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ORIGINAL ARTICLE

Oligomeric proanthocyanidins (OPCs) from grape seed extract suppress the activity of ABC transporters in overcoming chemoresistance in colorectal cancer cells

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Abstract

Multidrug resistance is a major hindrance in managing cancer. By performing a series of experiments in chemoresistant colorectal cancer cell lines, we demonstrate that oligomeric proanthocyanidins (OPCs) from grape seed extracts can sensitize both acquired (HCT116-FOr cells) and innately chemoresistant (H716 cells) cancer cells to chemotherapeutic drugs, 5-fluorouracil (5FU) and oxaliplatin, by inhibiting adenosine triphosphate-binding cassette (ABC) transporter proteins. When combined with chemotherapeutic drugs, OPCs significantly inhibited growth of the chemoresistant cells ($P < 0.05$ to < 0.001) and decreased the expression of several key ABC transporters. Moreover, the activity of the ABC transporters was also significantly decreased by OPCs in the cell lines ($P < 0.05$). We further confirmed that co-treatment with OPCs sensitized the chemoresistant cells to 5FU and oxaliplatin, as observed by improvement in cell cycle arrest, double-strand breaks and p53 accumulation in these cells. In addition, we confirmed that co-administration of OPCs with chemotherapeutic drugs significantly decreased chemoresistant xenograft tumor growth in mice ($P < 0.05$). Together, our study illuminates the downregulation of multiple ABC transporters as a mechanism by which OPCs overcome chemoresistance in cancer cells and may serve as adjunctive treatments in patients with refractory colorectal cancer.

Introduction

The various mechanisms by which oligomeric proanthocyanidins (OPCs) extracted from grape seeds exert anticancer effects have recently been an area of active research (1–6). By using a comprehensive, RNA-sequencing approach in colorectal cancer (CRC) cells, we have previously provided an unprecedented view of the genome-wide effects of OPCs in CRC (7,8). Our data supported some of the previous reports that OPCs modulate cell cycle, DNA replication and other key cancer-associated pathways. A closer analysis of our whole transcriptome results revealed that one of the key pathways that was distinctly and predominantly affected by the OPCs in colorectal cells was the adenosine triphosphate-binding cassette (ABC) transporter system. As ABC transporters play a central role in the development of drug resistance in cancer (9,10), we postulated that OPCs could potentially inhibit otherwise an overactive ABC transporter pathway in cancer cells, thereby offering additional insights on ways to overcoming chemoresistance in cancer.

Overexpressed in several chemoresistant cancer types, ABC transporters confer resistance to different chemotherapeutics including taxanes, alkaloids and doxorubicin, primarily through rapid elimination, thereby decreasing their overall accumulation within the cancer cells (11–17). Recognizing the potential clinical significance of ABC transporters in chemoresistance, over the years, concerted efforts have been made to develop therapeutic approaches to inhibit their activity. Consequently, various inhibitors of ABC transporters, including verapamil and quinine, were developed, which initially showed promise but failed clinically, due to high toxicity and other undesirable side effects (18,19). Likewise, other more specific ABC inhibitors, such as valspodar, biricodar, tariquidar and zosuquidar, also seemed attractive initially but failed

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Abbreviations

ABC	adenosine triphosphate-binding cassette
CRC	colorectal cancer
DMSO	dimethyl sulfoxide
5FU	5-fluorouracil
OPC	oligomeric proanthocyanidin

to significantly improve patient outcomes in clinical studies (20–25). Hence, the quest for developing safe and effective compounds that can inhibit the activity of ABC transporters for overcoming chemoresistance in cancer is still on. Our discovery that OPCs can resensitize chemoresistant cancer cells to such drugs in a safe and effective manner is quite promising as an adjuvant therapy in refractory patients.

In this study, we undertake a series of assays to evaluate the anticancer efficacy of OPCs and demonstrate that these compounds potently inhibit progression of chemoresistant CRC by blocking overactive ABC transporters. The tumor-inhibitory properties of OPCs in cells and mice xenografts derived from chemoresistant HCT116 cells significantly associated with the decreased expression of key ABC enzymes such as MRP2, MDR1 and ABCG1. Taken together, our data present OPCs as potential adjuvant therapeutic options in combination with conventional chemotherapeutic drugs for overcoming drug resistance and improving therapeutic outcome in patients with CRC.

Materials and methods

Cell culture and materials

CRC cell lines, HCT116 and H716, were purchased from the American Type Culture Collection (Manassas, VA). These cell lines were tested and authenticated using a panel of genetic and epigenetic markers and tested for mycoplasma on a regular basis. The cells were grown in Dulbecco's modified Eagle's medium (Gibco, Carlsbad, CA), supplemented with 10% fetal bovine serum, 1% penicillin and streptomycin, and maintained at 37°C in a humidified incubator at 5% CO_2. The drug-resistant cell line HCT116-FOr was established by maintaining the cells in increasing concentrations of 5-fluorouracil (5FU) and then oxaliplatin for several months. Both the cell lines were obtained from the American Type Culture Collection during the past 4–6 years; they were periodically authenticated every 4–6 months using a panel of short tandem repeats markers and a panel of genes with known genetic and epigenetic signatures; and the last authentication was performed in July 2018.

Grape seed-OPCs (VX1 extract; EuroPharma; 530 000 p.p.m. OPC concentration) were dissolved in dimethyl sulfoxide (DMSO) and diluted to appropriate experimental concentrations in culture medium. 5FU and oxaliplatin were bought from Sigma–Aldrich (Atlanta, GA) and dissolved in DMSO and water, respectively.

Cell viability and proliferation

Cells were plated in 96-well dishes at a density of 2000 cells per well in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and antibiotics, and allowed to attach overnight. Cell proliferation was measured in cells treated with a combination of OPCs (100 ng/µl), which was the most effective dose that was previously determined in these cell lines (7,8,26); and 5FU (10 µM) or oxaliplatin (5 µM) for 72 h using water-soluble tetrazolium-1 assay (a colorimetric cell proliferation assay, from Sigma–Aldrich) per manufacturer's instructions. Each experiment was performed in triplicate.

Cell cycle and apoptosis analysis

Cells plated in 24-well dishes were treated with OPCs or curcumin for 48 h in triplicates. Cell cycle and apoptosis assays were performed using Muse Cell Cycle Assay Kit (MCH100106; Millipore, Chicago, IL) and Muse Caspase-3/7 Kit (Millipore), respectively, on a Muse Cell Analyzer (Millipore), per manufacturer's instructions. Activation of Histone 2A was measured using Muse H2A.X activation detection kit (Millipore).

Evaluation of ABC transporter activity

Cells plated in P-100 dishes were treated with vehicle or OPCs for 48 h and then harvested in phosphate-buffered saline by trypsinization. EFLUXX-ID Green multidrug resistance assay kit from Enzo Life Sciences (Uniondale, NY) was used to assess the ABC pump enzymes, as activity per manufacturer's protocol.

mRNA expression analysis

RNA from cells treated for 18 h with DMSO (vehicle), OPCs (100 ng/µl), 5FU (10 µM), oxaliplatin (5 µM), OPCs (100 ng/µl) + 5FU(10 µM) and OPCs (100 ng/µl) + oxaliplatin (5 µM) were isolated using mRNeasy Kit (Qiagen, Carol Stream, IL). RNA from mice xenograft tumors collected in RNAlater solution (Qiagen) was extracted using mRNeasy Kit (Qiagen). Extracted RNA was used as a template for complementary DNA synthesis using a High-Capacity cDNA Reverse Transcription Kit (ThermoFisher Scientific, Dallas, TX) according to manufacturer's protocol. Real-time quantitative PCR was performed using SensiFAST SYBR mix (Bioline, London, UK) using the primer sequences listed in Supplementary Table 1, available at *Carcinogenesis* Online. All real-time quantitative PCR target genes were calculated using ΔΔCt method normalized to β-actin.

Genome-wide RNA-sequencing analysis

RNA from cell lines treated with DMSO or 100 ng/µl of OPCs were single-end sequenced. Next-generation sequencing library construction was performed using the TruSeq RNA Library Kit (Illumina, Chicago, IL) with up to 1 µg of total RNA input according to manufacturer's protocol. The quality of individual libraries was assessed using a High Sensitivity DNA Kit (Agilent, Los Angeles, CA). Libraries were pooled together using a PippinHT instrument (Sage Science, Beverly, MA). Efficiency of size selection was assessed using the High Sensitivity DNA Kit (Agilent). Pooled libraries were quantitated via quantitative PCR using the KAPA Library Quantification Kit, Universal (KAPA Biosystems, Philadelphia, PA) prior to sequencing on an Illumina HiSeq 2500 with single-end 75 base read lengths. For the analysis of RNA sequencing, Fastq files were trimmed using Flexbar to remove 3′ bases with quality scores lower than 30 before alignment, as described previously (27). The trimmed reads were mapped to human genome version GRCh38 downloaded from GENCODE (28) using HISAT2 (29) to generate alignment files in bam format. Samtools name-sorted bam files (30) were processed using htseq-count to summarize gene level counts as described previously (31). DESeq2 was used for differential gene expression analysis of RNA-sequencing read counts (32). All sequencing data have been deposited to the GEO database (GSE109607).

Meta-analysis was performed using Stouffer's *P*-value combination method (33) to identify genes that are homogenously up or downregulated independently in OPC-treated cells. In addition, kyoto encyclopedia of genes and genomes pathway enrichment analysis was performed on genes whose fold change expression (with respect to untreated controls) in cells treated with OPCs with respect to untreated controls.

Xenograft animal experiments

Seven-week-old male athymic nude mice (Envigo, Houston, TX) were housed under controlled conditions of light and fed *ad libitum*. Approximately 1 × 10^6 HCT116-FOr cells were suspended in Matrigel matrix (BD Biosciences) and subcutaneously injected into mice using a 27-gauge needle (n = 15 per group). Mice were randomly assigned to different treatment groups and orally gavaged with vehicle (water) or OPCs (100 mg/kg body weight dissolved in vehicle). The mice were intraperitoneally injected with 5FU or oxaliplatin on alternative days for 6 weeks. Tumor size was measured each day by calipers. Tumor volume was calculated using the following formula: 1/2 (length × width × width). The investigator was not blinded to the group allocation during the experiment and/or when assessing the outcome. The animal protocol was approved by the Institutional Animal Care and Use Committee, Baylor Scott and White Research Institute, Dallas, TX, USA.

Statistical analysis

All experiments were repeated three times. All data are expressed as mean ± standard deviation with statistical significance indicated when $P < 0.05$. Statistical comparisons between control and treatment groups were determined using paired *t*-test.

Results

Selection of appropriate models of chemoresistant CRC cells

Previously, we had identified the genome-wide effects of OPCs in CRC through comprehensive RNA-sequencing-based gene expression profiling (7). A striking observation we made when we explored the data in-depth was that several ABC transporters were downregulated by OPCs (Figure 1A). As ABC transporters have long been inculpated in the development of drug resistance, we were curious to examine the effects of OPCs in overcoming chemoresistance in CRC. To select the appropriate cell line models for this study, we evaluated the chemoresistance of several CRC cells to 5FU and oxaliplatin—the first-line chemotherapeutic drugs in CRC, by performing cell proliferation assays. We found HCT116 cell line to be sensitive to 5FU and oxaliplatin, and hence decided to use it as a representative model for a chemosensitive CRC. On the other hand, H716 cell line, derived from the ascites of patient with CRC undergoing 5FU treatment (Figure 1B), was found to be inherently resistant to 5FU and oxaliplatin, and was selected as a model for inherently chemoresistant CRC. To study the effect of OPCs on acquired chemoresistance, we developed a chemoresistant HCT116 cell

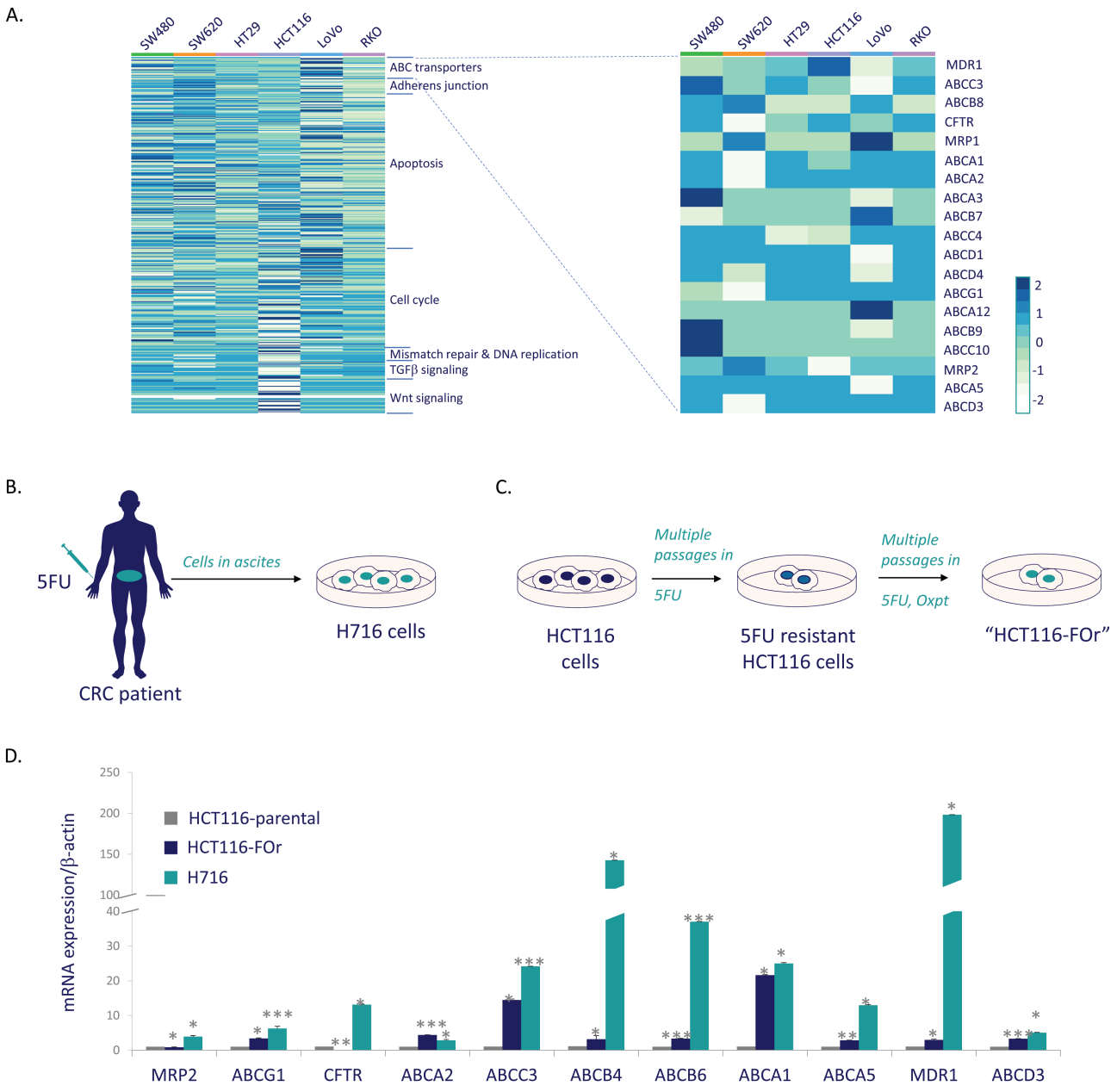

Figure 1. Selection of appropriate cell models of chemoresistant CRC cells. **(A)** Heat map showing various cancer-associated pathways that were affected by OPCs in six different CRC cell lines (left). A zoomed-in illustration showing how the expression of specific ABC transporter genes was affected by OPCs in these cell lines (right). **(B)** Schematic showing the establishment of inherently chemoresistant cell line H716 by culturing cells in ascites from colorectal patient undergoing 5FU treatment. **(C)** Schematic showing the establishment of chemoresistant cell line HCT116-FOr from parental chemosensitive HCT116 cells line by culturing these with increasing doses of 5FU for several passages, and then serially with oxaliplatin. **(D)** Comparison of mRNA levels of ABC transporters in chemosensitive HCT116 cells versus chemoresistant HCT116-FOr and H716 cells. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control treatments.

line, named 'HCT116-FOr' by maintaining HCT116 cells serially in increasing doses of 5FU and then in oxaliplatin for repeated passages for >9 months (Figure 1C). Reassuringly, as shown in Figure 1D, several key ABC transporters implicated for inducing chemoresistance (e.g. MDR1, MRP2 and ABCG1), were found to be overexpressed in the chemoresistant HCT116-FOr and H716 cells vis-à-vis chemosensitive HCT116 cells (34–37). These results provide a unique models to study chemoresistance, both in *de novo* and acquired settings, for evaluating the effects of OPCs in chemoresistant CRC.

OPCs sensitize chemoresistant CRC cells to 5FU and oxaliplatin

The proliferation of chemosensitive parental HCT116 cells significantly decreased when treated with 10 μM of 5FU ($P = 0.0037$) or 5 μM of oxaliplatin ($P = 0.01$), and reduced even further when combined with 100 ng/μl of OPCs (Figure 2A, left; $P = 0.005$ for OPCs + 5FU, $P = 0.013$ for OPCs + Oxpt). However, the HCT116-FOr cells were more resistant to the both chemotherapeutic drugs, with their proliferation rates decreasing only to ~93% when treated with 5FU ($P = 0.029$), and lowered to 59% when treated with oxaliplatin ($P = 0.0018$) (Figure 2A, middle), when compared to controls. Interestingly, OPCs alone effectively decreased their proliferation (6% live cells compared to untreated cells, $P = 0.00037$), and further decreased cell viability when combined with 5FU ($P = 0.0001$) or oxaliplatin ($P = 0.0018$). Similarly, although 5FU ($P < 0.05$) and oxaliplatin ($P < 0.01$) alone did not decrease cell viability in the inherently chemoresistant H716 cells (Figure 2A, right), combining them with OPCs significantly improved the sensitivity of these drugs in these cells ($P < 0.01$ for both OPCs + 5FU and OPCs + Oxpt).

We then assessed the rate of apoptosis in the cells treated with OPCs in combination with the both chemotherapy drugs to determine if programmed cell death accounted for the decreased cell viability observed in the cell proliferation assays. As expected, although parental HCT116 cells were markedly sensitive to individual treatment with 5FU (Figure 2B, left; 15% apoptotic cells, $P < 0.01$), oxaliplatin (20% apoptotic cells, $P < 0.01$) and OPCs (20% apoptotic cells, $P < 0.01$), a combined treatment comprising of OPCs together with both drugs significantly increased the fraction of apoptotic cells in these cells (33% apoptotic cells in OPCs + 5FU, $P < 0.001$; 38% apoptotic cells in OPCs + Oxpt, $P < 0.01$). In contrast, HCT116-FOr cells were relatively insensitive to 5FU and oxaliplatin when treated alone; however, treatment with OPCs sensitized them to both 5FU (17% apoptotic cells, $P < 0.01$; Figure 2B, middle) and oxaliplatin (24% apoptotic cells, $P < 0.01$; Figure 2B, middle). Likewise, treatment with OPCs led to significantly enhanced rate of apoptosis by 5FU and oxaliplatin in inherently chemoresistant H716 cells (Figure 2B, right) as well. Taken together, these data suggest that OPCs have the ability to overcome both inherent and acquired chemoresistance in colon cancer cells.

OPCs downregulate ABC transporters in chemoresistant CRC cell lines

We next evaluated the effect of OPCs on the expression of ABC transporters by measuring the mRNA levels of key genes that were identified to be upregulated in chemoresistant HCT116-FOr and NCI-H716 cell lines. Interestingly, the expression of *MRP2*, a gene notoriously connected with multidrug resistance in gastrointestinal (38), liver (39), ovarian (40–42) and several other cancers (43), increased in response to treatment with 5FU or oxaliplatin in both the chemoresistant cell lines (Figure 2C, top), but was decreased when combined with OPCs. The downregulation of MRP2 by OPCs was especially pronounced in the inherently chemoresistant H716 cells (Figure 2C, top-right; decreased to 10% of control treatment in 5FU + OPCs-treated cells, $P < 0.05$; decreased to 30% of untreated treatment in oxaliplatin + OPCs-treated cells, $P < 0.05$). Similarly, the expression of MDR1, a transporter frequently described in relation to drug resistance in cancer (44–46), was also decreased to about a 10th of control cells when combined with 5FU ($P < 0.01$) or oxaliplatin ($P < 0.01$) in H716 cells. Similarly, we also observed the downregulation of other overexpressed ABC transporters in the chemoresistant cell lines HCT116-FOr and H716 such as ABCG1, ABCC3 and ABCA5 (Figure 2C, bottom).

OPCs block ABC transporter activity in chemoresistant CRC cells

As OPCs decreased the overall expression of several ABC transporters, we next wanted to evaluate the effect of OPCs on the general functionality of ABC transporters. For this, we used a hydrophobic non-fluorescent molecule that easily penetrates the cell membrane, which is later broken down into hydrophilic fluorescent dye by cellular enzymes. Cells that have overactive ABC transporters are able to pump out these fluorescent dye molecules, resulting in low fluorescence of the cells. However, if the ABC transporter activity is inhibited, the dye molecules will be trapped inside the cells, thereby resulting in highly fluorescent cells (Figure 3A). When the cells were sorted (Figure 3B, top) and quantified (Figure 3B, bottom) based on their fluorescence using fluorescence-activated cell sorting, we observed a conspicuous shift toward higher fluorescence in cells treated with a combination of OPCs and the fluorescent dye in HCT116-FOr and H716 cell lines. Although there was not a noticeable difference in the high fluorescent cells in the chemosensitive HCT116 cells ($P < 0.05$), the count of the high fluorescent cells increased considerably in chemoresistant cell lines HCT116-FOr ($P < 0.01$) and H716 ($P < 0.001$), suggesting accumulation of dye within them.

OPCs improve cellular response to 5FU and oxaliplatin

Next, we evaluated whether the decrease in the activity of ABC transporters induced by OPCs truly improved response to the chemotherapeutic drugs 5FU and oxaliplatin. As 5FU and oxaliplatin inhibit the movement of cells through the cell cycle, we checked the level of cell cycle arrest in cells treated with OPCs. As expected, co-treatment with OPCs significantly increased cells arrested in their S-phase in both HCT116-FOr ($P < 0.001$ for OPCs + 5FU, and for OPCs + Oxpt) and H716 cells ($P < 0.01$ for OPCs + 5FU, $P < 0.001$ for OPCs+ Oxpt; Figure 4A). Double-strand breaks, as measured by the level of phosphorylation of γH2AX increased in cells co-treated with OPCs and 5FU or oxaliplatin, further confirmed the sensitization of chemoresistant cells to chemo drugs by OPCs in HCT116 ($P < 0.05$ for OPCs + 5FU and OPCs + Oxpt) and H716 cells ($P < 0.001$ for OPCs + 5FU, $P < 0.01$ for OPCs + Oxpt; Figure 4B).

As cells undergoing DNA damage in response to chemotherapeutic agents respond by elevating their p53 protein levels (47–49), we checked levels of p53 and its target genes p21 and PCNA as a measure of the cells responsiveness to 5FU and oxaliplatin. In line with our previous results, we observed an accumulation of p53 protein in cells treated with both OPCs and chemotherapeutic drugs. This was even more pronounced in the levels of p53-target genes, p21 and PCNA levels, which

Figure 2. OPCs sensitize chemoresistant CRC cells to 5FU and oxaliplatin, and downregulate ABC transporters. **(A)** Top: Cell proliferation assay showing the effect of OPCs in combination with 5FU or oxaliplatin in chemosensitive HCT116 cells (left), chemoresistant cells 'HCT116-FOr' (middle) and NCI-H716 (right) cells. **(B)** Apoptosis in chemosensitive HCT116 cells (left), chemoresistant cells 'HCT116-FOr' (middle) and NCI-H716 (right) cells treated with 100 ng/µl of OPCs with or without 10 µM 5FU or 5 µM oxaliplatin. **(C)** mRNA expression levels of ABC transporter genes in parental HCT116 (left), HCT116-FOr (middle) and H716 (right) relative to β-actin levels. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control treatments.

did not increase when treated only with 5FU or oxaliplatin, but increased drastically when treated in combination with the OPCs (Figure 4D). These data together attest that OPCs improve the response of the cells to chemo drugs.

OPCs resensitize chemoresistant CRC tumor growth to 5FU and oxaliplatin in an animal model

We evaluated the effect of OPCs in resensitizing chemoresistant cancer cells *in vivo* to 5FU and oxaliplatin by following the

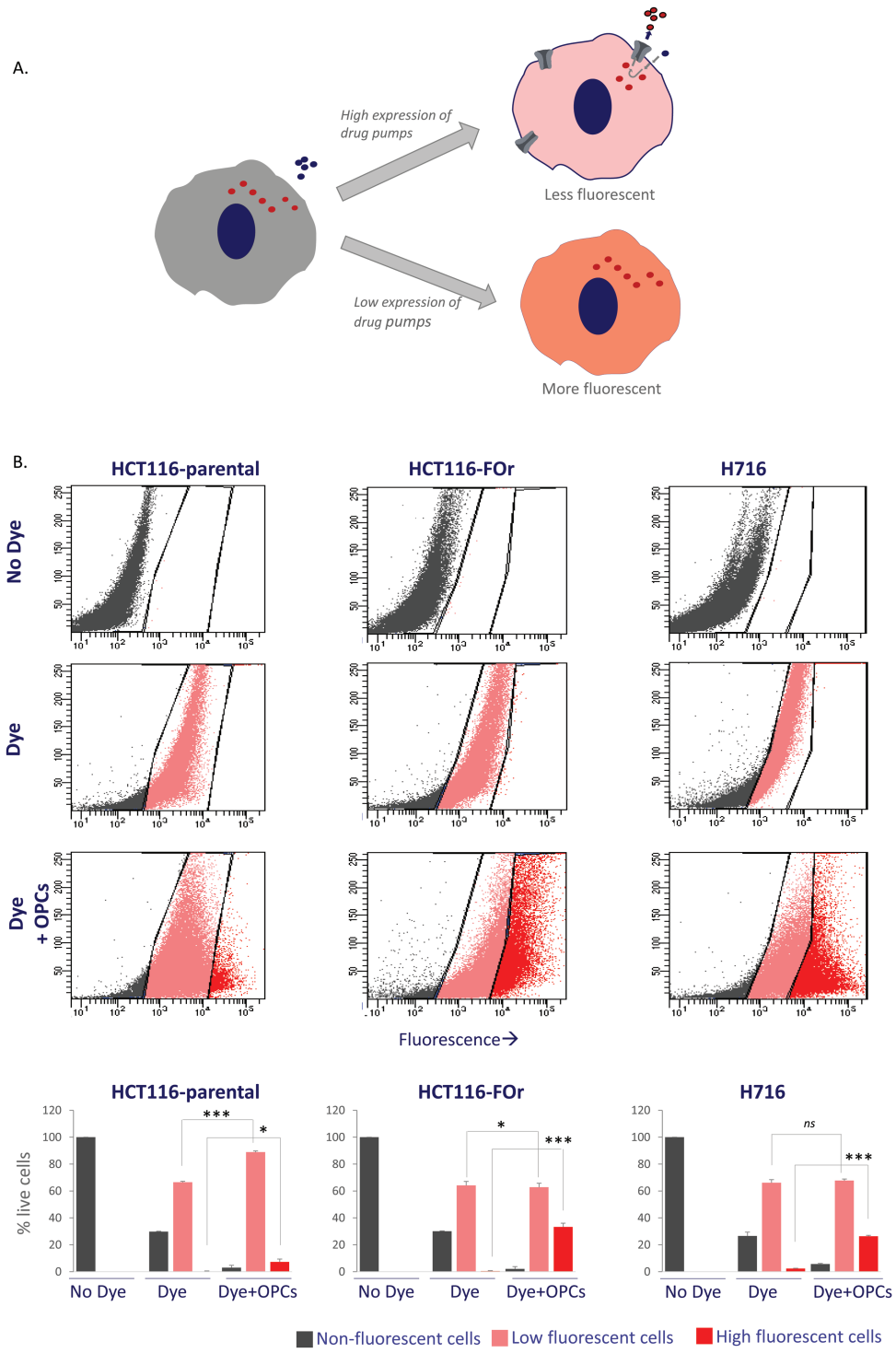

Figure 3. OPCs block ABC transporter activity in chemoresistant CRC cells. **(A)** A schematic of the principle of the functional detection of ABC transporter proteins: a non-fluorescent dye enters the cell and is converted to a fluorescent dye by intracellular esterases. The dye will be pumped out by overactive drug pumps, resulting in less fluorescent cells. If the drug pumps are inhibited, the dye will stay inside the cells making them more fluorescent. **(B)** Fluorescence-activated cell sorting-based measurement of fluorescent cells treated with or without OPCs (top).Quantitation of the number of fluorescent cells in different treatments (bottom). *P < 0.05, **P < 0.01, ***P < 0.001 compared to control treatments.

tumor growth in athymic mice with subcutaneous xenografts of HCT116-FOr cells that were orally administered OPCs in combination with intraperitoneal injections of 5FU or oxaliplatin (Figure 5A). There was no significant change in the weight of the mice during the course of the treatment (Supplementary Figure 1, available at *Carcinogenesis* Online). Although the tumors continued to grow in mice that were administered 5FU or oxaliplatin alone, co-administration of OPCs significantly attenuated tumor growth ($P < 0.05$ for tumor volume, $P < 0.05$ for tumor weight for OPCs + 5FU group; $P < 0.01$

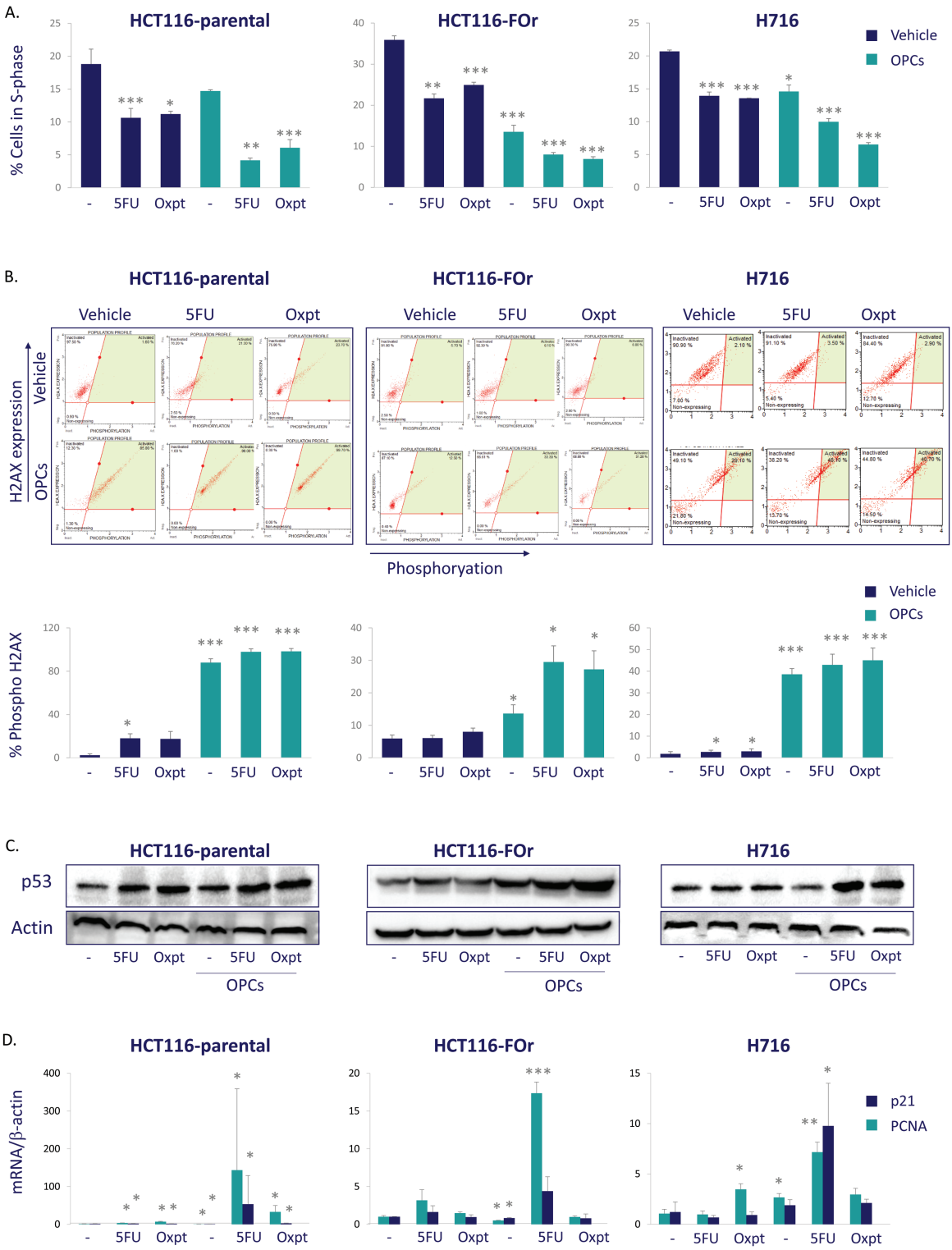

Figure 4. OPCs improve cellular response to 5FU and oxaliplatin. Effect of OPCs on **(A)** cell cycle arrest, **(B)** phosphorylation of γH2AX (top: dot plot with total H2Ax levels on the *y*-axis and phosphorylation of H2AX on the *x*-axis; bottom: quantification of percentage phosphorylation compared to total H2AX expression in cells), **(C)** protein levels of p53 and **(D)** and mRNA levels of the downstream targets of p53 (p21 and PCNA) as measures of response to 5FU and oxaliplatin. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ compared to control treatments.

for tumor volume, $P < 0.05$ for tumor weight for OPCs + Oxpt group; Figure 5A). Besides, in line with results obtained in cell lines, co-administration of OPCs with chemo drugs significantly decreased the expression of key drug transporters MRP2, MDR1, ABCG1, ABCC3 and ABCA5 more effectively than individually (Figure 5B).

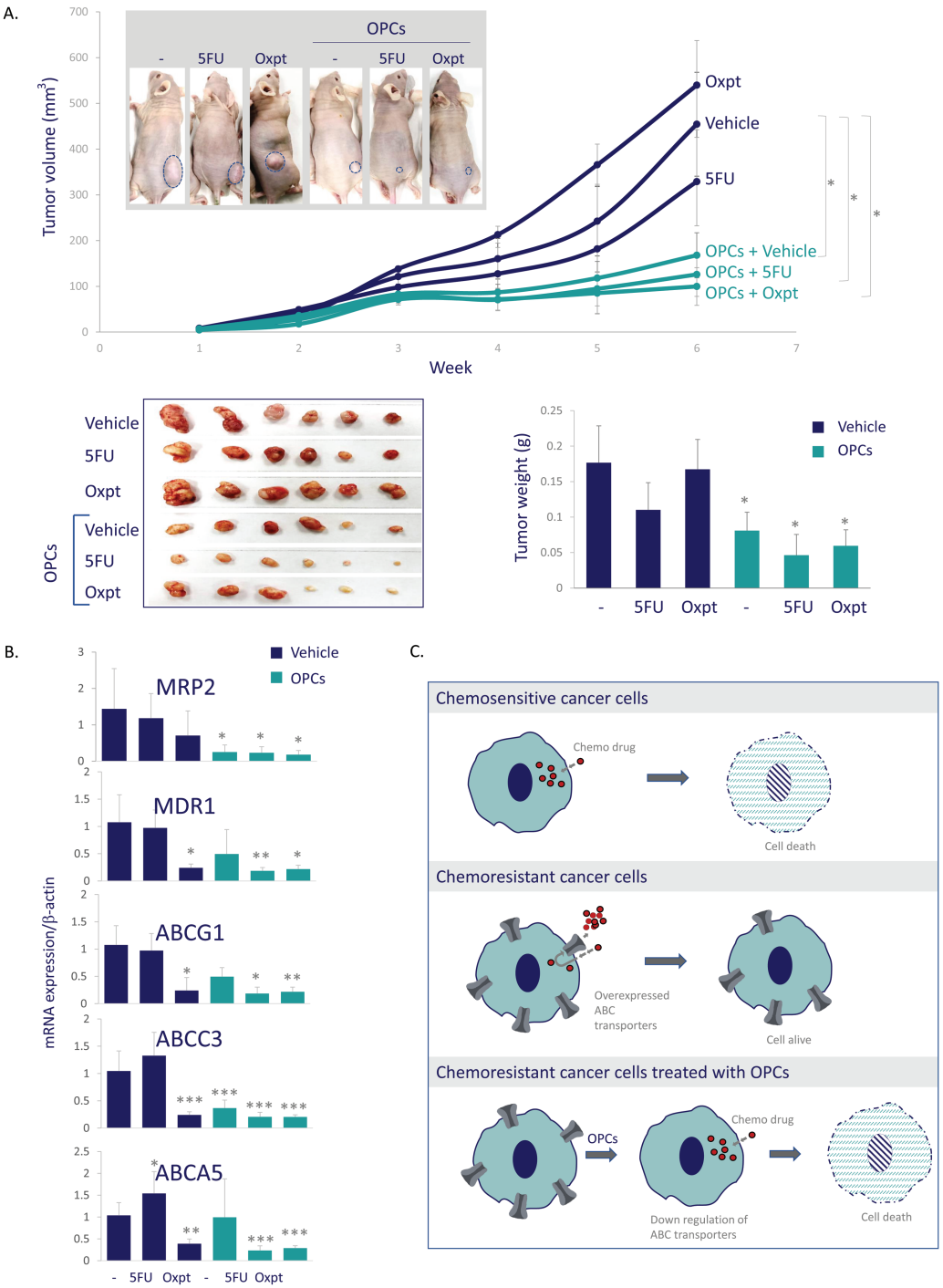

Figure 5. OPCs resensitize chemoresistant CRC tumor growth to 5FU and oxaliplatin *in vivo*. **(A)** Progressive tumor volume in mice orally gavaged with OPCs, individually and in combination with intraperitoneal administration of 5FU or oxaliplatin (top). *Inset*: Representative images of mice with subcutaneous tumors 6 weeks after administering orally with OPCs alone or in combination with intraperitoneal administration of 5FU or oxaliplatin. Xenograft tumors collected from killed mice at the end of the 6-week treatments (bottom). Quantification of tumor weights from different treatment groups (right). **(B)** qPCR analysis of mRNA levels of ABC transporter genes normalized to control group. *P < 0.05, **P < 0.01, ***P < 0.001 compared to control treatments. **(C)** Illustration of the mechanism by which OPCs resensitize chemoresistant cancer cells to chemo drugs.

Discussion

In our previous studies, we demonstrated the tumor-suppressive effects of OPCs derived from grape seeds on a variety of CRC and cancer stem cells (7,26). In this study, we for the first time provide an additional layer of mechanistic evidence for the anticancer effects of OPCs using a series of cell lines and a xenograft animal model, and demonstrate that these molecules play a key role in overcoming chemoresistant to first-line chemotherapeutic drugs such as 5FU and oxaliplatin, in CRC. Furthermore, we illustrate that these chemosensitizing effects of OPCs are mediated through the downregulation of several ABC transporters, which have long been known to play a causal role in the development of chemoresistance in cancer. We

demonstrate that OPCs block the activity of ABC transporters in chemoresistant cancer cells and facilitate resensitization of CRC cells to these drugs in cultured cells as well as in animal models.

Several tumors frequently overexpress multiple ABC transporters, which possess overlapping functions and substrates (50–52). ABC transporter activity is a crucial consideration when designing drugs as they greatly affect the pharmacokinetics and efficacy of all drugs, especially anticancer chemotherapeutic drugs (53). Pharmacologic inhibition or blocking the activity of a singular ABC transporter is not as effective, as the collective suppression of multiple ABC transporters. Studies have elegantly shown that blocking the activity of just two transporters, ABCG2 and MDR1, increased drug accumulation by 43-fold compared to knocking down either of these transporters individually (54). In this study, we showed the ability of OPCs to simultaneously block multiple efflux pathways, and hence markedly increase the efficacy of conventional cytotoxic chemotherapeutic drugs used as first-line treatments in patients with CRC. Our data suggest that suppression of a broad spectrum of ABC transporters is quite promising in reversing chemoresistance to a variety of chemo drugs, a concept that merits further validation and continued exploration. In addition, such an approach is not only beneficial in a preventive setting for minimizing development of chemoresistance in cancer but also provides an avenue to target preexisting chemoresistant cells by resensitizing them to the effects of such drugs owing to their increased dependency on the expression of ABC transporters.

In summary, we demonstrate that OPCs can reverse chemoresistance in refractory CRC by suppressing multiple ABC transporters. In addition to validating our results in cell lines, we also validated the sensitizing effects in an animal model. Therefore, as OPCs block various ABC transporters, it could prevent the emergence of acquired resistance in cancer patients undergoing targeted therapies. Taken together, our findings lay a platform for their use as adjunctive treatments in patients with CRC, based upon the premise that these are safe and inexpensive, and have the ability to target multiple cellular pathways involved in inducing chemoresistance in colorectal and possibly other cancers.

Supplementary material

Supplementary data are available at *Carcinogenesis* online.

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Conflict of Interest Statement: None declared.

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