Transcriptional Repression of Protein Kinase Cα via Sp1 by Wild Type p53 Is Involved in Inhibition of Multidrug Resistance 1 P-Glycoprotein Phosphorylation*

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The protein kinase C (PKC) family consists of serine/threonine protein kinases that play important roles in signal transduction, cell proliferation, and tumor formation. Recent studies found that PKCs are commonly overexpressed in human tumors, including soft tissue sarcoma (STS). Overexpression of PKCs contributes to invasion and migration of tumor cells and induction of angiogenesis. PKC can also phosphorylate the multidrug resistance (MDR) gene-encoded P-glycoprotein and induce MDR phenotype. Our previous studies showed that mutation of p53 enhanced STS metastasis and mediated the MDR phenotype. Restoring wild type (WT) p53 in STS cells containing mutant p53 sensitized the cells to chemotherapy. In the present study, we found that PKCα protein expression is inhibited by WT p53 partly due to reduced PKCα mRNA expression in STS cells, but p53 does not affect PKCα mRNA stability. Deletion and mutation analysis of the PKCα promoter fused to the luciferase reporter gene identified a Sp1 binding site (–244/–234) in the PKCα promoter that is required for p53-mediated inhibition of PKCα promoter activity. More importantly, PKCα phosphorylates and activates MDR1 P-glycoprotein, whereas inhibition of PKCα by p53 leads to decreased MDR1 phosphorylation in STS cells, which sensitizes STS cells to chemotherapeutic agents. These data indicate that WT p53 may resensitize STS to chemotherapeutic agents by reducing MDR1 phosphorylation via transcriptional repression of PKCα expression. Thus, molecular-based therapies targeting mutant p53 and PKCα may be an effective new strategy to improve chemotherapeutic efficacy in STS.

The protein kinase C (PKC) family consists of a large number of serine/threonine kinases that are activated by extracellular signals. In mammalian cells, the PKC family is further divided into three subfamilies, conventional PKCs (α, βI, βII, γ), novel PKCs (δ, ε, η, θ), and atypical PKCs (ι, λ, ζ) (1). PKCμ is an additional PKC family member that was discovered recently and is called as protein kinase D (2). A distantly related PKC family includes three isoforms of the PKC-related kinases, known as PKN1, PKN2, and PKN3 (3). Although PKC family members share primary sequence similarities, the subfamily members have different enzymatic activation profiles. Specifically, conventional PKC isoforms are activated by 1,2-diacylglycerol and phosphatidyserine (PS) in a calcium-dependent manner, novel PKC members are activated only by 1,2-diacylglycerol and PS, and atypical PKC members are activated by PS alone as a co-factor (4). In resting cells, PKC predominantly resides in the cytosol in an inactive state and, upon stimulation, translocates as an active kinase to discrete subcellular locations, such as the plasma membrane, membranous vesicles, cytoskeleton, mitochondria, and nucleus (5).

PKC functions have been the subject of intense study for many years after identification of PKC as the intracellular receptor for tumor-promoting phorbol esters such as tissue-type plasminogen activator. Studies have shown that PKC activity is elevated in some human tumors when compared with that in adjacent normal tissues (6) and that elevated PKC activity is associated with increased metastatic or invasive potential in some human carcinoma cells (7). More recent studies indicate that PKCα plays an important role in promotion of tumor invasion, migration, enhanced vascular endothelial growth factor secretion, and development of the multidrug resistance (MDR) phenotype (8–11). Because overexpression of PKCs has been found in many disorders, these kinases have become major targets for therapeutic intervention in a wide range of diseases, including cancers (12), and sensitization of tumors to radiotherapy and chemotherapy has been achieved with PKC inhibitors (13, 14). Although many studies have focused on dysregulation of PKC functions, it is still unclear how expression of PKCs is regulated.

Mutation of the tumor suppressor gene p53 is commonly found in a wide variety of human tumors and is one of the most common genetic alterations in soft tissue sarcoma (STS), occurring in 30–60% of these tumors (15). Interestingly, p53 missense mutations occur more frequently than non-sense mutations (16). Cancer cells harboring missense mutant (mut) p53 appear to have a gain of function with enhanced oncogenic properties and tumorigenic potential when compared with cells that merely lose p53 function through point mutations (16, 17). Also, our previous study showed that loss of WT p53 function enhances metastasis of STS (18). Wild type (WT) p53 functions as a transcription factor and, by binding to specific DNA sequences, exerts its tumor suppressor activity by stimulating transcription of growth-inhibitory genes while preventing expression of proliferation-promoting genes (19). In contrast, mut
p53 activates genes repressed by WT p53. Recent reports support this model by showing that transcription of the MDR1 gene and the proliferating cell nuclear antigen is activated by mut p53 but repressed by WT p53 (20, 21).

More recently, global phosphorylation and function of p53 were shown to be inversely related to PKC activity (22). Further, we investigated whether WT p53 may revert to MDR phenotype through inhibition of PKCα.

**EXPERIMENTAL PROCEDURES**

**Cell Lines**—The cell lines SKLMS-1 (a human leiomyosarcoma cell line harboring a p53 point mutation at codon 245) (24) and HT1080 (a fibrosarcoma cell line deficient in p53) (25) were obtained from the American Type Culture Collection and were cultured in Dulbecco's modified Eagle's medium/F-12 medium with 10% fetal bovine serum (complete culture medium; Invitrogen) in 5% CO2 at 37 °C. Also, the cell lines Saos-2 (a p53-null osteosarcoma cell line) and U2-OS (an osteosarcoma cell line with WT p53) (26) were cultured in the same medium supplemented with 10% fetal bovine serum. Clones of SKLMS-1 cells stably transfected with Ala-143 temperature-sensitive mut p53 were designated SKA1a, SKA2a, SKA1b, and SKNeo (vector control) (24, 26) and cultured in complete culture medium in 5% CO2 at 37 °C. These cells expressed WT p53 at 32 °C and mut p53 at 38 °C. Cells were passaged by treatment with 0.05% trypsin and 1 mM EDTA (Invitrogen).

**Western Blot Analysis**—Western blotting was carried out as described previously (27). Briefly, cells were rinsed with phosphate-buffered saline before lysis with radioimmune precipitation assay buffer (20 mM Tris-HCl, pH 8.0, 137 mM NaCl, 2 mM EDTA, 0.1% SDS, 5 mM NaOH at 100 °C for 10 min, neutralized with ammonium acetate to 0.1% SDS, 5% SDS, 5 mM NaF, 1 mM sodium vanadate, 2 mM EDTA, 10 μg/ml leupeptin, 10 μg/ml pepstatin A, 200 μg/ml aprotinin, 0.5 mM phenylmethylsulfonyl fluoride; 1 μl/plate), and lysed on ice for 20 min. Lysates were centrifuged at 13,800 × g for 15 min, and the supernatants were collected.

Immunoprecipitation of P-gp was carried out according to a described previously method (29). Briefly, 5 μg of the anti-P-gp antibody 219 (Centocor) was added to 200 μg of protein and incubated overnight in a sample rotator. Fifty microliters of protein A Sepharose 4B (Amersham Biosciences) was added to the supernatants and incubated in the sample rotator for 30 min. The beads were collected by centrifugation for 1 min and washed once in 1 ml of NaCl and 0.1% Nonidet P-40 and twice in an extraction buffer containing 1 mM urea. The immune complexes were dissociated from the beads by incubation in 100 mM NaF. Cell monolayers for 15 h at 37 °C. Sucrose gradient centrifugation was performed on 5% acrylamide gels, and autoradiography was performed.

**PKCα Promoter Deletion and Mutation Construction**—Human PKCα promoters (−1571/+77 and −227/+77) were generously provided by Dr. Robert Glazer (Georgetown University). All other constructs were made by amplifying the PKCα promoter with the reverse primer 5′-GAGAGTCGGGCTGGTGCTG-3′ (complement to the transcription start site) and the sequence for H11001 (5′-GGAGAGTCGGGCTGGTGCTG-3′). PKCα deletion constructs and 0.5 μg of pGL3-basic containing the various promoter deletion constructs in the TA vector were cotransfected with 1 μg of pSV40-lacZ and further incubated for 48 h. Using FuGENE 6, cells were then cotransfected with 1 μg of pGL3-basic containing the various PKCα deletion constructs and 0.5 μg of the internal control pSV40-β-galactosidase. Twenty-four hours later, SKLMS-1 cells were harvested, and luciferase activity was determined and normalized to β-galactosidase activity (15).

**Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay**—The nuclear extracts from SKLMS-1 cells were prepared accord-
WT p53 Transcriptionally Represses PKCa Expression

RESULTS

WT p53 Inhibits PKCs Expression—To investigate the effects of p53 on PKCα in STS, we first analyzed PKCα expression in STS cells with different p53 functional status by Western blot analysis. PKCα was highly expressed in SKLMS-1 cells, which contain mut p53, and at very low levels in U2-OS cells, which contain WT p53 (Fig. 1A). Similarly, high PKCα expression was found in HT1080 cells, which are deficient in p53, and Saos-2 cells, which are p53 null.

To further define the role of p53 in modulation of PKCα expression, we used the SKAla cells, which are SKLMS-1 cells that express the Ala-143 temperature-sensitive mut p53 (26). The Ala-143 point mutation allows for production of WT p53 at 32 °C but mut p53 at 38 °C. Western blot analysis of PKCα in these SKAla cells showed that PKCα expression was higher when p53 was in the mutant conformation at 38 °C than when it was in the WT conformation at 32 °C (Fig. 1B), whereas the parental sarcoma cells SKLMS-1 and vector control cells SKNeo showed no significant changes in PKCα expression when they were shifted between 32 and 38 °C culture conditions.

Therefore, the difference in PKCα expression between 32 and 38 °C in these SKAla cell lines was induced specifically by the change in functional p53 status, not by the temperature variance itself. We used the p21 expression level as a marker for functional WT p53.

To further determine the effect of WT p53 on PKCα expression in a p53 null background, we also transfected the Saos-2 cell line with the Ala-143 temperature-sensitive p53 mutant expression vector and observed the effect of WT and mut p53 on PKCα expression. Six hours after transfection, the transfected cells were shifted to 32 or 38 °C and cultured for an additional 36 h, then PKCα expression was analyzed by Western blotting. PKCα expression was inhibited when the transfected Saos-2 cells were cultured at 32 °C with WT p53 function that induced p21 expression (Fig. 1C). In contrast, PKCα level remained high when the transfected Saos-2 cells were cultured at 38 °C with mut p53 function, and the induction of p21 expression was negligible. These results clearly indicated that WT p53 inhibits PKCα expression.

p53 Inhibits PKCα mRNA Expression—To determine whether p53 inhibited PKCα expression at the mRNA level, we performed Northern blot analysis of PKCα mRNA level using a PKCα-specific probe (American Type Culture Collection) in the SKLMS-1, SKNeo, and SKAla cell lines cultured at 32 or 38 °C. In SKAla-1, SKAla-2, and SKAla-3 cells, PKCα mRNA (9.3 kilobases) was low at 32 °C with the WT p53 conformation but high at 38 °C with the mut p53 conformation. No comparative changes were found in either parental SKLMS-1 or vector control SKNeo cells (Fig. 2A). A similar result was found in Saos-2 cells transfected with Ala-143 and incubated at 32 versus 38 °C (Fig. 2B). In addition, SKAla-2 expressing the temperature-sensitive mut p53 showed a time-dependent inhibition of PKCα mRNA expression when shifted from 38 to 32 °C and vice versa (Fig. 2C). Therefore, p53 inhibits PKCα expression at the mRNA level.

p53 Inhibits PKCα mRNA Expression through Transcriptional Repression Not Affecting PKCα mRNA Stability—Inhibition of PKCα mRNA by p53 may result from either transcriptional repression or reduced mRNA stability. To investigate the mechanism by which p53 inhibits PKCα mRNA expression, we examined the rate of transcription of PKCα by nuclear runoff assays. Although there was not a significant difference in PKCα transcription between 38 and 32 °C in parental SKLMS-1 cells, we reproducibly detected an approximate 30% reduction in expression of PKCα transcript at 32 °C (WT p53) compared with that at 38 °C (mut p53) in SKAla-2 cells (Fig. 3A). Therefore, reduced transcription may at least partly account for the decrease in PKCα mRNA level by p53.

We next investigated whether p53 also inhibits PKCα mRNA stability in SKLMS-1 and SKAla cells. New RNA synthesis was blocked by treating cells with actinomycin D (5 μg/ml). Total RNA was extracted at different time points after treatment. Northern blot analysis using the PKCα-specific probe showed that there was not a significant difference in the measured half-life of PKCα mRNA from both SKLMS-1 and SKAla-2 cells cultured at 32 and 38 °C (Fig. 3B). Thus, the inhibited RNA expression level at 32 °C compared with that at 38 °C in SKAla-2 cells was not due to a decreased mRNA stability.

WT p53 Inhibits PKCα Promoter Activity via Sp1 Binding Site—To further understand the mechanisms by which WT p53 inhibits PKCα transcription, we fused the 1.6-kilobase PKCα promoter to the firefly luciferase reporter gene (Fig. 4A) to determine the effect of WT p53 on PKCα promoter activity. We preinfected SKLMS-1 cells with various doses of adenoviruses expressing WT p53 (Ad-p53) or expressing the LacZ gene (Ad-LacZ) for 48 h and subsequently transiently transfected the cells with the PKCα promoter-luciferase reporter construct (−1571/+77); we assayed reporter gene expression 48 h later. Compared with Ad-LacZ-infected cells, Ad-p53-infected cells showed markedly inhibited PKCα promoter activity at different cell:virus particle ratios in a viral particle concentration-dependent manner (Fig. 4B).

To localize the promoter region responsible for WT p53-mediated suppression of PKCα transcription, we generated a series of PKCα promoter deletion constructs that were composed of various lengths of 5′-promoter sequences fused to the luciferase gene (Fig. 4A). We transiently transfected the PKCα promoter-luciferase constructs and pGL3-basic vector (vector control) into SKLMS-1 cells preinfected with the Ad-p53 or Ad-LacZ (1000 virus particles/cell). The promoter activities of the various deletion constructs were not inhibited by Ad-LacZ, but they were markedly inhibited by Ad-p53 (Fig. 4C). However, the inhibitory effect of WT p53 was abolished when PKCα promoter was deleted down to −227, which also had dramatically reduced basal promoter activities. The data indicated that the −260/+77 construct is sufficient to confer WT p53-induced inhibition and that this 34-bp promoter region upstream to the transcription start site, from −260 to −227, may contain the p53-responsive element. As expected, the promoterless luciferase pGL3-basic vector showed no promoter activity and could not be repressed by WT p53 (data not shown).

To further identify the p53-responsive element for the PKCα inhibition in this 34-bp PKCα promoter region, we mutated the only known transcription factor binding site in this region. The Sp1 site (−244/−234, CCGCCTCCCCC) (32), as shown in Fig. 4D, and evaluated the transcriptional activity of this mutated construct. The −260 mut PKCα promoter with Sp1 site mutation retained basal promoter activity as the wild type −260 construct. However, the −260 mut with Sp1 site mutation was not inhibited by p53 as significantly as was the wild type −260 reporter gene (17 versus 62%) (Fig. 4E). To directly test Sp1...
binding to the Sp1 site in this region, we also performed electrophoretic mobility shift assay using the $^{32}$P-labeled oligonucleotides of the 34-bp PKCα promoter region. Although no specific Sp1 binding was detected using the mutant Sp1 probe (Fig. 4F, lane 1), a specifically shifted band can be detected in SKLMS-1 nuclear extracts using wild type Sp1 containing oligonucleotides (Fig. 4F, lane 2), which was supershifted by anti-Sp1 antibody (Fig. 4F, lane 3). The specific binding of Sp1 could be abolished by unlabeled wild type oligonucleotides (Fig. 4F, lane 4). These data indicated that WT p53 repression of the
PKCα promoter activity is mediated through the Sp1 binding site located in the −244/−234 region of the PKCα promoter (Fig. 4D).

PKCα Inhibition-induced Decrease in Phosphorylation of MDR1 P-glycoprotein—Previous reports showed that PKCα is selectively overexpressed in human STS, and PKCα catalyzes MDR1 P-glycoprotein phosphorylation and activation, which contributes to the MDR phenotype (29). To investigate the biological significance of p53-mediated PKCα transcriptional repression, we examined the effect of PKCα inhibition by p53 on phosphorylation of MDR1 P-glycoprotein and expression. We found a lower level of phosphorylation of MDR1 P-glycoprotein in SKAla-1, SKAla-2, and SKAla-3 cells when they were cultured at 32 or 38 °C for 72 h. The cells were then shifted from 32 to 38 °C or from 38 to 32 °C, and total RNA was prepared on days 0, 2, 4, and 6 and analyzed by Northern blotting for PKCα expression.

To assure that the decreased phosphorylation of MDR1 P-glycoprotein was due to specific inhibition of PKCα by wild type p53 but not other downstream effects of the wild type p53, we treated the SKAla-1 and SKAla-2 cells growing at 38 °C with PKCα inhibitor G6976 (Calbiochem) (33) to inhibit PKCα kinase activity. After 24 h of treatment, the phosphorylation of MDR1 P-glycoprotein was dramatically inhibited, which is associated with lower PKCα kinase activity, as indicated by decreased PKCα phosphorylation, whereas there was no significant change in total PKCα and total P-glycoprotein (Fig. 5B). The results clearly showed that inhibition of PKCα itself is sufficient to decrease phosphorylation of P-glycoprotein. Taken together, our data demonstrated that wild type p53 inhibits PKCα expression, which subsequently leads to decreased MDR1 P-glycoprotein phosphorylation and, thus, increased chemosensitivity; loss of p53 function results in increased P-glycoprotein phosphorylation because of the increased PKCα level, which contributes to chemo-resistance.

DISCUSSION

The functions of PKCs in signal transduction, cell proliferation, and tumor formation have been extensively investigated (34). Recent studies have shown that PKCs are also involved in tumor migration through regulation of matrix metalloproteinases and their inhibitors (9), potentiation of vascular endothelial growth factor induction (10), and proliferation of tumor cells (35). Because overexpression of PKCs has been found in many diseases, PKCs have become one of the major targets for interventions of tumor progression (12). However, the precise mechanism of PKC regulation is largely unknown. There have been only two studies of transcriptional control of PKCs; one
showing that 1,25-dihydroxyvitamin D3 transcriptionally regulates PKCβ (36) and the other showing that fibroblast growth factor-2 up-regulates PKCɛ mRNA 1.6–3.0-fold and PKCδ mRNA 1.7-fold (37). To the best of our knowledge our study has provided a novel link between WT p53 tumor suppressor and the inhibition of PKCα, a critical signaling component involved in multiple aspects of oncogenesis.

One question has arisen. Which cis-DNA element is responsible for the WT p53 inhibition effect on PKCα promoter activity? Because there is no conservative p53 DNA binding sequence in the PKCα promoter, other unknown cis (or trans) elements or protein factors may be responsible for the suppres-
WT p53 Transcriptionally Represses PKCa Expression

WT p53 transcriptionally represses PKCa expression by inhibiting the transcription of PKCa via the Sp1 binding site in its promoter. The promoter region responsible for WT p53-mediated suppression of PKCa transcription was localized using a series of 5' deletion constructs of the PKCa promoter. Twenty-four hours after transfection, luciferase activity was measured in SKLMS-1 cells transfected with wild type or mutant PKCa promoter constructs. The relative luciferase activities were determined and normalized to β-galactosidase activity. Values are expressed as the percentage of the relative luciferase activity (100%) in untreated SKLMS-1 cells transfected with PKCa promoter constructs. Mean ± S.E. of duplicate of three independent assays. *, p < 0.05. D, Sp1 binding site of the PKCa promoter sequence responsible for p53-mediated inhibition. E, relative luciferase activity was measured in wild type -260/+77 and mutant -260/+77 PKCa constructs as in C. F, identification of specific Sp1 binding to the 34-bp PKCa promoter region by electrophoretic mobility shift assay. Nuclear extracts from SKLMS-1 cells were incubated with radiolabeled mutant Sp1 oligonucleotides (lane 1) or wild type Sp1 oligonucleotides alone (lane 2) or plus anti-Sp1 antibody (lane 3). SKLMS-1 nuclear extracts were also preincubated with 100× unlabeled wild type Sp1 oligonucleotides and then were incubated with radiolabeled wild type Sp1 oligonucleotides (lane 4). The reactions were analyzed on 4% polyacrylamide gels containing 0.25× Tris borate/EDTA buffer. Ab, antibody.

Fig. 4. Localization of the PKCa promoter region responsible for WT p53-mediated suppression of PKCa transcription. A, PKCa promoter deletion constructs were generated by polymerase chain reaction followed by cloning as described under “Experimental Procedures.” B, dose-dependent inhibition of PKCa promoter-luciferase activity by Ad-p53. SKLMS-1 cells were pretreated with various doses of Ad-p53 or Ad-LacZ for 48 h. The cells were then cotransfected with 1 μg of the PKCa promoter-luciferase construct (−1571/+77) with 0.5 μg of pSV40-β-galactosidase. Twenty-four hours later the luciferase activity was measured as described under “Experimental Procedures.” C, PKCa promoter deletion constructs were transiently transfected into SKLMS-1 cells that were pretreated with Ad-p53 or Ad-LacZ (1000 virus particles/cell), and luciferase activities were determined on cells extracts and normalized to β-galactosidase activity. Values are expressed as the percentage of the relative luciferase activity (100%) in cell extracts from untreated SKLMS-1 cells transfected with PKCa promoter constructs. Mean ± S.E. of duplicate of three independent assays. *, p < 0.05. D, Sp1 binding site of the PKCa promoter sequence responsible for p53-mediated inhibition. E, relative luciferase activity was measured in wild type −260/+77 and mutant −260/+77 PKCa constructs as in C. F, identification of specific Sp1 binding to the 34-bp PKCa promoter region by electrophoretic mobility shift assay. Nuclear extracts from SKLMS-1 cells were incubated with radiolabeled mutant Sp1 oligonucleotides (lane 1) or wild type Sp1 oligonucleotides alone (lane 2) or plus anti-Sp1 antibody (lane 3). SKLMS-1 nuclear extracts were also preincubated with 100× unlabeled wild type Sp1 oligonucleotides and then were incubated with radiolabeled wild type Sp1 oligonucleotides (lane 4). The reactions were analyzed on 4% polyacrylamide gels containing 0.25× Tris borate/EDTA buffer.
be phosphorylated by PKCα (44), which suggests that PKCα is indirectly involved in the development of the MDR phenotype. Our current study shows that in addition to direct down-regulation of MDR1 P-glycoprotein by wild type p53 (23), another important mechanism by which WT p53 reverts the MDR phenotype and exerts its anticancer activity is that transcriptional repression of PKCα by WT p53 leads to decreased phosphorylation of P-glycoprotein. This inhibitory effect on PKCα and subsequent decreased phosphorylation of P-glycoprotein by WT p53 is consistent with our previous reports that reinstitution of WT p53 sensitizes STS to chemotherapeutic agents and inhibits STS growth in vitro and in vivo (23, 45).

In summary, we demonstrated here that restoration of WT p53 into human leiomyosarcoma cells containing mut p53 markedly suppresses PKCα transcription through the Sp1 binding site located -244/-234 in the promoter upstream of transcription start site. Importantly, inhibition of PKCα contributes to the decreased phosphorylation of MDR1 P-glycoprotein. Our data suggest that the use of molecular-based therapies targeting mut p53 and PKCα will be a new effective strategy for reverting the MDR phenotype and controlling the progression of STS.

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FIG. 5. Decreased phosphorylation of MDR1 P-glycoprotein after down-regulation of PKCα expression. A, SKLMS-1, SKNeo, SKAla-1, SKAla-2, and SKAla-3 cells were labeled with [32P]Pi for 3 h. Cell lysates were prepared, and immunoprecipitation was carried out with a C219 antibody as described under “Experimental Procedures.” The beads were collected, and the immune complexes were recovered and separated on 6% acrylamide gels before autoradiography. The quantitation was done by Scion Image Software and normalized to loading control. B, SKAla-1 and SKAla-2 cells were cultured at 38 °C and treated with PKCα inhibitor Gö6976 for 24 h, then labeled with [32P]Pi, for 3 h. The phosphorylation of P-glycoprotein was measured as A. Total PKCα, phosphorylated PKCα, total P-glycoprotein were determined by Western blot as described under “Experimental Procedures.” DMSO, Me2SO.
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Transcriptional Repression of Protein Kinase Cα via Sp1 by Wild Type p53 Is Involved in Inhibition of Multidrug Resistance 1 P-Glycoprotein Phosphorylation
Maocheng Zhan, Dihua Yu, Juehui Liu, Jonathan Hannay and Raphael E. Pollock

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Additions and Corrections

Vol. 279 (2004) 44327–44334

Carbon monoxide promotes Fas/CD95-induced apoptosis in Jurkat cells.

Ruiping Song, Zhihong Zhou, Peter K. M. Kim, Richard A. Shapiro, Fang Liu, Christiane Ferran, Augustine M. Choi, and Leo E. Otterbein

Page 44328, Fig. 1B: The +/- labeling for carbon monoxide is in reverse order. See the revised Fig. 1B below.

| Control | Etoposide | Fas/CD95 | Trail |
|---------|-----------|-----------|--------|
| CO      | -         | +         | -      |
|         | -         | -         | +      |
|         | -         | -         | -      |

Fig. 1B

Vol. 280 (2005) 4825–4833

Transcriptional repression of protein kinase Cα via Sp1 by wild type p53 is involved in inhibition of multidrug resistance 1 P-glycoprotein phosphorylation.

Maocheng Zhan, Dihua Yu, Juehui Liu, Robert I. Glazer, Jonathan Hannay, and Raphael E. Pollock

Dr. Robert I. Glazer was inadvertently omitted from the author list. His affiliation is Department of Pharmacology and Oncology and Lombardi Cancer Center, Georgetown University, Washington, D. C. 20007. The correct author list is shown above.

Vol. 280 (2005) 17512–17519

Identification of four adenosine kinase isoforms in tobacco By-2 cells and their putative role in the cell cycle-regulated cytokinin metabolism.

Zuzanna Kwade, Agnieszka Świątek, Abdelkrim Azmi, Alain Goossens, Dirk Inzé, Harry Van Onckelen, and Luc Roef

Page 17512: One of the GenBank™ accession numbers was misprinted in the footnote at the bottom of the page: “Y695055” should read “AY695055.”

FIG. 1B
Gating-enhanced accessibility of hydrophobic sites within the transmembrane region of the nicotinic acetylcholine receptor's δ subunit. A time-resolved photolabeling study.

Enrique Arevalo, David C. Chiara, Stuart A. Forman, Jonathan B. Cohen, and Keith W. Miller

Page 13638, Fig. 8: The original figure was determined to be of low resolution. See the revised Fig. 8 below.