pRb2/p130 Decreases Sensitivity to Apoptosis Induced by Camptothecin and Doxorubicin but not by Taxol

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ABSTRACT

Purpose: In addition to their original function as cell cycle regulators, retinoblastoma (Rb) family members were recently reported to modulate the sensitivity of cancer cells to chemotherapeutic agents. The purpose of this study is to investigate the possible role of pRb2/p130 in the sensitivity of ovarian cancer to camptothecin, doxorubicin, and taxol.

Experimental Design: pRb2/p130 was overexpressed in the CAOV-3 ovarian cancer cell line, and the effect of pRb2/p130 overexpression on sensitivity to apoptosis trigged by IC50 doses of different drugs was evaluated by various methods, including 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, flow cytometry, and Western blot analyses.

Results: The results reported in this study support the conclusion that overexpression of pRb2/p130 in the CAOV-3 ovarian cancer cell line lacking wild-type p53 is able to inhibit apoptosis triggered by camptothecin and doxorubicin through the c-Jun NH2-terminal kinase signaling transduction pathway. Conversely, taxol-induced cell death is not influenced by the pRb2/p130 protein level.

Conclusions: A careful analysis of pRb2/p130 expression in tumor specimens could help to identify the best clinical protocol to be used for each patient, improving efficacy and tolerance and therefore offering additional progress in the treatment of advanced ovarian cancer.

INTRODUCTION

To date, ovarian cancer remains a disease with a poor prognosis, even though improvements in earlier diagnosis and novel therapies over the past 20 years have led to significant survival rates. Several studies have demonstrated that different types of genetic and epigenetic modifications are involved in gynecologic malignancy (1, 2). pRb2/p130, together with pRB/p105 and p107, is a member of the retinoblastoma (Rb) family (3, 4) and is known to play an important role as a negative regulator of cell proliferation (5). We recently found (6) that pRb2/p130 expression is frequently lost or decreased in ovarian carcinoma and that overexpression of pRb2/p130 is able to arrest cell growth in ovarian cancer cells. Interestingly, in a high percentage of primary ovarian carcinomas, loss of pRb2/p130 expression inversely correlates with tumor grade, which is consistent with the studies performed on prostate and lung cancer (7, 8). This suggests the growth-suppressive role of pRb2/p130 in this malignancy and loss of pRb2/p130 expression could represent an important marker for the diagnosis and therapy of ovarian cancer. A better understanding of the correlation between pRb2/p130 expression and tumor response to chemotherapy is an important issue that could lead to optimization of treatment strategies in patients. Several studies highlight an antiapoptotic function of pRb/p105 (9–12), but little is known about the role of the other two Rb family members, pRb2/p130 and p107, in apoptosis. A study conducted in mice supports an antiapoptotic role of p107 (13). Our recent findings suggest a role of pRb2/p130 in glioblastoma γ-radiation–induced apoptosis (14), whereas restoring the function of pRb/p105 in a pRb/p105-null osteosarcoma cell line inhibits radiation-induced apoptosis (15), thus indicating different roles of the Rb family members in cell apoptosis. The present study was carried out to assess the role of pRb2/p130 in apoptosis driven by camptothecin (CPT), doxorubicin (DOX), and taxol (TX) in human ovarian carcinoma CAOV-3 cells, which exhibit a lack of wild-type p53, the most common genetic alteration in human cancer. The chemotherapeutic agent TX (paclitaxel) binds preferentially to α/β-tubulin heterodimers, inhibiting polymer assembly (16, 17) and leading to G2-M cell cycle arrest and apoptotic cell death (18, 19). Although TX is among the most successful drugs used for the treatment of advanced ovarian cancer, the emergence of clinical drug resistance remains the major obstacle to improving the overall survival of cancer patients. Therefore,
Despite the impressive therapeutic progress that has been made in cancer treatment, a number of possible alternative agents or drug combinations should be taken into consideration. CPT is an alkaloid derived from the Chinese tree *Campotheca acuminata* Decne. CPT and its derivatives are unique in their ability to inhibit DNA topoisomerase I by stabilizing the cleavable complex between topoisomerase I and DNA, which ultimately causes DNA double-strand breaks (20). In clinical studies, it has been widely established that CPT analogs exhibit remarkable antitumor activity (21). DOX (Adriamycin) is an anthracycline antibiotic widely used in the treatment of a variety of human malignancies, including ovarian cancer (22). Different mechanisms of action of this drug include intercalation into DNA affecting DNA and RNA synthesis, free radical formation, and the induction of DNA single-strand break damage via inhibition of topoisomerase II (23). Numerous cellular stresses including DNA-damaging agents such as topoisomerase I and II inhibitors initiate the apoptotic pathway through the activation of c-Jun NH2-terminal kinase (JNK) with a consequent increase in the phosphorylation of c-Jun and induction of activator protein-1–mediated transcription (24–26). Consistent with these data, we demonstrate that both CPT and DOX induce apoptosis in CAOV-3 cells through control of the c-Jun expression level and c-Jun phosphorylation status. Conversely, TX seems to change c-Jun phosphorylation status, but not the c-Jun expression level. In addition, pRb2/p130 overexpression causes resistance to CPT- and DOX-mediated apoptosis, whereas no effects have been detected with TX. The different apoptotic signaling pathways for CPT, DOX, and TX could also contribute to a greater effectiveness in chemotherapy when these agents are used in combination rather than alone. These findings highlight an antiapototic role of pRb2/p130 in CPT- and DOX-mediated cell death and suggest the importance of analyzing pRb2/p130 expression in tumor specimens because it is likely to influence the outcome of chemotherapy.

**MATERIALS AND METHODS**

**Cell Culture and Drugs.** The CAOV-3 ovarian carcinoma cell line was obtained from American Type Culture Collection (Manassas, VA) and grown at 37°C in a 5% CO2,95% air atmosphere in Dulbecco’s modified Eagle’s medium (DMEM; Mediatech Inc., Herndon, VA) supplemented with 10% fetal bovine serum (FBS; Mediatech Inc.). CPT and TX were purchased from BioVision (Mountain View, CA), and DOX was purchased from Sigma (St. Louis, MO).

** Colony Forming Assay.** Drug dilutions were freshly prepared before each experiment. Exponentially growing cells were seeded in 100-mm dishes at a density of 3 × 105 cells per dish. After 24 hours, cells were treated for 1 hour with different doses of CPT or DOX, ranging from 0.01 to 0.5 μmol/L, and for 4 hours with different doses of TX, ranging from 5 to 30 mmol/L. To evaluate cell colony forming ability, aliquots of cell suspension obtained from each treatment were seeded at a fixed density (400 cells per dish) in triplicate into 60-mm dishes with complete medium. Cells were then incubated at 37°C for 10 days. Colonies, which were defined as groups of a minimum of 50 cells, were counted after staining with 2% methylene blue in 95% EtOH. Surviving fractions were calculated as the ratio of absolute survival of the treated sample/absolute survival of the untreated sample.

**Adenoviral Production and Transduction.** Ad-RB2/p130 generation has been described previously (27). CAOV-3 cells were plated at 2.5 × 104 cells per mL in 10% FBS DMEM and then transduced in 2% heat-inactivated FBS DMEM with Ad-RB2/p130 at a multiplicity of infection of 400. The medium was changed after 24 h of transduction and replaced with 10% FBS DMEM. The Ad-CMV-Link1 (Ad-CMV) vector alone was used as a negative control at the same multiplicity of infection. The cells were either harvested or treated with the drugs at 48 hours after transduction. The efficiency of the adenoviral transduction was evaluated by direct fluorescence after transduction of the cells with Ad-CMV-GFP, as described previously (6). An increase in the pRb2/p130 protein level was already evident at 48 hours and was detected until 120 hours after adenoviral transduction.

**Flow Cytometry Analyses.** Cells were detached with trypsin and washed once with PBS. The pellets were then resuspended in 0.5 mL of PBS and fixed by adding ice-cold 70% EtOH while vortexing. Fixed cells were stored at 4°C for at least 30 minutes and then washed once with PBS. Cells were then stained with 10 μg/mL propidium iodide (Roche Applied Science, Indianapolis, IN) and 250 μg/mL RNase (Sigma) in PBS and incubated at 37°C for 30 minutes in the dark. Reactive oxygen species (ROS) content evaluation was performed by incubating the cells with dihydroethidium (Sigma) as described previously (28). All flow cytometry acquisitions were performed using a FACSCalibur instrument (Becton Dickinson, San Jose, CA), and the data obtained were analyzed by WinMDI 2.8 software.

**Statistical Analysis.** A paired Student’s *t* test was used to assess the differences in apoptotic rate and cell cycle distribution after drug treatment in pRb2/p130-overexpressing cells versus empty vector-transduced samples during different hours after exposure.

**3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium Bromide Analysis.** Cells at 48 hours after viral transduction were plated in 96-well plates at a density of 5 × 104 cells per plate. At 24 hours after plating, cells were treated with CPT and DOX for 1 hour and with TX for 4 hours, using different doses of these drugs. Untreated and drug-treated cells were plated in octuplicate, and plates were processed 120 hours after treatments. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), purchased from Sigma, was dissolved in PBS and added to culture media at a final concentration of 0.5 mg/mL. After incubation at 37°C for 5 hours, media were removed, and 200 μL of isopropanol were added to each well to dissolve purple crystals of formazan. Absorbance at 550 nm was evaluated using a scanning multiwell spectrophotometer (enzyme-linked immunosorbent assay reader). Cell viability was calculated as the ratio between the average absorbance of the treated samples versus that of the untreated control.

**Western Blot Analysis.** Cells were lysed in lysis buffer [50 mmol/L Tris-Cl (pH 7.4), 5 mmol/L EDTA, 250 mmol/L NaCl, 50 mmol/L NaF, 0.1% Triton X-100, 0.1 mmol/L Na2VO4, 1 mmol/L phenylmethylsulfonfluoride, 10 μg/mL leupeptin]. Equal amounts of protein extracts (50 μg) were loaded into 7–15% SDS polyacrylamide gels and then immu-
noblotted on a nitrocellulose membrane. Immunodetections were performed using anti–poly(ADP-ribose) polymerase [PARP (clone F-2)], anti–c-jun (clone D), anti–p-c-jun (clone KM-1), anti-JNK2 (clone D-2), anti-Bax (clone N-20), anti-Bcl-2 (clone C-2), anti-p73 (clone S-20), and anti-p21 (clone H-164), all purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The anti-pRb2/p130 antibody was purchased from Transduction Laboratories (Lexington, KY). pRb/p105 and p107 antibodies were produced as described previously (29–31). The relative amounts of proteins in all Western blots were normalized to the corresponding HSP70/72 protein amount with an anti-Hsp72/73 antibody from Oncogene Science, Inc. (Cambridge, MA).

JNK Activation Assay. CAOV-3 cells were transduced with Ad-Rb2/p130 and treated with the drugs 48 hours after transduction. Cells were collected for JNK activation assays at 24, 48, and 72 hours after treatment. After regular immunoprecipitation of endogenous JNK2 with an anti-JNK2 monoclonal antibody (clone D-2, Santa Cruz Biotechnology) from 300 μg of total lysate, autophosphorylation detections were essentially performed as described previously (32). The kinase buffer used in the reaction contained 20 mmol/L HEPES (pH 7.6), 20 mmol/L β-glycerolphosphate, 10 mmol/L p-nitrophenylphosphate, 10 mmol/L MgCl2, 1 mmol/L dithiothreitol, 50 μmol/L sodium orthovanadate, 10 μmol/L ATP, and 10 μCi of [γ-32P]ATP. The products were then resolved by 10% SDS-PAGE. The gel was dried and subjected to radiography.

RESULTS

Clonogenic Assays on CAOV-3 Cells Treated with Camptothecin, Doxorubicin, and Taxol. To demonstrate the effects of the chemotherapeutic agents on CAOV-3 cells, exponentially growing cells were treated over a range of concentrations for 1 hour (CPT and DOX) and 4 hours (TX) and then analyzed by colony formation assay to determine their survival capability. As shown in Fig. 1, treatment with the three drugs reduced CAOV-3 cell viability in a dose-dependent manner. Treatment with 0.25 μmol/L CPT reduced CAOV-3 cell viability by 50% (IC50). Similar results were obtained when the cells were treated with 0.25 μmol/L DOX, whereas treatment with 10 nmol/L TX reduced cell viability by about 50%.

pRb2/p130 Overexpression Inhibits Apoptosis Induced by Camptothecin and Doxorubicin but not Apoptosis Induced by Taxol. Consistent with previous results (33), our analysis of the cell cycle by flow cytometry indicated a G2-M accumulation that is evident 24 hours after both DOX and CPT treatment at the IC50 doses (Table 1). Furthermore, at 72 hours after treatment, we observed the progressive appearance of a subdiploid G0-G1 peak, whereas treatment with the IC50 dose of

<table>
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<th>G0-G1 (%)</th>
<th>S (%)</th>
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NOTE. The cell cycle distribution shown in the table is representative of three independent experiments.
Taxol resulted in the appearance of a subdiploid G0-G1 peak at only 24 hours after treatment (Fig. 2A). Analyses of longer time periods after TX treatment were not evaluated because the apoptotic fraction at 24 hours after treatment was already significant and comparable with that seen with the other two drugs. To investigate the effect of pRb2/p130 overexpression in drug-induced apoptosis, we transduced CAOV-3 cells with Ad-RB2/p130 virus or with Ad-CMV as a negative control. An increase in pRb2/p130 protein levels was detected at 48 hours after transduction with Ad-RB2/p130, but it was not enough to achieve a significant increase in the G0-G1 population analyzed by flow cytometry (Table 1). Therefore, the effect of pRb2/p130 on drug-triggered apoptosis did not depend on alteration of the cell cycle driven by the pRb2/p130 growth-suppressive function. Forty-eight hours after transduction, cells were treated with the drugs and collected for cell cycle analysis between 24 and 120 hours after treatment. Notably, overexpression of pRb2/p130 led to a decrease in the apoptotic fraction caused by CPT and DOX treatment, but not by TX treatment (Fig. 2A). PRb2/p130 caused inhibition of CPT-induced apoptosis by an average of 54% and inhibition of DOX-induced apoptosis by an average of 45%. Interestingly, pRb2/p130 overexpression did not influence the modifications of the cell cycle distribution induced by these drugs before the appearance of the sub-G1 population (Table 1), indicating that its role is specific in interrupting the cells from undergoing apoptosis. This suggests a differential involvement of pRb2/p130 in the inhibition of apoptosis triggered by CPT and DOX versus TX. We performed an analysis of cell cycle distribution and apoptosis in the untransduced parental cell line also, and we found that adenoviral transduction alone has no effect on CAOV-3 cells: There is no statistical difference between the cells transduced with Ad-CMV and the parental cell line (data not shown).

**Evaluation of the Effects of pRb2/p130 on Drug-Induced Cytotoxicity.** The effects of pRb2/p130 on the proliferation of the drug-treated cells were also investigated in vitro with the MTT assay, a nonradioactive method to investigate cell viability. As shown in Fig. 2B, pRb2/p130 reduced the cytotoxicity triggered by CPT and DOX but did not influence TX induced cytotoxicity, in accord with previous results obtained while analyzing the apoptotic rate. The proliferation rates were calculated from the absorbance readings, using the untreated cells as 100%. The half-maximal cytotoxicity effect in CPT-treated cells was around 0.20 μmol/L, whereas in treated cells overexpressing pRb2/p130, it was 0.5 μmol/L. Similarly, in DOX-treated cells, the dose that induces the 50% cytotoxicity was 1.25 μmol/L, and in treated cells overexpressing pRb2/p130, this dose increased to 2 μmol/L, indicating that pRb2/p130 specifically diminishes cell cytotoxicity consequent to either CPT or DOX treatment.

**Analysis of Various Regulators of Apoptosis in Response to Drug Treatment in Cells Overexpressing pRb2/p130.** Western blot analysis was used to evaluate changes in the expression of genes known to be involved in apoptosis or drug resistance, such as PARP, caspase-3, Bax, Bcl-2, p21^{WAF1}, and p73. In Fig. 3A, we demonstrate that treatment with the IC₅₀
dose of CPT and DOX, based on colony assay experiments, induced cleavage of 116-kDa PARP and the formation of an 89-kDa fragment at 72 hours after treatment, which is indicative of caspase activation and apoptotic induction (34). Overexpression of pRb2/p130 reduced the cleavage of PARP induced by both CPT and DOX treatment. Fig. 3B shows that treatment with the IC50 dose of TX for 4 hours induced the appearance of the cleaved fragment at 24 hours after treatment, which was not reduced by overexpression of pRb2/p130; this indicated that the effects of pRb2/p130 are specific in CPT- and DOX-induced cell death and do not influence TX-induced cell death. To understand the mechanisms through which pRb2/p130 exerts its antiapoptotic properties as a consequence of CPT and DOX treatment, we also evaluated changes in the protein level of p73, a member of the p53 family, because it has been found to exhibit an activity similar to that of p53, such as growth arrest and apoptosis induction (35). A slight decrease in p73 protein level was detected when cells overexpressed pRb2/p130, but treatment with both CPT and DOX did not induce changes in p73 expression, independently of pRb2/p130 protein level (Fig. 3C). Steady levels of p21wat1, Bax, and bcl-2, whose functions in apoptosis have been studied extensively and associated with both p53-dependent and p53-independent pathways (36–40), have been similarly observed (Fig. 3, C and D). To evaluate the protection by pRb2/p130 from apoptosis induced by CPT and DOX, we also studied the production and variation of ROS, which have been shown to trigger apoptotic cell death (41). Whereas DOX was not associated with ROS production, only at 120 hours after CPT treatment did the ROS form significantly, indicating that the ROS formation could not account for an early induction of apoptosis; besides, ROS formation was not reduced by pRb2/p130 overexpression (data not shown). Finally, given that the Rb family members have been reported to have a role in programmed cell death, we aimed to determine whether changes in their expression level or phosphorylation status occur during drug-induced apoptosis and whether their status is somehow altered by overexpression of pRb2/p130. We show that whereas treatment with DOX (Fig. 4A) and TX (Fig. 4B) did not cause significant changes in the Rb family members, CPT treatment provoked an increase in pRb/p105 phosphorylation, consequently leading to its inactivation, and a decrease in p107 protein level (Fig. 4A). Because these phenomena are still present in cells overexpressing pRb2/p130, we can exclude the possibility that the antiapoptotic effects of pRb2/p130 are a consequence of an altered status of the other two members of the Rb family.

**pRb2/p130 Exerts Antiapoptotic Effects through Inhibition of c-Jun Phosphorylation.** Several antineoplastic agents induce cell death through the stress-activated protein kinase cascade that ultimately phosphorylates the c-Jun transcription factor NH2-terminal region, resulting in induction of activator protein-1–mediated transactivation (24). Treatment with both CPT and DOX produced an increase in the c-Jun protein level, which was already detected at 48 hours after treatment (data not shown), and an increase in c-Jun phosphorylation status, which became visible at 72 hours after treatment (Fig. 5A). Interestingly, overexpression of pRb2/p130 did not interfere with an increase in c-Jun protein level but inhibited c-Jun activation through phosphorylation, as shown in Fig. 5A.

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**Fig. 3** Western blot analysis of PARP cleavage in CAOV-3 cells transduced with Ad-RB2/p130 or Ad-CMV control vector and collected 72 hours after a 1-hour treatment with CPT (0.25 μmol/L) and DOX (0.25 μmol/L; A) or 24 hours after a 4-hour treatment with TX (10 nmol/L; B). Western blot analysis of p73, p21 (C), Bcl-2, and Bax (D) in CAOV-3 cells transduced with Ad-RB2/p130 or Ad-CMV control vector and collected 72 hours after a 1-hour treatment with CPT (0.25 μmol/L) or DOX (0.25 μmol/L).
This effect is not due to adenoviral transduction; as shown in Fig. 5B, the level of phosphorylated c-Jun protein in transduced samples is comparable with that in the parental cell line. Because pRb2/p130 overexpression did not influence the status of c-Jun phosphorylation in untreated cells, its activity is therefore specific in preventing c-Jun phosphorylation consequent to drug treatment, thus interrupting the pathway through which the cells undergo apoptosis. Treatments with Taxol induced the appearance of another form of phosphorylation, slightly higher in molecular mass. These phosphorylation forms, highlighted with arrows in Fig. 5C, have also been noted with other cell stresses (42). The bottom band corresponds to the c-Jun phosphorylated form on the Ser-63 residue, whereas the top band represents the Ser/Thr-phosphorylated form. Neither of the two phosphorylation forms significantly varied when pRb2/p130 was overexpressed in treated cells.

Evaluation of the Influence of pRb2/p130 in JNK-Involved Cell Death. Because JNK2 is one of the best-studied upstream effectors of c-Jun phosphorylation (43), we investigated whether pRb2/p130 has an effect on the intrinsic auto-phosphorylation activity of JNK2, resulting in its constitutive activation (44). Fig. 5D shows that JNK2 protein levels did not vary after CPT and DOX treatment and were not influenced by pRb2/p130 overexpression. Interestingly, immunoprecipitation of endogenous JNK2 followed by autophosphorylation assays demonstrated that treatment with both CPT and DOX caused an increase in JNK2 autophosphorylation activity at 72 hours after treatment (Fig. 5E). This increment was greatly diminished by pRb2/p130 overexpression in CPT-treated cells and, surprisingly, was stable in DOX-treated cells.

DISCUSSION

Ovarian cancer is considered the second leading cause of death from gynecologic malignancies worldwide. Nevertheless, scientific findings are being rapidly translated to clinical therapies, making a considerable impact on survival among women with ovarian cancer. Genetic alterations leading to tumor development are likely to determine the sensitivity of the tumor to cytotoxic agents. Diagnosing such alterations and relating them to specific drug responses should provide the rationale for predicting the most effective individual therapeutic strategy. Several reports describe that pRb2/p130, a member of the Rb family, is deregulated or lost in several tumors (7, 29, 45, 46), and we previously detected its down-regulation and loss of expression in a number of ovarian carcinomas (6). Although the Rb family members have been widely known to induce cell growth arrest, their involvement in drug-induced cell death requires further clarification (9, 10, 12, 13, 47). In this study, we show an antiapoptotic role of pRb2/p130 triggered by the two anticancer drugs, CPT and DOX, whereas TX-induced apoptosis does not seem to be influenced by pRb2/p130. In particular, flow cytometry analyses indicated that pRb2/p130 overexpression reduced the apoptotic fraction by an average of 50% when cells were treated with CPT and DOX, and we noted a significant decrease in the cytotoxicity, evaluated by MTT assays, when pRb2/p130 was overexpressed in cells treated with the same drugs: this indicates that a much higher dose of these drugs is needed to provoke an effect comparable with that in cells not overexpressing pRb2/p130. In both analyses, pRb2/p130 did not change cell response to Taxol treatment. Consistent with these results, pRb2/p130 specifically reduced cleavage of the damage response factor PARP induced by CPT and DOX, leading to protection from cell death. Furthermore, we analyzed various factors known to modulate the apoptotic response to DNA damage, and no changes in expression levels of Bax, Bcl-2, p21\(^{waf1}\), and p73 were noted as a consequence of drug treatment alone or after pRb2/p130 overexpression. Furthermore, the suggested role of the Rb family members in apoptosis led us to analyze the protein level of Rb family members in our experiments. Interestingly, we noted that whereas DOX and TX did not affect the Rb family protein level, CPT treatment caused an increase in pRb/p105 phosphorylation, leading to its inactivation, and a decrease in p107 protein level. Because these results persist in cells overexpressing pRb2/p130 before drug treatment,
we conclude that pRb2/p130 leads to the inhibition of apoptosis with mechanisms presumably independent of the other two members of the Rb family. Intriguingly, neither pRb2/p130 protein level nor its status of phosphorylation is affected by CPT treatment, suggesting another function of pRb2/p130 distinctive from the other two members of the Rb family. It remains to be seen whether the loss of p107 and a functional pRb/p105 in CPT-induced apoptosis are elements of additional pathways that could intersect or act in parallel with the pathway described here for pRb2/p130. Finally, in this study we demonstrate that with CPT and DOX treatment, pRb2/p130 exerts its antiapoptotic function through control of the stress-activated protein kinase cascade. Specifically, with CPT and DOX treatments, pRb2/p130 inhibits the NH₂-terminal phosphorylation of c-Jun induced by the drugs, which is crucial because it inactivates c-Jun transactivation ability, leading to an arrest of the downstream apoptotic pathway (48, 49). We also show that even though TX-triggered apoptosis does not depend on c-Jun expression, it induces the appearance of another phosphorylation form of c-Jun. This, however, is not influenced by pRb2/p130 overexpression before TX treatment, indicating that pRb2/p130 does not interfere with CAOV-3 sensibility to this drug. Moreover, we demonstrate that pRb2/p130 inhibits the autophosphorylation activity of JNK2 in CPT-treated cells, therefore inactivating its ability to phosphorylate c-Jun. This does not appear to happen with DOX treatment, and thus we are led to the conclusion that different pathways, other than JNK, act as upstream effectors for c-Jun phosphorylation and are, indeed, influenced by pRb2/p130 overexpression. This is also consistent with the finding that DOX-induced apoptosis is hardly reversed by JNK inhibitors (50). Overall, these data suggest a significant impact of pRb2/p130 in inhibiting CPT- and DOX-induced apoptosis. Conversely, TX-induced cell death is not influenced by pRb2/p130 protein level. The antiapoptotic effect of pRb2/p130 is consistent with previous studies proving a protective role of the pRb/p105 member of the Rb family in apoptosis induced by CPT (12). Nevertheless, the effects of pRb2/p130 may vary under different conditions, as seen in a previous study (14), in which a proapoptotic role of pRb2/p130 in γ-radiation-induced cell death is demonstrated. The decrease in pRb2/p130 expression detected in many tumors as well as in ovarian cancer is likely to cause a greater effect of CPT and DOX on the tumor and a lesser effect on the normal tissue, consequently leading to milder side effects in patients. The choice of drug treatment in ovarian carcinoma after the analysis of pRb2/p130 protein level could have pivotal clinical implications. The analysis of pRb2/p130 expression on tumor specimens could be a useful diagnos-

![Fig. 5](https://cancers.aacrjournals.org/files/fig5.jpg) Western blot analysis of c-Jun protein level and c-Jun phosphorylation level at Ser-63 in CAOV-3 cells transduced with Ad-RB2/p130 and Ad-CMV control vector collected 72 hours after a 1-hour treatment with CPT (0.25 μmol/L) and DOX (0.25 μmol/L; A) or 24 hours after a 4-hour treatment with TX (10 nmol/L; C). Evaluation of adenoviral transduction effect on phosphorylated c-Jun protein level by Western blot (B). Evaluation of protein levels by Western blot analysis (D) and autophosphorylation activity by activation assay (E) of JNK2 in CAOV-3 cells transduced with Ad-RB2/p130 and Ad-CMV control vector collected 72 hours after a 1-hour treatment with CPT (0.25 μmol/L) and DOX (0.25 μmol/L).
Role of pRb2/p130 in Drug-Induced Apoptosis

A unique tool to tailor unique treatments based on each patient’s pattern. Optimization of the use of chemotherapeutic drugs may offer great opportunities for further improving the management of ovarian cancer because it could help to enhance the patients’ benefit from the therapy and minimize toxicity.

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REFERENCES


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pRb2/p130 Decreases Sensitivity to Apoptosis Induced by Camptothecin and Doxorubicin but not by Taxol

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