NF-κB activity compared to parental and cisplatin sensitive A549 cells [6] . On the other hand, Min and colleagues claimed that cisplatin induced microtubule depolymerization in A549 cell line compared with oxaliplatin [7]. Kim et al. demonstrated the role of NF-κB in activation of cisplatin-induced apoptosis in human head and neck squamous cell carcinoma (HNSCC) cell lines [8]. Taken together, these findings emphasize the role played by NF-κB in the determination that resistance is, at minimum, cancer-type dependent [9, 10].

The findings by Patel et al., suggest novel possibilities for therapeutic intervention. There is a need to explore the benefit from including NF-κB inhibitors in ovarian cancer standard therapy. This would also allow for the assessment of whether it is possible to revert resistance toward anti-micro tubular agents. It could also help to shed a light on the discrepancies between data regarding the effect of different types of anti-micro tubular agents on subpopulation resistant to high concentrations of cisplatin; i.e. ones that induce polymerization versus others that cause depolymerization.

Interestingly, an increasing number of studies provide evidence that continuous cisplatin exposure has been associated with generation of cancer stem-like cells (CSCs) [11-14], this was accompanied by multidrug resistance [14]. Thus, we can speculate that the selected clone OVCAR8-CP5 in Patel et al., 2021 might be a result of enrichment of stem-like cells that are known to be much more resistant to chemotherapy with broad-range resistance to variety of chemotherapeutics including anti-micro tubular agents as reported by the current manuscript.

The authors stated that "since anti-microtubule drugs preferentially target rapidly proliferating cells it is possible that the OVCAR8-CP5 cells are evading anti-microtubule-mediated killing by proliferating slowly". Indeed, this might be the mechanism and CSCs are not only proliferating slowly, they also acquire multiple mechanisms to confer cross-resistance to many chemotherapeutic agents, including overexpression of ABCG2 and other transporters.

“Regular” cancer cells might acquire characteristics of CSCs as a result of stimulation of epithelial-mesenchymal transition (EMT) process [15]. Although the tumor microenvironment was identified as the principle inducer of EMT process, chemotherapy agents including cisplatin were reported to stimulate EMT process, as well [3]. One of the hallmarks of the EMT is the change of morphology of epithelial cells to mesenchymal-like morphology which is consistent with changes in cells’ morphology during the selection for the OVCAR8-CP5 resistant cells as reported here. Stimulation of EMT and CSC generation is associated with alteration in gene expression including Snail, Twist, Nanog, Oct 4, NF-κB and many others, which provide a good marker set for EMT.

It appears that EMT, along with acquisition of a CSC-like phenotype mediated, at least in part, by NF-κB signaling pathway plays a decisive role in the determination of a resistant phenotype in ovarian cancer [16]. Consequently, inhibition of NF-κB activity blocked EMT and acquisition of a CSC-like phenotype would represent a compelling therapeutic approach for this type of cancer [16]. It would also address the active role of NF-κB-TNFα-PIK3CA pathway in cisplatin-mediated promotion and maintenance of CSC-like population in platinum-resistant cells as shown by Thakur et al [13].

The study presented by Patel et al., includes most of these considerations and indicates novel therapy approaches would provide a substantial impact in the field of cancer therapy.

Acknowledgments: The authors would like to thank Mr. Imran Siddiq for English editing.

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