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LETTER TO THE EDITOR

WILEY Cancer Science

Multidrug-resistant transporter expression does not always result in drug resistance

Dear Editor,

With great interest, we have read the paper by Sasaki et al[1] evaluating the role of ATP-binding cassette (ABC) subfamily G member 2 (ABCG2) in chemoresistance and pluripotency in pancreatic ductal adenocarcinoma (PDAC).[1] Selection for ABCG2-positive cells did not result in increased chemoresistance, even though these cells were able to efflux fluorescent dye more efficiently than unsorted cells. Furthermore, epithelial-to-mesenchymal (EMT) or cancer stem cell (CSC) expression was not increased in ABCG2-expressing cells in adherent cultures. These unexpected results indicate that the expression of ABC transporters does not always cause chemoresistance or confer stem cell-like features, and, in fact, other mechanisms likely play an important role in the aggressive nature of PDAC.

The ABC transporters have been studied extensively for their correlation between CSC and chemoresistance.[2,3] Their ability to efflux xenobiotics, such as chemotherapeutics, makes them interesting targets. In our unbiased proteomic screening of a gemcitabine-resistant population of PANC1 cells (ATCC), we identified another ABC transporter. Cells that withstood high-dose gemcitabine exposure showed increased ABCC1 expression and phosphorylation (Figure 1). These results led to the hypothesis that ABCC1 expression and post-translational modification contribute to gemcitabine resistance and could be a novel target to overcome chemoresistance in PDAC.

Pharmacological inhibition of ABCC1 by the drug MK-571 in combination with gemcitabine resulted in improved sensitivity in vitro (Figure 2A). Interestingly, MK-571 monotherapy resulted in significantly reduced viability of gemcitabine-resistant cells (Figure 2B), suggesting additional cellular functions of ABC transporters in carcinogenesis, as described previously.[2] Stable gene silencing of ABCC1 by shRNAs, however, did not enhance response to gemcitabine (Figure 2C). These contradictory results can be explained by functional redundancy in the ABC family[3] and the non-selectivity of ABC-targeting agents for specific ABC transporters.[5] Together with limitations due to toxicity and adverse drug interactions, this might explain why none of the studies aimed at overcoming drug resistance by ABC members translated into successful clinical application.[6]

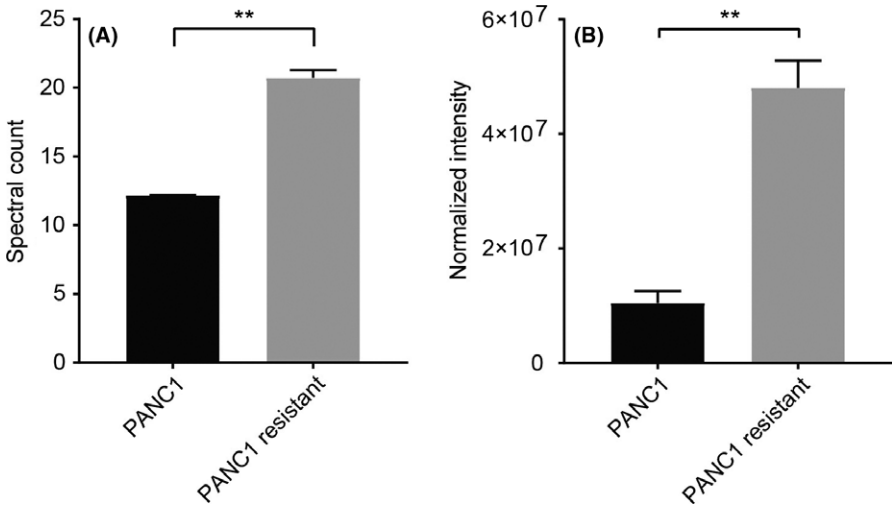

FIGURE 1 ATP-binding cassette subfamily C member 1 (ABCC1) A, expression is upregulated and B, peptide phosphorylation is increased in gemcitabine-resistant PANC1 cells (ATCC). Biological replicates were prepared from cell lysates of PANC1 and its resistant counterpart. In-solution digestion was performed, and samples were enriched for phosphopeptides with titanium dioxide beads, or directly measured on mass spectrometry. Raw data are deposited under PXD010112.[4] $^{**}P < .01$ (unpaired Student's *t*-test; error bars, SD, n = 2)

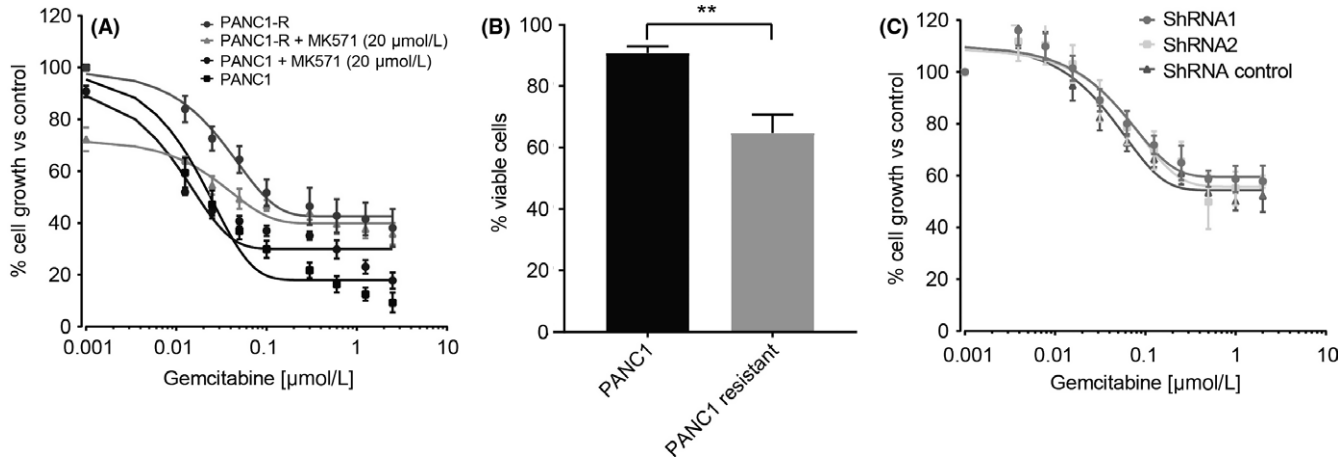

FIGURE 2 ATP-binding cassette subfamily C member 1 (ABCC1) inhibition and its effect on viability and gemcitabine sensitivity. A, Dose-response curves of gemcitabine in combination with ABCC1 inhibitor MK-571 (20 μmol/L) showed improved cytotoxicity in resistant cells upon 72 hours of drug exposure. B, Monotherapy with MK-571 (20 μmol/L) reduced viability of resistant cells. $^{**}P < .01$ (unpaired Student's *t*-test; error bars, SEM, n = 4). C, ABCC1 silencing with shRNAs (MISSION® shRNA Library) had no effect on cytotoxicity of gemcitabine

Moreover, specific drug efflux capacities of individual ABC transporters have not been fully explored.[2] Chemotherapeutics used by Sasaki et al[1] are not all standard of care for PDAC, precluding relevance for clinical practice. Thus, further studies should evaluate the correlation of ABCG2 expression with chemoresistance in a larger panel of cytotoxic agents used against PDAC, as well as report data on well-known gemcitabine determinants, such as ENT1.[7] Also, other potential ABCG2 drug resistance-related signaling pathways might be explored, such as SIRT1/CREB- or Wnt/b-catenin-ABCG2 pathways, which have been unraveled in recent studies with microRNA.[6]

The authors observed that cell growth in spheroids induced chemoresistance, regardless of prior ABCG2 expression. ABCG2 was upregulated in this cell culture system, leading to the conclusion that ABCG2 expression correlates to stemness in this model. The effect of model selection, however, needs to be taken into account for interpretation of results. For instance, Longati et al noted metabolism and gene expression shifts in tumor spheroids, inducing chemoresistance.[8] Also, gemcitabine-resistant populations have been shown to harbor CSC potential after in vivo selection,[9] emphasizing that the plasticity of cell phenotypes depends on experimental model. Another chemoresistance factor that can be responsible for divergent results between culture conditions is mechanobiology. This novel field has been suggested to play a pivotal role in PDAC,[10] and will need to be further explored with regard to gene and ABC expression, in order to understand its role in sphere and in vivo chemoresistance.[10,11]

Sasaki et al[1] tied the ABCG2 expression spheroids to observed drug resistance since verapamil treatment reversed chemoresistance.[1] Given that ABC transporters have other tumor-driving functions as well as transport, the effect of verapamil monotherapy should be considered as a control. As we have shown, inhibition of ABC transporters can affect viability by itself, overestimating the effect of drug transport inhibition. Moreover, verapamil was previously found to be inactive against ABCG2.[11] This inactivity might explain why verapamil was able to improve chemoresistance on spheroids of both origins, independent of ABCG2 expression, and why it most likely influenced other another oncogenic pathway in PDAC cells resulting in improved drug sensitivity. In-depth analysis with controlled gene modulation is needed to elucidate the true role of ABCG2 in PDAC progression and chemoresistance.

In conclusion, chemoresistance contributes to poor prognosis in PDAC patients, and understanding the mechanisms that underlie this phenomenon will pave the way for improved therapy response. The published results together with our results show that ABC transporters can influence drug resistance, possibly by initiating or mediating pluripotency. Further research, however, is needed to understand the multifactorial contributions of these transporters to chemoresistance in PDAC. More importantly, the Sasaki et al results underline the gap that exists between in vitro pre-clinical drug experiments and clinical effects in patients. Further studies are needed to explore the functionality of ABC transporters in 3D and in in vivo models to understand and improve the targeting of these transporters. These studies will hopefully translate into improved therapies and overall survival in PDAC patients.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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