SUPPLEMENTARY MATERIALS AND METHODS

Lentiviral shRNA production and knockdown validation

pGIPZ shRNA constructs for stable knockdown of human ABCB5 were obtained from Open Biosystems (Huntsville, AL) (clone identification: V2LHS_100516, catalog no. RHS4430-200216400). A nonsilencing pGIPZ shRNA (catalog no. RHS4346) was used as a control. Lentiviral particles were made via Lipofectamine 2000 (Invitrogen, Waltham, MA)-mediated triple transfection of 293T cells with pGIPZ shRNA plasmids, along with the lentiviral envelope plasmid (pMD2.G, Addgene number 12259) and the lentiviral packaging plasmid (psPAX2, Addgene number 12260). Melanoma cells were transduced with either nonsilencing or ABCB5-specific shRNA containing lentiviral particles in the presence of 8 μg/ml polybrene, and stable cells were selected with 3 μg/ml puromycin for 1 week and were pooled before determining knockdown efficiency. Knockdown efficiency was determined with semiquantitative reverse transcriptase PCR. Total RNA was collected, following the manufacturer's protocol for the RNeasy Mini Kit (Qiagen). cDNA was synthesized with the iScript cDNA Synthesis Kit (Bio-Rad, Hercules, CA), by using 1 μg of total RNA. To test for knockdown of the ABCB5 message, 2 μl of cDNA with a human ABCB5-specific primer pair (QuantiTect Primer Assay, QT02394679; Qiagen) and a human β-actin–specific primer set were amplified in a PCR reaction with iQ SYBR Green Supermix (Bio-Rad) at a final volume of 50 μl. The PCR reaction program consisted of an initial 3 minutes at 95 °C and 40 cycles of 95 °C for 10 seconds and 55 °C for 45 seconds. PCR reaction results were visualized via agarose gel. ABCB5 pGIPZ shRNA (catalog no. RHS4430-99297707; Dharmacon, Lafayette, CO) gave the best knockdown in both cell lines and was used in functional studies.

SUPPLEMENTARY REFERENCES

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