Expanding the Roles for Pregnane X Receptor in Cancer: Proliferation and Drug Resistance in Ovarian Cancer

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Abstract

Purpose: We examined the presence of the pregnane X receptor (PXR) and its effects on ovarian cancer cells after activation by its cognate ligand.

Experimental Design: SKOV-3 and OVCAR-8 ovarian carcinoma cells were analyzed for expression of PXR by quantitative reverse transcription-PCR and Western blot. Human ovarian cancer tissue was also analyzed for PXR expression by immunohistochemistry. Ligand (agonist)–induced PXR target genes were analyzed in SKOV-3 cells by quantitative reverse transcription-PCR. SKOV-3 cell proliferation was assessed by MTT assay. In vivo confirmation of in vitro effects of PXR ligands were done in NOD.Scid mice carrying SKOV-3 xenografts.

Results: PXR is expressed in ovarian cancer cells. In SKOV-3 cells, PXR is functional and its activation by cognate ligands induces PXR target genes (CYP2B6, CYP3A4, and UGT1A1) but not MDR1 and MRP2. PXR activation in SKOV-3 cells induces cell proliferation and drug resistance. In mice harboring SKOV-3 xenografts, rifampicin (PXR agonist) induces cell proliferation and tumor growth.

Conclusion: PXR activation, regardless of the type of ligand agonist present, promotes the "malignant" phenotype of cancer cells. These data serve as the basis for finding novel nontoxic inhibitors of PXR activation as a method to control cell growth and prevent induction of drug resistance.

Epithelial ovarian carcinoma is a difficult disease to control using conventional chemotherapy (1). Novel biology and targets need to be defined to better treat the disease. In this effort, there is a paucity of data regarding orphan (or adopted orphan) nuclear receptors and ovarian tissues (2–4). Furthermore, very little data are published regarding orphan nuclear receptors [e.g., pregnane X receptor (PXR)] and this malignancy.

Our laboratory has shown previously that the PXR is activated by several different microtubule-binding drugs (e.g., paclitaxel) and that its presence and activation may be responsible for altered drug metabolism in mice (5, 6). Others have shown that PXR activation regulates P-glycoprotein in the blood-brain barrier (PXR activation tightens the blood-brain barrier; ref. 7). Indeed, recent reports suggest that down-regulation of PXR may have an apoptotic and drug sensitivity phenotype (8–10). Others show that PXR activation may in fact enhance drug sensitivity through alteration of oxidant stress (10). It is intriguing that microarray studies of PXR activation in rats and LS180 colon cancer cells show that genes up-regulated by PXR in general have more to do with cell cycle proliferation and inhibition of apoptosis and conversely those genes down-regulated with cell cycle inhibition and proapoptosis (11, 12). PXR clearly induces CYP3A4 and MDR1 in multiple cell types and these enzymes/transporters are known to detoxify microtubule-binding and topoisomerase-binding drugs (5, 6, 13, 14).

Animal data provide further evidence of the functional role of PXR in cell cycle, cell growth, and carcinogenesis. Mouse liver carcinogens, phenobarbital and polychlorinated biphenyls, activate PXR and induce PXR target genes (15, 16). Phenobarbital may also mediate liver tumorigenesis via the constitutive active/androstane receptor, another member of the orphan nuclear receptor superfamily (17). Because constitutive active/androstane receptor and PXR cross-talk in receptor-mediated gene transcription, we hypothesize that PXR may be similarly implicated in carcinogenesis (18). Finally, PXR may regulate cell cycle because it associates with condensed chromatin during mitosis and regulates cell cycle genes (11, 19). Based on all these reports, we investigated the effects of PXR activation in ovarian cancer cell lines. In this study, we show data that PXR activation indeed...
induces ovarian cancer cell proliferation (growth) and multi-drug resistance.

Materials and Methods

Plasmids and reagents. Cremophor EL and rifampicin were obtained from Sigma. Hyperforin was purchased from Cayman Chemical. GlaxoSmithKline (Dr. J. Collins) supplied T0901317 (T1317) and GSK1385, respectively. Enzon Pharmaceuticals (Dr. O. Myers Squibb (Dr. F. Lee) supplied BMS-247550 (ixabepilone). Bristol-T1317 and GSK1385, respectively. Enzon Pharmaceuticals (Dr. O. Sachdev) supplied SN-38 (7-ethyl-10-hydroxycamptothecin). Bristol-Chemical. GlaxoSmithKline (Dr. J. Collins) supplied T0901317 obtained from Sigma. Hyperforin was purchased from Cayman.

Cell culture. HepG2 (American Type Culture Collection) and OVCAR-8 (kind gift from I. David Goldman) cells were maintained in RPMI 1640 supplemented with 10% fetal bovine serum. SKOV-3 cells were a gift from Dr. Gloria Huang and were maintained in MEM-a supplemented with 13% fetal bovine serum. When indicated, cells were propagated in charcoal-adsorbed sera and phenol-free medium.

Immunohistochemistry. HepG2 cells were processed into slides using histogel (Richard-Allan Scientific). The slides were placed in a 59°C oven in the morning of the experiments. For cells and tissue specimens, after dewaxing and rehydrating the slides, slides were placed in a sodium citrate solution (pH 6.0; Vector Labs) for 20 min. The cells were then sequentially blocked with a 3% peroxidase blocking solution, 2% bovine serum albumin, and 5% donkey serum in TBS for 1 to 2 h. Cells were incubated with 1:40 dilution of the primary polyclonal PXR antibody as described previously (19) and developed using the LI-COR Odyssey Infrared Imager. The Western blot analysis was done in duplicate.

Immunoblotting. The presence of PXR in SKOV-3 and HepG2 nuclear protein fraction was determined by Western blot analysis. Protein concentration was determined via the modified Bradford assay using the NanoDrop ND-100 Spectrophotometer. Nuclear fraction was isolated using the BioSource Nuclear Extraction Kit (Invitrogen) per manufacturer’s instructions. Nuclear protein (40-200 μg) was resolved by 12% SDS-PAGE and transferred to nitrocellulose. The blot was probed with a 1:5,000 dilution of a polyclonal PXR antibody as described previously (19) and developed using the LI-COR Odyssey Infrared Imager. The Western blot analysis was done in duplicate.

Semi-quantitative reverse transcription-PCR and real-time reverse transcription-PCR. These experiments have been described previously (5, 6). In brief, total RNA was isolated using the Qiagen RNeasy Mini kit (Qiagen). Reverse transcription was determined via the modified Bradford assay using the NanoDrop ND-100 Spectrophotometer. Protein concentration was determined via the modified Bradford assay using the NanoDrop ND-100 Spectrophotometer. Nuclear fraction was isolated using the BioSource Nuclear Extraction Kit (Invitrogen) per manufacturer’s instructions. Nuclear protein (40-200 μg) was resolved by 12% SDS-PAGE and transferred to nitrocellulose. The blot was probed with a 1:5,000 dilution of a polyclonal PXR antibody as described previously (19) and developed using the LI-COR Odyssey Infrared Imager. The Western blot analysis was done in duplicate.

Expression of PXR in SKOV-3 and OVCAR-8 ovarian cancer cell lines. A. RT-PCR of PXR and peptidylprolyl isomerase A or cyclophilin A, which served as an internal control in SKOV-3 and OVCAR-8 cells. The PCR was repeated twice from independent RNA isolations. The cDNA was run on 1% agarose gel. B. singleplex real-time (quantitative RT-PCR) PXR mRNA expression in SKOV-3 and OVCAR-8 cells. These experiments were done three separate times each assayed in triplicate. Peptidylprolyl isomerase A served as the internal control. C. protein immunoblot of PXR (~ 50 kDa band) using nuclear fractions isolated from HepG2, OVCAR-8, SKOV-3, and MDA-MB-468 breast cancer cells. [Note: These cells are known to have undetectable protein levels for PXR (23)]. HepG2 (40 μg) and MDA-MB-468 (100 μg) nuclear fractions served as a positive and negative control, respectively. Immunoblots were repeated three times using nuclear fractions isolated from three independent experiments. The best image is shown.
serial dilutions of each drug. Following incubation for 24 to 120 h, the MTT assay was done as described previously (5, 20, 21). Medium with drug was changed every 72 h. The vehicle was 0.2% DMSO.

**Chemotherapeutic sensitivity assay.** SKOV-3 cells were incubated in complete medium with and without 20 μmol/L rifampicin for 48 h. At 48 h, cells were trypsinized and aliquots of 5 × 10^5 cells were plated in 96-well plates in triplicates with or without 20 μmol/L rifampicin. After 24 h of incubation at 37°C, cells were treated with serial dilutions of the chemotherapeutic agent. After a further 48 h of incubation, the MTT assay was done as described previously (5, 20, 21).

**In vivo SKOV-3 xenograft studies.** SKOV-3 cells (2 × 10^6) were implanted by s.c. route into both flanks of NOD.SCID mice. On day 35 after inoculation, the mean ± SD (n = 32) volume of implanted tumor was 8 ± 3.5 mm^3 (range, 4.3-18.3). Sixteen mice were selected from this pool (mean ± SD tumor volume, 6.1 ± 3.4) for rifampicin injections and another 16 (mean ± SD tumor volume, 10.1 ± 4.8) for control injections. Rifampicin was formulated in 30% polyethylene glycol and dosed by direct venous (tail vein) injection at 40 mg/kg/d over 3 consecutive days repeated every 7 days. Control i.v. injections consisted of 30% polyethylene glycol. The tumor volume was calculated twice a week using the following formula: length (mm) × width (mm)^2 × π / 6 (22).

**High-pressure liquid chromatography analysis.** Analytical high-pressure liquid chromatography was done using a C18 reverse-phase column (Jupiter, 5 μm, 150 × 2 mm; Phenomenex) under gradient conditions. Methanol-fixed or frozen cell pellets were resuspended in 200 μL of 20 mmol/L ammonium acetate and sonicated (Heat Systems Ultrasound) for 3 min using a microprobe set to 32% power output and a 50% duty cycle. Cell homogenate (100 μL) was deproteinized with 200 μL acetonitrile containing 0.5% acetic acid and 0.1 μg/mL 7-ethylcamptothecin (internal standard). The extraction mixture was clarified by centrifugation at 14,000 × g for 5 min and the supernatant (200 μL) was transferred to high-pressure liquid chromatography sample vial. The sample was evaporated to dryness in a Speed-Vac (ThermoScientific) for 2 h at 45°C and reconstituted in 100 μL of 20 mmol/L ammonium acetate (pH 3.5) containing 10% acetonitrile and 1% tetrahydrofuran. The sample (50 μL) was applied to a column preequilibrated with 90% 20 mmol/L ammonium acetate (pH 3.5, mobile phase A) and 10% acetonitrile containing 1% tetrahydrofuran (mobile phase B). The column was initially eluted at 45°C at a flow rate of 0.5 mL/min for 1 min with 10% B. The column was eluted for an additional 4 min with a linear gradient to 25% B followed by a 3-min linear gradient to 30% B and a 3-min linear gradient to 65% B. The column was reequilibrated by eluting the column for 3 min with 10% B. Eluted peaks were detected using fluorescence detection (Agilent Technologies; λex = 368 nm, λem = 515 nm). Cell extract concentrations were calculated using the peak area ratios (analyte/internal standard) interpolated from a 1/y^2 weighted linear regression fit of peak area ratios versus nominal concentration of calibrators. The lower limits for detection of SN-38 and SN-38 glucuronide was 2.5 and 3.1 ng/mL, respectively.

**Statistical analysis.** The significance of numerical comparisons (values) between groups were made using the Student’s t and nonparametric tests (SigmaPlot 9.0; Systat Software). Descriptive statistics were used to calculate mean, SD, and SE.

**Results**

**Expression of PXR in SKOV-3 cell line and ovarian tissue.** PXR is expressed in two ovarian cancer cell lines, SKOV-3 and OVCAR-8. Figure 1A represents the data from

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Fig. 3. Expression of PXR target genes in rifampicin-treated SKOV-3 cells. Quantitative RT-PCR for human PXR and PXR target genes (CYP2B6, CYP3A4, UGT1A1, MRP2, and MDR1) in SKOV-3 cells treated with 20 μmol/L rifampicin for 48 h. Total RNA was isolated and subject to quantitative RT-PCR. For these PCR experiments, 18S served as an internal control. These experiments were done two separate times each assayed in triplicate. Columns, mean; bars, SD.
semiquantitative reverse transcription-PCR (RT-PCR) for PXR expression in SKOV-3 and OVCAR-8 cells. MDA-MB-468 and SKOV-3 cells express PXR mRNA. In the quantitative RT-PCR studies, SKOV-3 cells express PXR mRNA 3.8-fold over that observed in OVCAR-8 cells (Fig. 1B). PXR is expressed at the protein level in both SKOV-3 and OVCAR-8 cells; however, no such expression is observed for MDA-MB-468 cells (Fig. 1C). The latter cell line has been shown to have very low or undetectable levels of PXR mRNA (23). PXR is clearly detected by immunochemistry as speckled bodies within the nucleus of human ovarian carcinoma tissue (Fig. 2).

**PXR activation induces PXR target genes in SKOV-3 cells.** We did quantitative RT-PCR to determine which of known target genes of PXR were amplified in the ovarian cancer cell line. When SKOV-3 cells were treated with 20 μmol/L rifampicin, >10-fold increase was detected in CYP3A4 and CYP2B6 and ~8.5-fold increase in UGT1A1 mRNA (Fig. 3). Importantly, there was no increased transcription of MDR-1 or MRP-2 genes. The same experiments were repeated using a 0 to 1.0 μmol/L concentration range for T1317 and GSK1385 (EC50 > 190 nmol/L). The latter is an investigational anticholesterol drug that is a dual PXR/liver X receptor agonist (24, 25) but, at a concentration range between 0 and 1.0 μmol/L, serves as a more potent PXR agonist. Rifampicin (0.49-60 μmol/L), a known PXR agonist, induces a significant increase in cell survival, and the longer the duration of exposure to rifampicin, the greater is the effect on cell survival (compare 48, 72, and 96 h exposures; Fig. 4A; ref. 26). Similar data using rifampicin have been shown for OVCAR-8 cells (data not shown). Hyperforin (0.05-1 μmol/L), a known potent PXR agonist, also induces a significant increase in cell survival, and the longer the duration of exposure to hyperforin, the greater is the effect on cell survival (compare 48, 72, and 96 h exposures; Fig. 4B; ref. 27). To further confirm the effect of PXR activation on cell survival, SKOV-3 cells were treated with T1317 or GSK1385 (0-1.0 μmol/L) for 48 h and MTT assay was done to determine...
cell survival fraction. The data were then expressed as fold survival when treated with the PXR agonist, T1317, normalized to survival at the same concentration of GSK1385. There is a significant increase in cell survival with T1317 over the concentration range 0.05 to 1.0 μmol/L, suggesting that PXR activation directly contributes to cell survival (Fig. 4C).

HepG2 cells were treated with rifampicin, ketoconazole, or a concentration range of ketoconazole in the presence of rifampicin for 48 h. The MTT survival assay was done to determine viability of cells. We confirm that, in HepG2 cells, rifampicin induces a concentration-dependent proliferation of cells. Ketoconazole has minimal effects until a concentration of 12.5 μmol/L, when ~15% of cells are nonviable, and by 50 μmol/L, >35% of cells are nonviable. However, when a combination of ketoconazole and rifampicin is tested, cell proliferation is clearly decreased (Fig. 5). Although these data cannot completely exclude off-target effects of ketoconazole, it is likely that inhibition of rifampicin-activated PXR by ketoconazole contributes toward decreased cell proliferation.

In comparing all the data, specifically, the ability of different PXR agonists to enhance cell proliferation of SKOV-3 cells, it appears that the relative known potency of each agent mirrors its potency in enhancing cell proliferation.

**PXR activation induces SKOV-3 cell proliferation in vivo.** To validate the *in vitro* effects of PXR activation on SKOV-3 cancer

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**Fig. 5.** HepG2 cell proliferation in the presence of rifampicin and/or ketoconazole. HepG2 cells were treated with (A) rifampicin (0–50 μmol/L), (B) ketoconazole (0–50 μmol/L), or (C) both drugs each at the same concentration over a 48 h duration. At the end of each time point of drug exposure, cells were subjected to MTT assay (see Materials and Methods). The fractional survival was calculated from $A_{490}$ ratios of treated divided by control (vehicle-treated) wells (minus the blank). Experiments were repeated four separate times each in triplicate. Points, mean; bars, SD.

**Fig. 6.** SKOV-3 mouse xenografts treated with rifampicin or vehicle. Sixteen NOD:SCID mice carrying SKOV-3 xenografts in both flanks were treated with rifampicin three times per week (black arrows) continuously from days 35 to 60. Tumor volumes were assessed as described in Materials and Methods. A, photomicrograph of the first five tumors dissected from the control and rifampicin-treated group. B, tumor weights (mg) as assessed on day 60 for rifampicin-treated ($n=32$ tumors) and control group ($n=32$ tumors). Columns, mean; bars, SE. C, tumor volumes ($n=32$) measured on day 5 after the first injection of each week. Points, mean; bars, SE. D, Ki-67 staining of SKOV-3 xenografts. Multiple (8–10) sections per tumor ($n=3$ tumors per treatment group) were assessed. The image ($\times20$) is representative of the Ki-67 staining observed in rifampicin-treated versus control-treated xenografts (H&E staining of an adjacent section of the tumor is also shown). Quantitation of Ki-67-positive cells ($n=32$) was done under $\times10$ magnification visually by counting cells (red arrows) randomly over 10 high-power fields ($\times10$). Columns, mean; bars, SD.
cell growth, NOD.SCID mice carrying SKOV-3 xenografts were treated with or without rifampicin. Rifampicin-treated mice consistently had significantly larger tumors on both visual inspection at necropsy (day 60) and by tumor weight (Fig. 6A and B). The tumor volumes were assessed twice weekly and these values were significantly higher for the rifampicin-treated group compared with controls (days 45-60; Fig. 6C). Assessment for proliferation in tumor cells was done using Ki-67 antibody. The immunohistochemistry shows that there is a significant increase in cell proliferation (increased Ki-67-stained cells) in tumors from mean (SE) rifampicin-treated versus control mice [937.0 (37) versus 577.0 (21); P < 0.00001; Fig. 6D]. These data validate our in vitro findings that PXR activation induces cell growth (proliferation) and survival.

**PXR activation induces multidrug resistance in SKOV-3 cells.** The role of PXR in cancer cell drug resistance is unclear. In SKOV-3 cells, rifampicin (20 μmol/L) significantly decreased the cytotoxicity of ixabepilone over a concentration range (0.1-50 μmol/L; Fig. 7A), paclitaxel over a concentration range (0.05-50 μmol/L; Fig. 7B), and SN-38 (active metabolite of CPT-11) over a concentration range (0.05-20 μmol/L; Fig. 7C). The metabolites of ixabepilone, paclitaxel, and SN-38 are...
nontoxic to cells. As an example, this has been shown for the metabolites of paclitaxel [see Fig. 7B, (i) and (ii)]. Because IC₅₀ values have not been reached for ixabepilone in SKOV-3 cells, the resistance to ixabepilone (0.1-50 μmol/L) in the presence of rifampicin is best represented as percent increased survival relative to ixabepilone without rifampicin. This averages to ~20% (Fig. 7A). For paclitaxel, the resistance index induced by rifampicin is ~10-fold (IC₅₀ with rifampicin/IC₅₀ without rifampicin ~40/4 μmol/L; Fig. 7B). For SN-38, the resistance index induced by rifampicin is ~17.5 (IC₅₀ with rifampicin/IC₅₀ without rifampicin ~3.5/0.2).

**PXR activation significantly decreases SN-38 concentration in SKOV-3 cells.** In SKOV-3 cells incubated with SN-38 for 8 h, rifampicin significantly decreases the mean ± SD concentrations of SN-38 (1.47 ± 0.43 μg/mL) when compared with vehicle-treated cells (2.249 ± 1.7 μg/mL; P < 0.0001). Similarly, with longer exposure of SN-38 for 24 h, rifampicin also decreased the mean ± SD concentrations of SN-38 (0.32 ± 0.19 μg/mL) when compared with vehicle-treated cells (3.096 ± 1.96 μg/mL; P < 10⁻³). There was a significant decline in SN-38 concentrations between 8 and 24 h (mean ± SE, 1.466 ± 0.1 versus 0.316 ± 0.04 μg/mL, respectively; P < 0.0001; Fig. 8A) of rifampicin-treated samples. However, in the vehicle-treated samples, the SN-38 concentrations between 8 and 24 h incubation was not significantly different (mean ± SE, 2.25 ± 0.35 versus 3.1 ± 0.4 μg/mL, respectively; P = 0.117; Fig. 8A). The samples incubated with SN-38 for 8 h, regardless of pretreatment with rifampicin, did not show SN-38 glucuronide accumulation. However, at 24 h, SN-38 glucuronide was detectable above the lower limit of quantitation (3.1 ng/mL) in the rifampicin-pretreated samples only (mean ± SE, 0.004 ± 0.0008 μg/mL; Fig. 8B). Taken together, these data suggest that rifampicin-mediated activation of PXR significantly lowers cellular SN-38 concentrations while increasing SN-38 metabolism to its glucuronide conjugate presumably through induction of UGT1A1.

**Discussion**

We have shown that PXR has a novel function in an ovarian cancer cell line, SKOV-3. PXR activation induces cell proliferation and multidrug resistance. Drug resistance is likely mediated through transcriptional induction of genes involved in drug metabolism. Although the effects on cell proliferation and drug resistance are modest (albeit a statistically significant difference), we hypothesize that the net effect of both phenotypes makes them more “malignant” in character (e.g., antimetabolites target rapidly proliferating cells, but in a situation where PXR is activated the resistance phenotype may overcome this cytotoxic phenotype and vice versa).

Chemicals are known to have multiple targets in cells and can induce growth proliferation in cancer cell lines. This is a mechanism that may not be directly related to PXR activation. To measure more accurately the true contribution of PXR to cell proliferation, we used a chemical probe set (T1317/GSK1385, agonist/inert analogue pair). T1317 is active on PXR, but GSK1385 is not; however, both compounds activate liver X receptor (all isoforms) within the dose range of <1 μmol/L (24, 25). Therefore, conclusions regarding the absolute contribution of PXR activation to cell proliferation need further
evaluation. However, collectively, all PXR agonists tested (rifampicin, hyperforin, and T1317) induced cell proliferation, which suggests that PXR activation contributes to cell proliferation. We have further confidence in these data as others have recently published that down-regulation of PXR can inhibit endometrial cancer cell growth and induce apoptosis (8, 9). It also must be noted that other orphan receptors, like liver receptor homologue-1, is also associated with colon cell proliferation and tumorigenesis, whereas more closely related receptors to PXR, farnesol X receptor and liver X receptor, induce proliferation or apoptosis in cancer cells (28–31). This observation is in keeping with the notion that PXR signaling is both ligand and promoter (tissue) specific (32, 33). Specific ligands for a given tissue type may induce one phenotype of the receptor, whereas the same ligand in another tissue type may have dramatically different effects.

Our data regarding PXR-mediated induction of drug resistance shows that PXR activation can increase the cytotoxic threshold of cells to chemotherapy. This observation supports the existing but scant literature on PXR-mediated sensitization of cellular cytotoxicity to xenocanctics (8, 10, 30, 33). We hypothesize that the method of resistance induced by PXR induction may be from up-regulation of drug-detoxifying enzymes. In support of this hypothesis is the observation that rifampicin-treated cells had lower SN-38 concentrations compared with vehicle-treated cells. Furthermore, SN-38 glucuronide was only observed in 24 h incubation samples that were pretreated with rifampicin. Indirectly, this implies that SN-38 metabolism to its glucuronide (presumably through UGT1A1) is enhanced in cells with PXR activation. The increased formation of inactive SN-38 glucuronide may explain the observed resistance to SN-38 cytotoxicity in PXR-activated cells. However, other mechanisms of resistance (e.g., down-regulation of apoptosis genes) may also play a dominant role. To dissect the mechanisms involved, further work needs to be done on PXR-induced metabolic and nonmetabolic pathways in cancer cells.

Our data collectively show a novel and new PXR-mediated phenotype in cancer cells. The clinical relevance of our finding is that PXR, which can be activated by at least 5% of our pharmacopoeia, can enhance the “malignant” state of tumors (34–36). This process may be blocked and effort is under way to define mechanisms involved in these phenotype (37).

Disclosure of Potential Conflicts of Interest

M. Sinz and S. Kim are employed by BMS; C. Longley is employed by Enzon.

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4. Kim JW, Havelock JC, Carr BR, et al. The orphan enzyme. In support of this hypothesis is the observation that PXR activation contributes to cell proliferation. This observation shows that PXR activation can increase the cytotoxic threshold of cells to chemotherapy. This observation supports the existing but scant literature on PXR-mediated sensitization of cellular cytotoxicity to xenocanctics (8, 10, 30, 33). We hypothesize that the method of resistance induced by PXR induction may be from up-regulation of drug-detoxifying enzymes. In support of this hypothesis is the observation that rifampicin-treated cells had lower SN-38 concentrations compared with vehicle-treated cells. Furthermore, SN-38 glucuronide was only observed in 24 h incubation samples that were pretreated with rifampicin. Indirectly, this implies that SN-38 metabolism to its glucuronide (presumably through UGT1A1) is enhanced in cells with PXR activation. The increased formation of inactive SN-38 glucuronide may explain the observed resistance to SN-38 cytotoxicity in PXR-activated cells. However, other mechanisms of resistance (e.g., down-regulation of apoptosis genes) may also play a dominant role. To dissect the mechanisms involved, further work needs to be done on PXR-induced metabolic and nonmetabolic pathways in cancer cells.

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