Antisense Suppression of Proline-directed Protein Kinase F_A Enhances Chemosensitivity in Human Prostate Cancer Cells[1]

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ABSTRACT

Initial clinic studies revealed that proline-directed protein kinase F_A (PDPK F_A) is overexpressed manyfold in various human cancerous tissues relative to the normal control. However, the role of overexpressed PDPK F_A in cancers remains unknown and needs to be established. To determine whether PDPK F_A is associated with drug sensitivity, we investigated the effects of partial inhibition of this kinase on the human prostate carcinoma cell line (PC-3). PDPK F_A antisense expression vector and its specific antibody were successfully developed. Two stable transfected antisense clones (PA7 and PA3) of human prostate carcinoma cell were subcloned, and they expressed ~75% and ~35% of the total PDPK F_A existing in the control-transfected clone as determined by both immunoprecipitate activity assay and immunoblot analysis. In sharp contrast, the PDPK F_A antisense clones expressed no significant suppression of any other related proline-directed protein kinase member expression, demonstrating the specificity of these two antisense clones. When compared with parental or control-transfected cells, the low-PDPK F_A-expressing antisense clones displayed an enhanced sensitivity to carboplatin, 5-fluorouracil, paclitaxel, and hydroxyurea. Estimation of the IC_{50} index further revealed that the antisense clones displayed up to >100-fold drug sensitivity, and there was a correlation between suppressed levels of PDPK F_A and drug sensitivity. Taken together, the results demonstrate that specific antisense suppression of overexpressed PDPK F_A in human prostate cancer cells is sufficient to enhance various drug sensitivity, indicating that PDPK F_A is an important regulator in controlling multiple drug resistance of human prostate cancer cells.

INTRODUCTION

One of the primary problems facing the treatment of cancer is the development of drug-resistant tumors (1, 2). For example, prostate cancer is the most commonly diagnosed neoplasm in men and the second leading cause of male cancer death in the Western world. Although androgens and estrogens play a pivotal role in the progression of metastatic prostate cancer, androgen ablation does not provide a durable response, and virtually all patients progress to an androgen-refractory state with a median survival time of 12–18 months. Surgery and radiation are effective only in the absence of metastatic disease, whereas chemotherapy has had no impact on overall survival (3). Furthermore, additional chemotherapy is rarely successful. Therefore, development of new and effective treatments for prostate cancer is needed.

PDPK F_A[3] was originally identified as type-1 protein phosphatase activating factor/glycogen synthase kinase-3α (4–6) but has subsequently been demonstrated as a multisubstrate PDPK possibly involved in the regulation of diverse cell functions (7, 8). Initial clinical studies revealed that PDPK F_A was overexpressed manyfold in various human cancerous tissues relative to normal controls (9–12), suggesting an association of PDPK F_A with human neoplastic diseases. However, the exact functional role of overexpressed PDPK F_A in cancer remains unknown and needs to be further established.

In this report, we use a more direct approach to investigate the potential role of PDPK F_A in the chemosensitivity of PC-3 cell line that was developed from a bone metastatic carcinoma cell of a prostate cancer patient by Kaighn *et al.* (13). We have successfully cloned a partial sequence of PDPK F_A cDNA and constructed a recombinant antisense expression vector. The stably transfected PC-3 cells with a specific suppression of PDPK F_A expression appeared to be more sensitive to anticancer drugs. A second human prostate carcinoma cell line (LNCaP) that has only half of the total PDPK F_A activity in the PC-3 cell due to less tyrosine phosphorylation of the protein (12) also displayed similar enhanced sensitivity to the anticancer drugs tested. Similarly, genistein that could block the activity of PDPK F_A activity in the PC-3 cell (12) also enhanced similar levels of chemosensitivity. The results presented here demonstrate that suppression of PDPK F_A expression is sufficient to enhance chemosensitivity of human prostate cancer cells. Suppression of overexpressed PDPK F_A may provide a useful

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[3] The abbreviations used are: PDPK F_A, proline-directed protein kinase F_A/type I protein phosphatase activating factor F_A/factor A/glycogen synthase kinase-3α; PDPK, proline-directed protein kinase; GSK-3β, glycogen synthase kinase-3β; MAPK, mitogen-activated protein kinase; G418, geneticin; CMV, cytomegalovirus; IC_{90}, 90% inhibitory concentration.

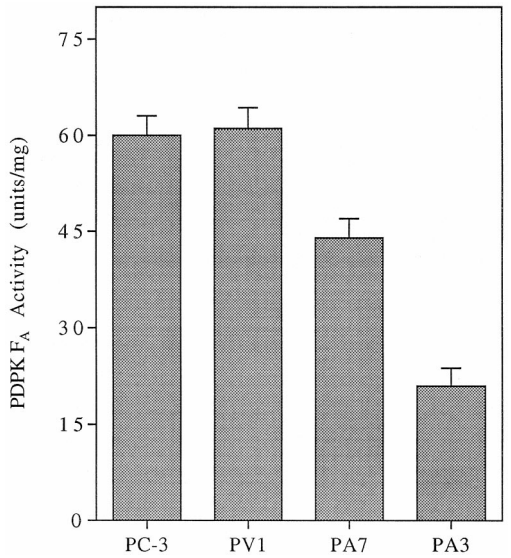

Fig. 1 Suppression of PDPK F_A activity in antisense clones of PC-3 cells. The activity of PDPK F_A in cell extracts (~300 μg of cell protein) of PDPK F_A antisense clones (PA7 and PA3) and the control-transfected clone (PV1) as indicated was immunoprecipitated by using ~1.5 μg of anti-PDPK F_A antibody. The kinase activity in the immunoprecipitate was measured using phospho GS-2 as a specific peptide substrate as described in "Materials and Methods." Data were taken from the averages of three independent experiments and expressed as means ± SD.

clinical target for therapeutic intervention aimed at potentiating anticancer drug sensitivity in chemotherapy of human prostate cancer.

MATERIALS AND METHODS

Materials. The PC-3 cell line, an androgen-independent human prostate carcinoma cell and the LNCaP cell line, an androgen-sensitive human prostate carcinoma cell derived from different metastatic legions were supplied by the American Type Culture Collection (Rockville, MD). The cells within passages 5–30 were used for all of the experiments in this text. All of the reagents used in this text were essentially as described in previous reports (12, 14–16).

Production of Anti-PDPK F_A Antibody. The peptide QAPDATPTLTNSS, corresponding to the carboxyl terminal region from amino acids 471–483 of the sequence of F_A (6), was synthesized by peptide synthesizer (model 9050, Milligen, Bedford, MA). The cysteine residue was added to the NH_2 terminus to facilitate coupling of the peptide to BSA according to the procedure described by Reichlin (17) using glutaraldehyde as the cross-linker. The detailed procedure for production and affinity purification of this antibody was as described in previous reports (12, 15).

Cloning of PDPK F_A cDNA and Construction of Recombinant Antisense Expression Vector. A partial sequence (~1.0-kb fragment starting from the 3′ end of F_A cDNA) was cloned from human fibroblast cells by a reverse transcriptase polymerization chain reaction using CGCGGCCTGGAAGAGGCCAG and ACTGGAGGTGGGGACAGGGA as the first pair of primers and AAGCTAGCGCCTGTGCTCGGCGCCATGA

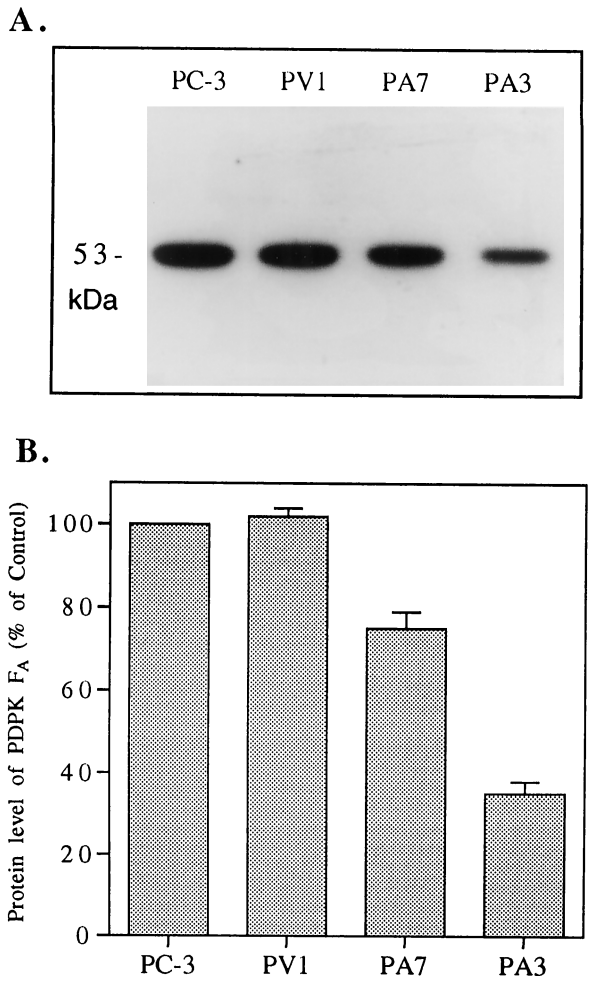

Fig. 2 Suppression of PDPK F_A protein expression in antisense clones of PC-3 cells. Cell extracts (~10 μg of cell protein) of PDPK F_A antisense clones (PA7 and PA3) and the control clone (PV1) as indicated were subjected to 10% SDS-PAGE and immunoblotted with 50 ng/ml of anti-PDPK F_A antibody (*A*) followed by densitometric quantification of the relative amount of PDPK F_A (percentage of the control) on the immunoblot (*B*) as described in "Materials and Methods." Data were taken from the representative results of three independent experiments and expressed as means ± SD.

and TTGAATTCGCCCTCAGGAGGAGTTAGTG as the second pair of primers (6, 18). The cloned cDNA fragment was constructed into the pBK-CMV vector in an antisense orientation downstream of the CMV promoter using *Eco*RI-*Nhe*I as the ligation site. The neomycin resistance gene placed downstream of the SV40 origin was used as the second open reading frame for the initial screening of the transfected clones. The developed antisense construct named as AtF_ApBK-CMV was put into mass production in *Escherichia coli*, and plasmid was purified by the alkaline lysis method.

Cell Culture and Selection of Stably Transfected Clones. Human prostate carcinoma cell lines (PC-3 and LNCaP) were cultured as described in a previous report (12). For transfection, pBK-CMV vector as the control or AtF_ApBK-CMV vector as the antisense construct as described above was

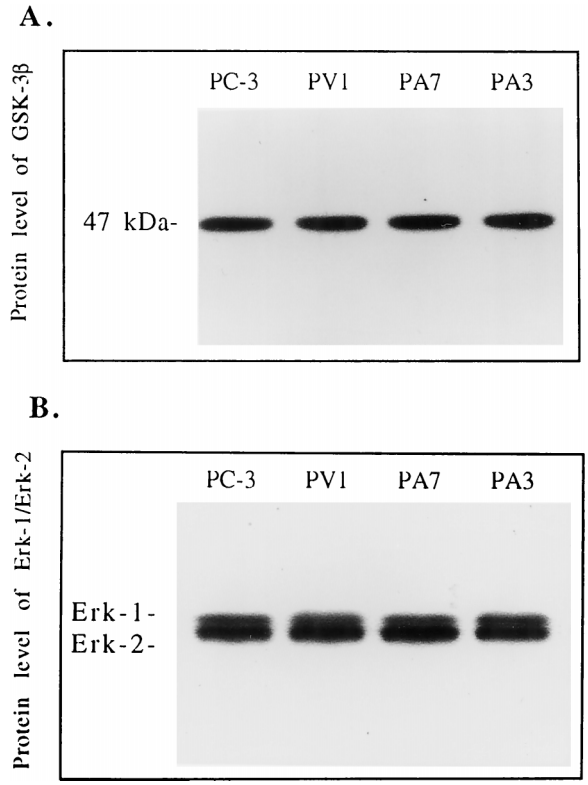

Fig. 3 Immunoblot analysis of GSK-3β and MAPKs in PDPK F_A antisense clones of PC-3 cells. The same cell extracts as described in the legend to Fig. 2 were subjected to immunoblot analysis with 50 ng/ml anti-GSK-3β antibody (*A*) or with 50 ng/ml anti-Erk-1/-2 antibody (*B*).

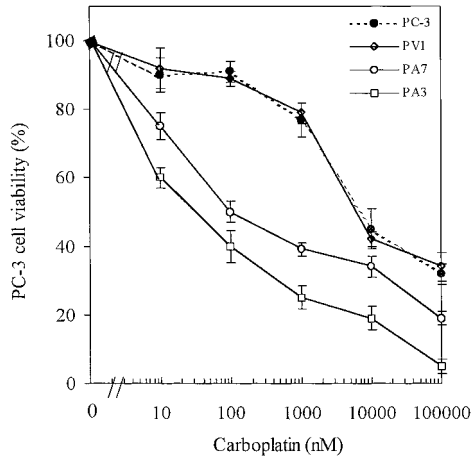

Fig. 4 Antisense suppression of PDPK F_A enhances carboplatin sensitivity in PC-3 cells. PDPK F_A antisense clones (PA7 and PA3) and parental or control-transfected (PV1) cells as described in Fig. 2 were continuously incubated with various concentrations of carboplatin as indicated at 37°C for 48 h. The antisense clones within passages 5–30 and 1×10^5 cells in a 60-mm culture dish were used for the experiments. Cell viability expressed as the percentage of control cells without any drug treatment was determined by the trypan blue exclusion method. Data were taken from the averages of three independent experiments and expressed as means ± SD.

introduced into cells by *N*-[1-(2,3-dioleoyloxyl)propyl]-*N,N,N*-trimethylammoniummethyl sulfate. Briefly, 10 μg of vector mixed with *N*-[1-(2,3-dioleoyloxyl)propyl]-*N,N,N*-trimethylammoniummethyl sulfate were incubated with ~1×10^6 cells in serum-free medium at 37°C for 6 h. The transfected cells were then seeded in a 96-well plate (~1×10^4 cells/well) with complete medium containing 400 μg/ml G418 for selection of recombinant clones expressing G418 resistance. After 4 weeks, individual clones surviving in the presence of G418 were further expanded to mass culture.

Cell Extract Preparation and PDPK F_A Immunoprecipitate Activity Assay. For cell extract preparation, ~1.0×10^6 human prostate cancer cells were homogenized, and the cell extracts were prepared as described in a previous report (12). For PDPK F_A activity assay in the immunoprecipitate, the total PDPK F_A activity in 300 μl of cell extracts (~300 μg of cell protein) was immunoprecipitated with anti-PDPK F_A antibody (~1.5 μg of pure IgG) and assayed with 60 μM phosphoGS-2 (YRRAAVPPSPSLSRHSSPHQ-pSEDEEE) as the specific peptide substrate as described in a previous report (12). A unit of PDPK F_A is that amount of enzyme that incorporates 1 pmol of phosphate/min into the peptide substrate.

Immunoblot Analysis. For immunoblot analysis, the cell extract containing ~20 μg of cell protein was subjected to 10% SDS-PAGE, electrotransferred to polyvinylidene difluoride membrane, and then immunoblotted with 50 ng/ml primary antibodies as indicated and then with goat antirabbit or antimouse IgG antibody conjugated with peroxidase (1:3000) essentially as described in previous reports (12, 15). Immunoblot was developed with the enhanced chemiluminescence system using peroxidase substrate at 25°C for chemiluminescence detection (19). The luminescent light emission was recorded on X-ray film and quantified by computing densitometer (Molecular Dynamics, Sunnyvale, CA).

RESULTS

Transfection of PC-3 cells was performed with the AtF_ApBK-CMV vector as the antisense construct or with pBK-CMV vector alone as the control following G418 selection as described in "Materials and Methods." Several G418-resistant clones were successfully subcloned, and expression levels of PDPK F_A were determined by immunoprecipitate kinase assay for cellular activity and by immunoblot analysis for protein expression. Similar protein and activity levels (60 ± 5 units/mg cell protein) of PDPK F_A were found in both untransfected parental and the control-transfected clones, indicating that neither transfection nor G418 treatment could affect the expression of PDPK F_A in PC-3 cells (see Figs. 1 and 2). In contrast, two antisense clones were obtained in which the cellular activities of PDPK F_A were decreased to the levels of 44 ± 3 units/mg cell protein (PA7) and 21 ± 3 units/mg cell protein (PA3), respectively (Fig. 1). Immunoblot analysis revealed that the protein levels of PDPK F_A in these two antisense clones were also suppressed in a similar manner (Fig. 2*A*). Computing densitometric analysis further revealed that the PDPK F_A protein levels of PA7 and PA3 were suppressed to ~75% and ~35% of the control level (Fig. 2*B*). The suppressed activity (Fig. 1) and

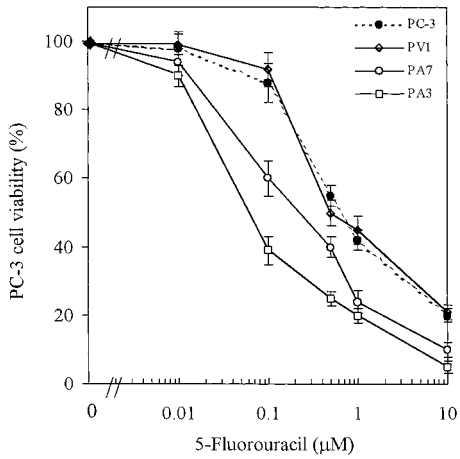

Fig. 5 Antisense suppression of PDPK F_A enhances 5-fluorouracil sensitivity in PC-3 cells. PDPK F_A antisense clones (PA7 and PA3) and parental or control-transfected (PV1) cells were treated with 5-fluorouracil at concentrations as indicated at 37°C for 72 h, and the cell viability was determined as described in the legend to Fig. 4. Data were taken from the averages of three independent experiments and expressed as means ± SD.

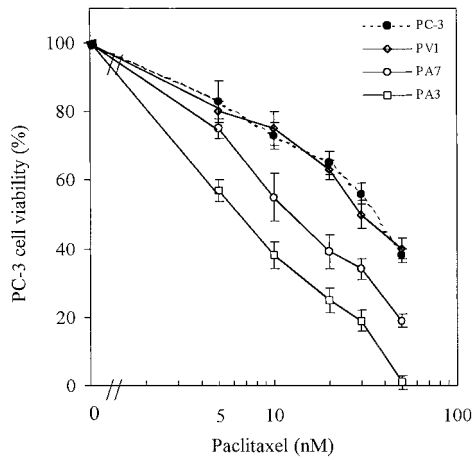

Fig. 6 Antisense suppression of PDPK F_A enhances paclitaxel sensitivity in PC-3 cells. PDPK F_A antisense clones (PA7 and PA3) and parental or control-transfected (PV1) cells were treated with paclitaxel at concentrations as indicated for 24 h, and the cell viability was determined as described in the legend to Fig. 4. Data were taken from the averages of three independent experiments and expressed as means ± SD.

protein (Fig. 2) levels of PDPK F_A in the antisense clones appeared to be very similar, demonstrating that inhibition of PDPK F_A activity in antisense clones is due to suppression of the protein expression. Moreover, although the DNA sequence of PDPK F_A has 85% identity with GSK-3β (6), the PDPK F_A antisense construct appeared to have no significant effect on the protein expression of GSK-3β in PC-3 cells as depicted in Fig. 3*A*. We also determined another well-established PDPK member, namely the MAPKs, and again no significant effect on the expression of Erk-1/Erk-2 in these two antisense clones could be observed (Fig. 3*B*). The results demonstrate that the antisense expression vector constructed here specifically suppressed PDPK F_A in PC-3 cells and had no effect on the expression of the other DNA sequence-homologous PDPK members, such as GSK-3β and MAPKs.

The two PDPK F_A antisense PA7 and PA3 cell clones, expressing low protein levels of PDPK F_A as described above, were found to display an enhanced sensitivity to various anticancer drugs. As shown in Fig. 4, the PDPK F_A antisense cell clones potentially enhanced the efficacy of carboplatin [*cis*-diamine-(1,1-cyclobutane-dicarboxylato)platinum(ii)] in human prostate carcinoma chemotherapy. The suppressed PDPK F_A levels appeared to be proportionally correlated with the enhanced drug sensitivity (Fig. 4), suggesting an association of PDPK F_A with drug resistance in the human prostate carcinoma cell. It is important to note that when the concentration of carboplatin was increased from 1 nM up to 1000 nM, the viability ratio of parental or control-transfected cells was not significantly affected (Fig. 4). In sharp contrast, under identical conditions, the viability ratio of PDPK F_A antisense clones was dramatically decreased to ~25% of the control level (Fig. 4). Moreover, carboplatin at the concentration of 100,000 nM could decrease the viability ratio of the antisense cell clone to <5% of the control level, whereas >35% of the parental cells remain viable under identical conditions (Fig. 4), demonstrating that specific antisense suppression of PDPK F_A could potently enhance carboplatin sensitivity in PC-3 cells. The PDPK F_A antisense clones also proportionally and potentially enhanced sensitivity to 5-fluorouracil (Fig. 5), paclitaxel (Fig. 6), and hydroxyurea (Fig. 7). It is noted that both untransfected parental cells and the control vector-transfected cells appeared to have a similar sensitivity toward all of the anticancer drugs tested (Figs. 4–7), indicating that neither transfection nor G418 treatment could affect the drug sensitivity in PC-3 cells. Estimation of IC_{50} index further revealed that

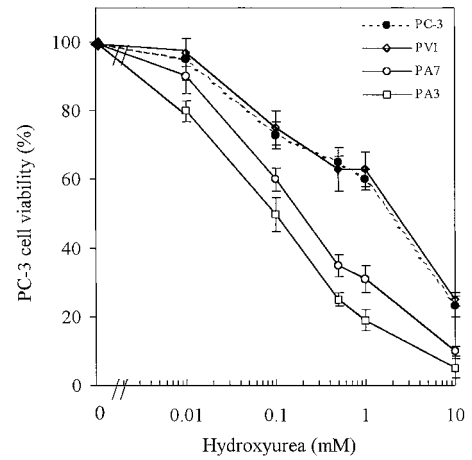

Fig. 7 Antisense suppression of PDPK F_A enhances hydroxyurea sensitivity in PC-3 cells. PDPK F_A antisense clones (PA7 and PA3) and parental or control-transfected (PV1) cells were treated with hydroxyurea at concentrations as indicated for 48 h, and the cell viability was determined as described in the legend to Fig. 4. Data were taken from the averages of three independent experiments and expressed as means ± SD.

Table 1 Enhanced drug sensitivity in PDPK F_A antisense clones (PA7 and PA3) of human prostate carcinoma PC-3 cell[a]

	Drugs							
	Carboplatin		5-Fluorouracil		Paclitaxel		Hydroxyurea	
Cells	IC_{50} (nM)	-Fold	IC_{50} (μM)	-Fold	IC_{50} (nM)	-Fold	IC_{50} (mM)	-Fold
PC-3	8000 ± 70	1.0	0.81 ± 0.07	1.0	36.6 ± 1.5	1.0	1.93 ± 0.19	1.0
PV1[b]	8100 ± 40	1.0	0.77 ± 0.11	1.0	34.4 ± 2.8	1.1	2.11 ± 0.07	0.9
PA7	125 ± 8	64.0	0.24 ± 0.03	3.4	15.1 ± 0.6	2.4	0.25 ± 0.03	7.7
PA3	55 ± 6	145.5	0.08 ± 0.01	10.0	7.8 ± 0.2	4.7	0.11 ± 0.01	17.5

[a] The drugs sensitivity in terms of IC_{50} was determined by cell viability analysis of parental (PC-3), control-transfected (PV1), and antisense (PA7 and PA3) clones. All the cell clones were seeded initially at 1.0×10^5 cells in different concentrations of drugs as indicated and incubated at 37°C for various time intervals. The viable cells were counted by trypan blue exclusion method in a haemocytometer.

[b] Not significantly different when compared to parental cells. All the other values are significant with $P < 0.01$ (Student's *t* test). Data were taken from the averages of three independent experiments and expressed as means ± SD.

Table 2 Enhanced drug sensitivity in the PDPK F_A antisense clone (PA3) of the human prostate carcinoma PC-3 cell[a]

	Drugs							
	Carboplatin		5-Fluorouracil		Paclitaxel		Hydroxyurea	
Cells	IC_{90} (μM)	-Fold	IC_{90} (μM)	-Fold	IC_{90} (nM)	-Fold	IC_{90} (μM)	-Fold
PV1	96.25 ± 7.95	1.0	0.62 ± 0.04	1.0	22.65 ± 1.50	1.0	710.15 ± 9.07	1.0
PA3	5.35 ± 0.63	18.0	0.09 ± 0.01	6.9	2.76 ± 0.33	8.2	68.38 ± 5.55	10.4

[a] The drugs sensitivity in terms of IC_{90} was determined by clonogenic assay, which was performed by plating control-transfected (PV1) and antisense (PA3) clones (~500 cells) in a 6-well plate and cultured in different concentrations of drugs as indicated at 37°C for 10 days. The cell colonies were stained by crystal violet. The formed colonies containing >50 cells were counted. Data were taken from the averages of three independent experiments and expressed as means ± SD. All the values are significant with $P < 0.01$ (Student's *t* test).

the antisense cell clones displayed >100-fold sensitivity toward carboplatin as compared with parental or control-transfected cells as summarized in Table 1. The enhanced drug sensitivity in antisense clones could also be observed when using 5-fluorouracil, paclitaxel, or hydroxyurea because the testing drugs and a correlation between suppressed PDPK F_A levels and drug sensitivity was consistently obtained (Table 1). In addition, a second cell culture assay, such as clonogenic assay, was also used to study these effects, and the enhanced drugs sensitivity in terms of IC_{90} in the antisense clone could also be observed, further supporting that the antisense effect is functionally significant (see Table 2). The relative sensitivity of the parental and transduced PC-3 cells to clinically relevant cisplatin concentration (~300 nM) was also tested, and the isodose sensitization enhancement could also be observed, supporting the clinical implication of the present study (see Fig. 8). A second human prostate carcinoma cell line (LNCaP), which has only half of the total PDPK F_A activity in PC-3 cell due to less tyrosine phosphorylation of the protein (12), was also tested as another model system and similar sensitization enhancement could be obtained (see Tables 1 and 3). In similarity, genistein, a tyrosine kinase inhibitor that could block the activity of PDPK F_A in the PC-3 cell (12), was also found to be able to enhance these anticancer drugs' sensitivity in the PC-3 cell (see Tables 1 and 3). The results taken together demonstrate that suppression of PDPK F_A expression is able to enhance various drug sensitivity in both PC-3 and LNCaP cells, indicating an essential primary role of PDPK F_A in the resistance of human prostate carcinoma cells to multiple anticancer agents.

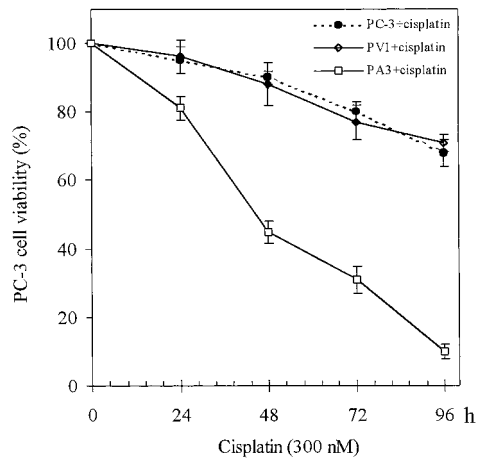

Fig. 8 The isodose sensitization enhancement of the PDPK F_A antisense clone to clinically relevant cisplatin concentration in PC-3 cells. PDPK F_A antisense clones (PA3) and parental or control-transfected (PV1) cells were treated with clinically relevant concentration of cisplatin (300 nM) at 37°C for various time intervals as indicated. The cell viability was determined as described in the legend to Fig. 4. Data were taken from the averages of three independent experiments and expressed as means ± SD.

DISCUSSION

In this study, the PDPK F_A-specific antisense expression vector and antibody were established. The antisense expression vector could be introduced stably into a human prostate cancer

Table 3 The drug sensitivity in the LNCaP cell and the genistein-treated PC-3 cell[a]

Cells	Drugs			
	Carboplatin (nM)	5-Fluorouracil (μM)	Paclitaxel (nM)	Hydroxyurea (mM)
LNCaP	400 ± 13	0.18 ± 0.02	5.9 ± 0.2	1.07 ± 0.05
PC-3 + 25 μM genistein[b]	360 ± 9	0.21 ± 0.01	9.3 ± 0.5	1.03 ± 0.13

[a] The drug sensitivity in terms of IC_{50} was determined as described in the legend to Table 1.

[b] Before the treatment with drugs, PC-3 cells were first treated with 25 μM genistein at 37°C for 24 h.

cell line (PC-3). Two stable antisense clones were successfully subcloned, which could constitutively suppress ~25% and ~65% of the total PDPK F_A activity associated with the PC-3 cell, respectively. Although the DNA sequence homology between PDPK F_A and GSK-3β is ~85% (6), the constructed antisense vector presented here displayed little effect on the expression of GSK-3β in the PC-3 cell. We also tested the effect of this antisense vector on the well-established PDPK member, namely MAPKs, and again no suppression of MAPK expression could be observed. The results taken together demonstrate that the antisense construct presented here specifically suppresses the endogenous PDPK F_A expression in the PC-3 cell.

The PA7 and PA3 antisense clones, which suppressed ~25% and ~65% of PDPK F_A, proportionally and potentially displayed an enhanced sensitivity to various anticancer agents, including carboplatin, 5-fluorouracil, paclitaxel, and hydroxyurea. The increased drug sensitivity observed in the first series of experiments appeared to have a correlation between PDPK F_A suppressed levels and drug sensitivity (IC_{50}). This, together with the facts that LNCaP cell with less PDPK F_A activity displayed similar enhanced chemosensitivity and genistein that could block PDPK F_A activity also potentiated similar chemosensitivity of these drugs in PC-3 cell, demonstrated an essential role of this PDPK in various drugs resistance (see Tables 1–3). It is important to note that the enhanced chemosensitivity presented here involved multiple chemotherapy agents with various mechanisms of action and resistance. This is also the reason why the enhanced drug sensitivity appeared disproportionate to the degree of PDPK F_A deactivation (for instance, a >100-fold increase in sensitivity to carboplatin with only a 25% decrease in PDPK F_A activity). All of these can be mainly due to the facts that PDPK F_A is a diverse multisubstrate PDPK and that the signal of a 25% decrease in this PDPK activity can be amplified and potentiated manyfold on various putative drug resistance-related target proteins (6–8, 20–24). PDPK F_A may, therefore, represent a general systemic protein kinase involved in regulating multiple drug resistance. This obviously presents an intriguing issue that remains to be further established. On the other hand, whether suppression of overexpressed PDPK F_A may provide a hopeful clinic target for therapeutic intervention potentiating chemosensitivity in human prostate cancer treatment obviously presents another intriguing issue deserving further investigation. Nevertheless, the present study clearly demonstrates that specific suppression of PDPK F_A is sufficient to enhance various anticancer drug sensitivity in human prostate carcinoma cells, providing an initial evidence for a critical role of this PDPK in regulating drug resistance in human cancer.

REFERENCES

1. Goldstein, L. J. Clinical reversal of drug resistance. Curr. Probl. Cancer (Phila.), *19:* 65–124, 1995.

2. Hickman, J. A. Apoptosis and chemotherapy resistance. Eur. J. Cancer, *32:* 921–926, 1996.

3. Dawson, N. A. Treatment of progressive metastatic prostate cancer. Oncology, *7:* 17–24, 1993.

4. Vandenheede, J. R., Yang, S-D., Goris, J., and Merlevede, W. ATP.Mg-dependent protein phosphatase from rabbit muscle. Purification of the activating factor and its regulation as a bifunctional protein also displaying synthase kinase activity. J. Biol. Chem., *255:* 11768–11774, 1980.

5. Hemmings, B. A., Yellowlees, D., Kernohan, J. C., and Cohen, P. Purification of glycogen synthase kinase 3 from rabbit skeletal muscle. Copurification with the activating factor (F_A) of the (Mg-ATP) dependent protein phosphatase. Eur. J. Biochem., *119:* 443–451, 1981.

6. Woodgett, J. R. Molecular cloning and expression of glycogen synthase kinase-3/factor A. EMBO J., *9:* 2431–2438, 1990.

7. Yang, S-D. Signal transduction and biological functions of protein phosphatase activating factor (protein kinase FA/GSK-3α). J. Chin. Biochem. Society, *23:* 123–134, 1994.

8. Plyte, S. E., Hughes, K., Nikolakaki, E., Pulverer, B. J., and Woodgett, J. R. Glycogen synthase kinase-3: functions in oncogenesis and development. Biochem. Biophys. Acta, *1114:* 147–162, 1992.

9. Lee, T-T., Ho, Y-S., Yu, J-S., and Yang, S-D. Overexpression of cellular activity and protein level of protein kinase F_A/GSK-3α correlates with human thyroid tumor cell dedifferentiation. J. Cell. Biochem., *58:* 474–480, 1995.

10. Yang, S-D., Yu, J-S., Lee, T-T., Ni, M-H., Yang, C-C., Ho, Y-S., and Tsen, T-Z. Association of protein kinase F_A/GSK-3α (a proline-directed kinase and a regulator of proto-oncogenes) with human cervical carcinoma dedifferentiation/progression. J. Cell. Biochem., *59:* 143–150, 1995.

11. Yang, S-D., Yu, J-S., Yang, C-C., Lee, S-C., Lee, T-T., Ni, M-H., Kuan, C-Y., and Chen, H-C. Overexpression of protein kinase F_A/GSK-3α (a proline-directed protein kinase) correlates with human hepatoma dedifferentiation/progression. J. Cell. Biochem., *61:* 238–245, 1996.

12. Yang, C-C., Hsu, C-P., Sheu, J-C., Mai, X-Y., and Yang, S-D. Differential tyrosine phosphorylation/activation of oncogenic proline-directed protein kinase FA/GSK-3α in well- and poorly-differentiated human prostate carcinoma cells. J. Protein Chem., *17:* 329–335, 1998.

13. Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., and Jones, L. W. Establishment and characterization of a human prostatic carcinoma cell line (PC-3). Invest. Urol., *17:* 16–23, 1979.

14. Lee, S-C., Kuan, C-Y., Yang, C-C., and Yang, S-D. Bioflavonoids commonly and potently induce tyrosine dephosphorylation/inactivation of oncogenic proline-directed protein kinase F_A in human prostate carcinoma cells. Anticancer Res., *18:* 1117–1121, 1998.

15. Yu, J-S., and Yang, S-D. Immunological and biochemical study on tissue and subcellular distributions of protein kinase F_A (an activating factor of ATP. Mg-dependent protein phosphatase). A simplified and efficient procedure for high quantity purification from brain. J. Protein Chem., *12:* 665–674, 1993.

16. Yu, J-S., and Yang, S-D. Tyrosine dephosphorylation and inactivation of protein kinase F_A/GSK-3α by genistein in A431 cells. J. Cell. Biochem., *56:* 131–141, 1994.

17. Reichlin, M. Use of glutaraldehyde as a coupling reagent for proteins and peptides. Methods Enzymol., *70:* 159–165, 1980.

18. He, X., Saint-Jeannet, J. P., Woodgett, J. R., Varmus, H. E., and Dawid, I. B. Glycogen synthase kinase-3 and dorsoventral patterning in *Xenopus* embryos. Nature (Lond.), *374:* 617–622, 1995.

19. Gillespie, P. G., and Hudspeth, A. J. Chemiluminescence detection of proteins from single cells. Proc. Natl. Acad. Sci. USA, *88:* 2563–2567, 1991.

20. Yang, S-D., Song, J-S, Yu, J-S., and Shiah, S-G. Protein kinase F_A/GSK-3 phosphorylates tau on Ser235-Pro and Ser404-Pro that are abnormally phosphorylated in Alzheimer's disease brain. J. Neurochem., *61:* 1742–1747, 1993.

21. Yu, J-S., and Yang, S-D. Protein kinase F_A/glycogen synthase kinase-3 predominantly phosphorylates the *in vivo* site Thr97-Pro in brain myelin basic protein: evidence for Thr-Pro and Ser-Arg-X-X-Ser as consensus sequence motifs. J. Neurochem., *62:* 1596–1603, 1994.

22. Yang, S-D., Huang, J-J., and Huang, T-J. Protein kinase F_A/glycogen synthase kinase 3α predominantly phosphorylates the *in vivo* sites of Ser502, Ser506, Ser603, and Ser666 in neurofilament. J. Neurochem., *64:* 1848–1854, 1995.

23. Boyle, W. J., Smeal, T., Defize, L. H. K., Angel, P., Woodgett, J. R., Karin, M., and Hunter, T. Activation of protein kinase C decreased phosphorylation of c-jun at sites that negatively regulate its DNA-binding activity. Cell, *64:* 573–584, 1991.

24. Sinha, B. K., Yamazaki, H., Eliot, H. M., Schneider, E., Borner, M. M., and O'Connor, P. M. Relationships between proto-oncogene expression and apoptosis induced by anticancer drugs in human prostate tumor cells. Biochim. Biophys. Acta, *1270:* 12–18, 1995.

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