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X-Rays in a Cisplatin-resistant Ovarian Tumor Cell Line Independent Pathways of p53 Induction by Cisplatin and

Zahid H. Siddik, Betsy Mims, Guillermina Lozano, et al.

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ICANCER RESEARCH 58. 698–703. February 15. 1998]
Independent Pathways of p53 Induction by Cisplatin and X-Rays in a
Cisplatin-resistant Ovarian Tumor Cell Line¹ CANCER RESEARCH 58. 698-703. February 15. 1998]
**Independent Pathways of p53 Induction by Cisplatin
Cisplatin-resistant Ovarian Tumor Cell Line¹ Independent Pathways of p53 Induction by Cisplatin
Cisplatin-resistant Ovarian Tumor Cell Line¹
Zahid H. Siddik,² Betsy Mims, Guillermina Lozano, and Gerald Thai
Penatments of Clinical Investigation 17 H.S. G. T. Land** *Cisplatin-resistant Ovarian Tumor Cell Line¹*
Zahid H. Siddik,² Betsy Mims, Guillermina Lozano, and Gerald Thai
Departments of Clinical Investigation [Z. H. S., G. T.] and Molecular Genetics [B. M., G. L.], Universit

ABSTRACT

partments of Clinical Investigation (Z. H. S., G. T.) and Molecular Genetics [B. M., G. L.], l
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The p53 tumor suppressor gene is critical in regulating cell prolifera
n following DNA damage, and disruption of p53 pro the p53 tumor suppressor gene is critical in regulating cell prolifera-
tion following DNA damage, and disruption of p53 protein function by
mutation has been implicated as a factor responsible for resistance of $\frac{1000$ as
 mutation following DNA damage, and disruption of p53 protein function by

mutation has been implicated as a factor responsible for resistance of

tumor cells to chemotherapeutic agents. Our studies were initiated by The p53 tumor suppressor gene is critical in regulating cell prolifera-
tion following DNA damage, and disruption of p53 protein function by
mutation has been implicated as a factor responsible for resistance of
tumor cell The $p53$ tumor suppressor gene is critical in regulating cell proliferation following DNA damage, and disruption of $p53$ protein function by mutation has been implicated as a factor responsible for resistance of tumor c tion following DNA damage, and disruption of p53 protein function by
mutation has been implicated as a factor responsible for resistance of
tumor cells to chemotherapeutic agents. Our studies were initiated by
asking whet mutation has been implicated as a factor responsible for resistance of tumor cells to chemotherapeutic agents. Our studies were initiated by also asking whether the translational product of the $p53$ gene is associated wit tumor cells to chemotherapeutic agents. Our studies were initiated by
asking whether the translational product of the $p53$ gene is associated with
cisplatin resistance in the 2780CP human ovarian tumor model. We have
demo asking whether the translational product of the $p53$ gene is associated with
cisplatin resistance in the 2780CP human ovarian tumor model. We have
demonstrated by single-strand conformation polymorphism analysis and
sequ cisplatin resistance in the 2780CP human ovarian tumor model. We have trademonstrated by single-strand conformation polymorphism analysis and case
quencing that $p53$ in parental cisplatin-sensitive A2780 cells was wild r demonstrated by single-strand conformation polymorphism analysis and
sequencing that $p53$ in parental cisplatin-sensitive A2780 cells was wild
tion of damaged DNA (12, 13). Increased levels of p53 protein
type. In 2780CP **ionizing that p53** in parental cisplatin-sensitive A2780 cells was wild represent type. In 2780CP cells, however, a mutation was found in exon 5 at codon can all 172 (Val to Phe). Interestingly, exposure to X-rays result 172 (Val to Phe). Interestingly, exposure to X-rays resulted in p53 induction in both A2780 and 2780CP tumor models. The p53 increases by the redionizing radiation were accompanied by concomitant increases in levels of th tion in both A2780 and 2780CP tumor models. The p53 increases by the
ionizing radiation were accompanied by concomitant increases in levels of
the p53-regulated p21^{Wart/Cip1} protein and led to arrest of cells in G_1 p ionizing radiation were accompanied by concomitant increases in levels of the $p53$ -regulated $p21^{Wafl/Cip1}$ protein and led to arrest of cells in G_1 phase of the cell cycle. A yeast functional assay confirmed that $p53$ the p53-regulated p21^{Waft/Cip1} protein and led to arrest of cells in G_1 phase
of the cell cycle. A yeast functional assay confirmed that p53 in A2780 was
wild type, but, more importantly, it provided evidence that th of the cell cycle. A yeast functional assay confirmed that $p53$ in A2780 was
wild type, but, more importantly, it provided evidence that the $p53$ inaction
mutation in 2780CP cells was temperature sensitive and heterozyg wild type, but, more importantly, it provided evidence that the $p53$ mutation in 2780CP cells was temperature sensitive and heterozygous. These experiments demonstrate that sensitive and resistant cells have the normal p mutation in 2780CP cells was temperature sensitive and heterozygous. These experiments demonstrate that sensitive and resistant cells have the normal p53 functions, despite the presence of p53 mutation in the 2780CP model These experiments demonstrate that sensitive and resistant cells have that

normal p53 functions, despite the presence of p53 mutation in the 2780CP medi

model. In parallel investigations using the Western technique, exp model. In parallel investigations using the Western technique, exposure of
A2780 cells to clinically relevant concentrations of cisplatin $(1-20 \mu M)$ re
resulted in time- and dose-dependent increases in p53, together with A2780 cells to clinically relevant concentrations of cisplatin $(1-20 \mu M)$ reportes resulted in time- and dose-dependent increases in p53, together with side coordinate increases in p21^{Wart/Clp1}. In contrast, cisplatin resulted in time- and dose-dependent increases in p53, together with
coordinate increases in p21^{Waf1/Cip1}. In contrast, cisplatin did not induce
these proteins in 2780CP cells to any significant degree. The results
indi coordinate increases in p21^{Waf1/Cip1}. In contrast, cisplatin did not induce
these proteins in 2780CP cells to any significant degree. The results
indicate that a defect exists in the signal transduction pathway for p53
i these proteins in 2780CP cells to any significant degree. The results indicate that a defect exists in the signal transduction pathway for p53 induction following cisplatin-induced DNA damage in 2780CP cells, and this may indicate that a defect exists in the signal transduction pathway for p53
induction following cisplatin-induced DNA damage in 2780CP cells, and
this may represent a significant mechanism of cisplatin resistance. Fur-
thermo induction following cisplatin-induced DNA
this may represent a significant mechanism
thermore, induction of p53 in 2780CP cells
trongly suggests that independent pathwa
tion for the two DNA-damaging agents.

INTRODUCTION

INTRODUCTION

p53

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Ovarian cancer is diagnosed in about 20,000 patients every year and

is one of the four leading causes of cancer deaths among women in the

United States (1). Although platinum-based antitumor a INTRODUCTION

United States (1). Although platinum-based antitumor agents (i.e.,

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United States (1). Although platinum-based antitumor agents (i.e., s
 EXECUTE INTERTURNATION
Covarian cancer is diagnosed in about 20,000 patients every year and
is one of the four leading causes of cancer deaths among women in the
Clinical States (1). Although platinum-based antitumor agen Ovarian cancer is diagnosed in about 20,000 patients every year and
is one of the four leading causes of cancer deaths among women in the
United States (1). Although platinum-based antitumor agents (*i.e.*, sai
cisplatin is one of the four leading causes of cancer deaths among women in the 275 c
United States (1). Although platinum-based antitumor agents (*i.e.*, samp
cisplatin and carboplatin) play critical roles in the treatment of this United States (1). Although platinum-based antitumor agents (*i.e.*, samp
cisplatin and carboplatin) play critical roles in the treatment of this have
disease, a major impediment concerns relapse in more than 80% of
patie cisplatin and carboplatin) play critical roles in the treatment of this disease, a major impediment concerns relapse in more than 80% of patients, who fail subsequent challenge with the platinum agent due to the onset of d disease, a major impediment concerns relapse in more than 80% of repatients, who fail subsequent challenge with the platinum agent due to the onset of drug resistance in their tumor cells (1, 2). Resistance to incisplatin discussel, a major impediment concerns relative in more than 60% of patients, who fail subsequent challenge with the platinum agent due to that the onset of drug resistance in their tumor cells (1, 2). Resistance to increa the onset of drug resistance in their tumor cells (1, 2). Resistance to cisplatin is characterized in general by a number of mechanisms, which include reduced drug accumulation, increased intracellular GSH,³ and/or incre cisplatin is characterized in general by a number of mechanisms, $p53$ increases only in sensitive cells
which include reduced drug accumulation, increased intracellular model.
GSH,³ and/or increased repair of cisplatin chanisms, individually or colle

ce of the cytotoxic DNA addi

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The costs of publication of this article v

ald Thai
J. University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030
activation of apoptosis (programmed cell death) that is normally seen
in tumor cells following drug exposure (7, 8). Conversely, down**in tumoral Thai**
I. University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030
activation of apoptosis (programmed cell death) that is normally seen
in tumor cells following drug exposure (7, 8). Conversely, *I. University of Texas M. D. Anderson Cancer Center, Houston, Texas 77030*
activation of apoptosis (programmed cell death) that is normally seen
in tumor cells following drug exposure $(7, 8)$. Conversely, down-
regulati activation of apoptosis (programmed cell death) that is normally seen
in tumor cells following drug exposure $(7, 8)$. Conversely, down-
regulation of the apoptotic process itself could render cells resistant
not only to activation of apoptosis (programmed cell death) that is normally seen
in tumor cells following drug exposure (7, 8). Conversely, down-
regulation of the apoptotic process itself could render cells resistant
not only to ci

translational product of which is stabilized on DNA damage and regulation of the apoptotic process itself could render cells resistant
not only to cisplatin but also to other chemotherapeutic agents $(9-11)$.
Apoptosis is normally an orderly process controlled by a number of
regulato not only to cisplatin but also to other chemotherapeutic agents $(9-11)$.
Apoptosis is normally an orderly process controlled by a number of
regulatory genes. The tumor suppressor $p53$ is one such gene, the
translational Apoptosis is normally an orderly process controlled by a number of regulatory genes. The tumor suppressor $p53$ is one such gene, the translational product of which is stabilized on DNA damage and causes cells to arrest i regulatory genes. The tumor suppressor $p53$ is one such gene, the translational product of which is stabilized on DNA damage and causes cells to arrest in the G_1 phase of the cell cycle to limit the replication of dam translational product of which is stabilized on DNA damage and causes cells to arrest in the G_1 phase of the cell cycle to limit the replication of damaged DNA (12, 13). Increased levels of p53 protein can also trigger causes cells to arrest in the G_1 phase of the cell cycle to limit the replication of damaged DNA (12, 13). Increased levels of p53 protein can also trigger programmed cell death through transactivation of the *Bax* gen replication of damaged DNA (12, 13). Increased levels of p53 protein
can also trigger programmed cell death through transactivation of the
Bax gene (10, 11, 14, 15). This is consistent with a recent report that
reduced can also trigger programmed cell death through transactivation of the *Bax* gene (10, 11, 14, 15). This is consistent with a recent report that reduced expression of the *Bax* gene is associated with cisplatin resistance Bax gene (10, 11, 14, 15). This is consistent with a recent report that reduced expression of the Bax gene is associated with cisplatin resistance in an *in vitro* model of ovarian cancer (16). The intricate control of ce reduced expression of the *Bax* gene is associated with cisplatin resistance in an *in vitro* model of ovarian cancer (16). The intricate control of cellular processes under $p53$, however, can be severely disrupted by in that drug resistance in an *in vitro* model of ovarian cancer (16). The intricate control of cellular processes under $p53$, however, can be severely disrupted by inactivation of the p53 protein through rearrangements, de of cellular processes under $p53$, however, car
inactivation of the p53 protein through rearra
missense mutations in the $p53$ gene (17). Th
that drug resistance can develop as a resul
mediated apoptotic signals (12, 18, inactivation of the p53 protein through rearrangements, deletions, and
missense mutations in the p53 gene (17). The consequence of this is
that drug resistance can develop as a result of an absence of p53-
mediated apopto maculation of the postphodulation in the and model real and model in the massense mutations in the $p53$ gene (17). The consequence of this is that drug resistance can develop as a result of an absence of $p53$ mediated a

strongly suggests that independent pathways are involved in p53 regula-
testicular cancer (21) and childhood acute lymphoblastic leukemia
tion for the two DNA-damaging agents.
(22), are highly responsive to chemotherapeut that drug resistance can develop as a result of an absence of $p53$ -
mediated apoptotic signals (12, 18, 19).
The crucial role of $p53$ in drug-induced cytotoxicity is supported by
reports that several anticancer agents, mediated apoptotic signals (12, 18, 19).

The crucial role of $p53$ in drug-induced cytotoxicity is supported by

reports that several anticancer agents, such as 5-fluorouracil, etopo-

side, and Adriamycin, are less acti The crucial role of $p53$ in drug-induced cytotoxicity is supported by
reports that several anticancer agents, such as 5-fluorouracil, etopo-
side, and Adriamycin, are less active against tumor cells containing
mutant rat Ine crucial role or p55 in artig-induced cytotoxicity is supported by
reports that several anticancer agents, such as 5-fluorouracil, etopo-
side, and Adriamycin, are less active against tumor cells containing
mutant rath side, and Adriamycin, are less active against tumor cells containing
mutant rather than wild-type $p53$ both in vitro (10) and in vivo (11).
Similarly, cisplatin is 2- to 3-fold more effective against wild-type
 $p53$ -cont mutant rather than wild-type $p53$ both in vitro (10) and in vivo (11).
Similarly, cisplatin is 2- to 3-fold more effective against wild-type
 $p53$ -containing Burkitt's lymphoma cells than against those with
mutant $p53$ Similarly, cisplatin is 2- to 3-fold more effective against wild-type $p53$ -containing Burkitt's lymphoma cells than against those with mutant $p53$ (20). Clinically, there is ample evidence to indicate that tumors that r p53-containing Burkitt's lymphoma cells than against those with mutant p53 (20). Clinically, there is ample evidence to indicate that tumors that rarely exhibit p53 mutations at presentation, such as testicular cancer (21 mutant $p53$ (20). Clinically, there is ample evidence to indicate that
tumors that rarely exhibit $p53$ mutations at presentation, such as
testicular cancer (21) and childhood acute lymphoblastic leukemia
(22), are highl tumors that rarely exhibit $p53$ mutations at presentation, such as testicular cancer (21) and childhood acute lymphoblastic leukemia (22), are highly responsive to chemotherapeutic agents, whereas $p53$ mutation in tumor testicular cancer (21) and childhood acute lymphoblastic leukemia (22), are highly responsive to chemotherapeutic agents, whereas $p53$ mutation in tumors correlates with poor prognosis (23). Mutations in $p53$ have also 22), are highly responsive to chemotherapeutic agents, whereas $p53$ mutation in tumors correlates with poor prognosis (23). Mutations in $p53$ have also been described in ovarian cancer (24), with mutations in Fédération mutation in tumors correlates with poor prognosis (23). Mutations in $p53$ have also been described in ovarian cancer (24), with mutations in Fédération Internationale des Gynaecologistes et Obstetristes grade 1–3 cancers p53 have also been described in ovarian cancer (24), with mutations
in Fédération Internationale des Gynaecologistes et Obstetristes grade
1–3 cancers being localized in exons 5–8 between residues 135 and
275 of the DNA-b in Fédération Internationale des Gynaecologistes et Obstetristes grade 1–3 cancers being localized in exons 5–8 between residues 135 and 275 of the DNA-binding domain in approximately 50% of the clinical samples examined 1–3 cancers being localized in exons 5–8 between residues 135 and 275 of the DNA-binding domain in approximately 50% of the clinical samples examined (25). As a result of these recent developments, we have undertaken an i 275 of the DNA-binding domain in approximately 50% of the clinical
275 of the DNA-binding domain in approximately 50% of the clinical
samples examined (25). As a result of these recent developments, we
have undertaken an samples examined (25). As a result of these recent developments, we have undertaken an investigation to define the role of $p53$ in cisplatin resistance in our 2780CP human ovarian tumor cells. We demonstrate that in thes have undertaken an investigation to define the role of $p53$ in cisplatinmodel. p53 increases only in sensitive cells but not in the cisplatin-resistant

Tumor Models. The A2780 and the corresponding 2780CP ovarian tumor cells were grown under conditions described in our previous report (26). The **MATERIALS AND METHODS**
 Tumor Models. The A2780 and the corresponding 2780CP ovarian tumor

cells were grown under conditions described in our previous report (26). The

A2780 line was established from a patient's biops THEMOTES TREVIS MELTINOUS
Tumor Models. The A2780 and the corresponding 2780CP ovarian tumor
cells were grown under conditions described in our previous report (26). The
A2780 line was established from a patient's biopsy p Tumor Models. The A2780 and the corresponding 2780CP ovarian tumor
cells were grown under conditions described in our previous report (26). The
A2780 line was established from a patient's biopsy prior to initiation of any
 cells were grown under conditions described in our previous report (26). The A2780 line was established from a patient's biopsy prior to initiation of any chemotherapeutic regimen (27). The cisplatin-resistant A2780/C30 ce A2780 line was established from a patient's biopsy prior to initiation of any chemotherapeutic regimen (27). The cisplatin-resistant A2780/C30 cells (27, 28) were used to derive the subcloned 2780CP line by growing A2780/C chemotherapeutic regimen (27). The cisplati
28) were used to derive the subcloned 2780
cells in cisplatin-free medium until the resis
A2780/C30 models were both provided kind
Chase Cancer Center, Philadelphia, PA).
Cytotox were used to derive the subcloned 2780CP line by growing A2780/C30 ls in cisplatin-free medium until the resistance stabilized. The A2780 and 780/C30 models were both provided kindly by Dr. Thomas Hamilton (Fox ase Cancer cells in cisplatin-free medium until the resistance stabilized. The A2780 and
A2780/C30 models were both provided kindly by Dr. Thomas Hamilton (Fox
Chase Cancer Center, Philadelphia, PA).
Cytotoxicity and Biochemical Phar

A2780/C30 models were both provided kindly by Dr. Thomas Hamilton (Fox
Chase Cancer Center, Philadelphia, PA).
Cytotoxicity and Biochemical Pharmacology Studies. Cells (A2780, 400
cells/well; 2780CP, 3000 cells/well) were Chase Cancer Center, Philadelphia, PA).
Cytotoxicity and Biochemical Pharmacology Studies. Cells (A2780, 400
cells/well; 2780CP, 3000 cells/well) were aliquoted in 96-well plates and
allowed to attach and grow for 2 days b Cytotoxicity and Biochemical Pharmacology Studies. Cells (A2780, 400 cells/well; 2780CP, 3000 cells/well) were aliquoted in 96-well plates and allowed to attach and grow for 2 days before being exposed to cisplatin (Sigma

The costs of the cytotoxic DNA adducts of cisplatin and may prevent

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^{2.} H. S.) and NIH Grant CA38929 (to G. L.). Presented in preliminary form at the Annual Meeting of the American Association for Cancer Research, Washington April 20–24, 1996.
April 20–24, 1996.
² To whom requests for rep nual Meeting of the American Association for Cancer Research, Washington, DC,

il 20–24, 1996.

² To whom requests for reprints should be addressed, at Department of Clinical

estigation, University of Texas M. D. Anders

April 20–24, 1996.

² To whom requests for reprints should be addressed, at Department of Clinical

Investigation, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe

Boulevard, Houston, TX 77030. Phone: (7 absorbance in MTT cytotoxicity assay to 50% of control; Anderson Cancer Center, 1515 Holcombe
Boulevard, Houston, TX 77030. Phone: (713) 792-7746; Fax: (713) 792-6759.
³ The abbreviations used are: GSH, glutathione; MTT, *niversity of Texas M. D. Anderson Cancer Center,* Boulevard, Houston, TX 77030. Phone: (713) 792-7746; Fax: (713) 79
³ The abbreviations used are: GSH, glutathione; MTT, 3-(4,5-dimen-
2,5-diphenyltetrazolium bromide; IC

DIFFERENTIAL p53 INDUCTION B
reported previously (26). Sensitivity to X-rays was determined in a similar p
manner. Briefly, cells (1,200 A2780 cells/well or 36,000 2780CP cells/well) DIFFERENTIAL p53 INDUCTION B
reported previously (26). Sensitivity to X-rays was determined in a similar
manner. Briefly, cells (1,200 A2780 cells/well or 36,000 2780CP cells/well)
were aliquoted in 24-well plates, incubat DIFFERENTIAL p53 INDUCTION BY C
reported previously (26). Sensitivity to X-rays was determined in a similar posti
manner. Briefly, cells (1,200 A2780 cells/well or 36,000 2780CP cells/well) was
were aliquoted in 24-well pl reported previously (26). Sensitivity to X-rays was determined in a similar postincul
manner. Briefly, cells (1,200 A2780 cells/well or 36,000 2780CP cells/well) washed v
were aliquoted in 24-well plates, incubated for 2 reported previously (26). Sensitivity to X-rays was determined in a similar manner. Briefly, cells (1,200 A2780 cells/well or 36,000 2780CP cells/well) were aliquoted in 24-well plates, incubated for 2 days, and then irra Ils receiving negligible amount $(<2\%)$ of the dose. Cytotoxicity was as-
sed 5 days later by the MTT assay. Evaluations in attached cells of cellular p53)
platin uptake, DNA adduct formation, DNA adduct repair, and endoge sessed 5 days later by the MTT assay. Evaluations in attached cells of cellular p5
cisplatin uptake, DNA adduct formation, DNA adduct repair, and endogenous 10
GSH levels were conducted as detailed in our previous reports

Determination of p53 Status by SSCP and DNA Sequencing. Exponencisplatin uptake, DNA adduct formation, DNA adduct repair, and endogenous 10% GSH levels were conducted as detailed in our previous reports (26, 29). ports
Determination of $p53$ Status by SSCP and DNA Sequencing. Exponen GSH levels were conducted as detailed in our previous reports (26, 29). ports
Determination of $p53$ Status by SSCP and DNA Sequencing. Exponentially growing cells were used to isolate DNA by the standard phenol-chloro-
f **Determination of p53 Status by SSCP and DNA Sequencing.** Exponentially growing cells were used to isolate DNA by the standard phenol-chloro-
form extraction procedure, as reported previously (30). Exons 4-9 of p53 in mem tially growing cells were used to isolate DNA by the standard phenol-chloro-
form extraction procedure, as reported previously (30). Exons 4–9 of $p53$ in mem
the DNA were amplified by PCR and were examined electrophoreti the DNA were amplified by PCR and were examined electrophoretically by p53;
SSCP analysis, essentially as reported (31). The 15- μ I reactions contained 0.1 inger
 μ g of genomic DNA; 10 pmol of primer; 0.2 mM dATP, dGT SSCP analysis, essentially as reported (31). The 15- μ l reactions contained C
 μ g of genomic DNA; 10 pmol of primer; 0.2 mM dATP, dGTP, dTTP, a
[³²P]dCTP; 0.1 unit of Amplitaq DNA polymerase (Perkin-Elmer Corp.); a
1 μg of genomic DNA; 10 pmol of primer; 0.2 mM dATP, dGTP, dTTP, and [³²P]dCTP; 0.1 unit of Amplitaq DNA polymerase (Perkin-Elmer Corp.); and 1.5 μl of PCR buffer (Perkin-Elmer). The primers used were: exon 5, GE5F (TGCCG [³²P]dCTP; 0.1 unit of Amplitaq DNA polymerase (Perkin-Elmer Corp.); and 1.5 µl of PCR buffer (Perkin-Elmer). The primers used were: exon 5, GE5F
(TGCCGTGTTCCAGTTGCTTT, forward) and GE5R (TCCAAATACTC-CACACGCAA, reverse); 1.5 µl of PCR buffer (Perkin-Elmer). The primers used were: exon 5, GE5 (TGCCGTGTTCCAGTTGCTTT, forward) and GESR (TCCAAATACTCCACACGCAA, reverse); exon 6, GE6F (CAGATAGCGATGGTGAGCAC forward) and GE6R (GCCACTGACAACCACCCTTA, (TGCCGTGTTCCAGTTGCTTT, forward) and GESR (TCCAAATACI
CACACGCAA, reverse); exon 6, GE6F (CAGATAGCGATGGTGAGCA
forward) and GE6R (GCCACTGACAACCACCCTTA, reverse); exon
GE7F (TGCCACAGGTCTCCCCAAGG, forward) and WG7R (AGGGC
CAGCG CACACGCAA, reverse); exon 6, GE6F (CAGATAGCGATGGTGAGCAG, RES
forward) and GE6R (GCCACTGACAACCACCCTTA, reverse); exon 7,
GE7F (TGCCACAGGTCTCCCCAAGG, forward) and WG7R (AGGGGT-
CAGCGGCAAGCAGA, reverse); exon 8, GE8F (ACCTGAT forward) and GE6R (GCCACTGACAACCACCCTTA, reverse); exon 7,
GE7F (TGCCACAGGTCTCCCCAAGG, forward) and WG7R (AGGGGT-
CAGCGGCAAGCAGA, reverse); exon 8, GE8F (ACCTGATTTCCTTACT-
GCCT, forward) and GE8R (GAGGCAAGGAAAGGTGATAA, rev CAGCGGCAAGCAGA, reverse); exon 8, GE8F (ACCTGATTTCCTTACT-
GCCT, forward) and GE8R (GAGGCAAGGAAAGGTGATAA, reverse); and
exon 9, GE9F (GTAAGCAAGCAGGACAAGAA, forward) and GE9R
(ACGGCATTTTGAGTGTTAGA, reverse). The reaction wa $GCCT$, forward) and GE8R (GAGGCAAGGAAAGGTGATAA, reverse); and varies on 9, GE9F (GTAAGCAAGCAGGAAAGGTGATAA, reverse); and varies and carrier at 72°C for 5 min. An aliquot of the PCR reaction was diluted with an equal volum exon 9, GE9F (GTAAGCAAGCAGGACAAGAA, forward) and GE9R (ACGGCATTTTGAGTGTTAGA, reverse). The reaction was heated at 94°C for 5 min, followed by 35 cycles at 94, 62, and 72°C for 1 min each, with a final extension at 72°C fo exon 9, GE9F (GTAAGCAAGCAGGACAAGAA, forward) and GE9R

(ACGGCATTTTGAGTGTTAGA, reverse). The reaction was heated at 94°C

for 5 min, followed by 35 cycles at 94, 62, and 72°C for 1 min each, with a

final extension at 72°C for 5 min, followed by 35 cycles at 94, 62, and 72°C for 1 min each, with a final extension at 72°C for 5 min. An aliquot of the PCR reaction was diluted with an equal volume of formamide, with 20 mM EDTA and 0.05% bromph final extension at 72°C for 5 min. An aliquot of the PCR reaction was diluted with an equal volume of formamide, with 20 mM EDTA and 0.05% bromphend blue; denatured at 95°C for 3 min; rapidly cooled on ice; and loaded on with an equal volume of formamide, with 20 mM EDTA and 0.05% bromphe-
nol blue; denatured at 95°C for 3 min; rapidly cooled on ice; and loaded on
0.5× mutation detection enhancement gel (FMC Bioproducts, Rockland, ME).
Ge nol blue; denatured at 95°C for 3 min; rapidly cooled on ice; and loaded on 0.5× mutation detection enhancement gel (FMC Bioproducts, Rockland, ME).
Gels were prepared in Tris-borate-EDTA, as reported by Orita *et al.* (3 Gels were prepared in Tris-Isamples were electrophores
dried and visualized by aute
eluted from the gel in water
directly or after cloning.
Determination of p53 S

dried and visualized by autoradiography. The SSCP variants were identified, 3-fold cieluted from the gel in water at 55°C for 2 h, amplified by PCR, and sequenced were als directly or after cloning. ing a 2.
Determination eluted from the gel in water at 55°C for 2 h, amplified by PCR, and sequenced
directly or after cloning.
Determination of p53 Status by a Yeast Functional Assay. The assay
was performed essentially as described (32, 33). directly or after cloning. In the Determination of p53 Status by a Yeast Functional Assay. The assay accuse access, NY) and consisted (32, 33). The mRNA was isolated from less exponentially growing cells using the Dynabead **Determination of p53 Status by a Yeast Functional Assay.** The assay
was performed essentially as described (32, 33). The mRNA was isolated from
exponentially growing cells using the Dynabeads mRNA Direct Kit (Dynal,
Lake was performed essentially as described (32, 33). The mRNA was isolated from less D
exponentially growing cells using the Dynabeads mRNA Direct Kit (Dynal, 1). Re
Lake Success, NY), and cDNA prepared using RT-1 (CGGGAGGTAG exponentially growing cells using the Dynabeads mRNA Direct Kit (Dynal, 1).
Lake Success, NY), and cDNA prepared using RT-1 (CGGGAGGTAGAC) as
the primer for reverse transcription and a First Strand cDNA Synthesis Kit
(Pha Lake Success, NY), and cDNA prepared using RT-1 (CGGGAGGTAGAC) as
the primer for reverse transcription and a First Strand cDNA Synthesis Kit
the primer for reverse transcription and a First Strand cDNA Synthesis Kit
(Pharm the primer for reverse transcription and a First Strand cDNA Synthesis Kit (Pharmacia Biotech, Uppsala, Sweden). The $p53$ cDNA was amplified by PCR using P3 and P4 primers (33), and the products were mixed with the pRDI-2 (Pharmacia Biotech, Uppsala, Sweden). The $p53$ cDNA was amplified by PCR
using P3 and P4 primers (33), and the products were mixed with the pRDI-22
gapped $p53$ expression vector (provided generously by Dr. Richard Iggo, exampled $p53$ expression vector (provided generously by Dr. Richard Iggo, Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland). The mixture was used to transfect a *Saccharomyces cerevisiae* strain y Swiss Institute for Experimental Cancer Research, Epalinges, Switzerland).
The mixture was used to transfect a *Saccharomyces cerevisiae* strain ylG397
that contained a *p53*-responsive promoter 5' of the adenine gene. Aft The mixture was used to transfect a *Saccharomyces cerevisiae* strain ylG397 that contained a $p53$ -responsive promoter 5' of the adenine gene. After 48–72 h at 35° C, the plates were scored for white, pink, or red co that contained a $p53$ -responsive promoter 5' of the adenine gene. After $48-72$ h at 35° C, the plates were scored for white, pink, or red colonies. As controls, EBV-transformed lymphoblastoid cell lines from patient in a 35°C, the plates were scored for white, pink, or red colonies. As controls, \overline{EBV} -transformed lymphoblastoid cell lines from patients with Li-Fraumeni syndrome were used: the A1-000 line contained a temperature-s EBV-transformed lymphoblastoid cell lines from patients with Li-Fraumeni syndrome were used: the A1-000 line contained a temperature-sensitive heterozygous mutation at codon 133 of $p53$ (34), A14-000 expressed an inactiva syndrome were used: the A1-000 line contained a temperature-sensitive het-
erozygous mutation at codon 133 of $p53$ (34), A14-000 expressed an inacti-
vating heterozygous mutation at codon 282 (35), and A15-010 was wild t

erozygous mutation at codon 133 of $p53$ (34), A14-000 expressed an inactivating heterozygous mutation at codon 282 (35), and A15-010 was wild type.⁴
Evaluation of Cell Cycle Arrest. Attached cells in an exponential-g vating heterozygous mutation at codon 282 (35), and A15-010 was wild type.⁴
Evaluation of Cell Cycle Arrest. Attached cells in an exponential-growth
phase in 100-mm tissue culture plates were exposed to X-rays (12.6 G phase in 100-mm tissue culture plates were exposed to X-rays (12.6 Gy) as
described above and postincubated at 37°C with or without the mitotic inhib-
itor nocodazole (0.4 μ g/ml) to prevent cells from recycling (24, 36 itor nocodazole (0.4 μ g/ml) to prevent cells from recycling (24, 36). After 24 h, cells (1 × 10⁶) were washed with ice-cold PBS, resuspended in 1 ml of cold PBS, fixed by dropwise addition of 2.6 ml of absolute ethan 24 h, cells (1×10^6) were washed with ice-cold PBS, resuspended in 1 ml of cold PBS, fixed by dropwise addition of 2.6 ml of absolute ethanol, and left at -20°C overnight. The fixed cells were then centrifuged cold PBS, fixed by dropwise addition of 2.6 ml of absolute ethanol, and left at -20°C overnight. The fixed cells were then centrifuged, rinsed in PBS, and resuspended in 1 ml of 0.04% pepsin (Sigma) in 0.01 N HCl. -20°C overnight. The fixed cells were then centrifuged, rinsed in PBS, and
resuspended in 1 ml of 0.04% pepsin (Sigma) in 0.01 N HCl. After 20 min at
room temperature, the cells were pelleted by centrifugation and washed For 2-3 h at 37°C in the dark, and analyzed on a Becton Dickinson flow
expansion of propidium in the data of DNA adducts
for 2-3 h at 37°C in the dark, and analyzed on a Becton Dickinson flow
expansion of propidium in the cytometer. S. Cells were finally resuspended in a solution of propidium iodide (10 /ml) containing 200 units/ml RNase A (Sigma) and 0.1% NP40, incubated $-2-3$ h at 37°C in the dark, and analyzed on a Becton Dickinson flow ometer.
W

Example 1989 Western Analysis. Cells were exposed to cisplatin $(1-20 \mu M)$ for 2 h at can component of the transference is defined as IC_{50} versus 2780CP/IC₅₀ versus A2780.
37°C) or X-rays (3.1, 6.3, or 12.6 Gy in a Western Analysis. Cells were

C) or X-rays (3.1, 6.3, or

4 G. Lozano, unpublished data.

manner. Briefly, cells (1,200 A2780 cells/well or 36,000 2780CP cells/well) washed with PBS and lysed for 10 min on ice with 1 ml of lysis buffer [50 mm were aliquoted in 24-well plates, incubated for 2 days, and then irr Gy/min) from a Philips RT 250 kV orthovoltage X-ray generator. A 3-mm 0.5% sodium deoxycholate, 100 μ g/ml phenylmethylsulfonyl fluoride, and 1 lead-shielding jig allowed four wells to be irradiated at a time, with adja lead-shielding jig allowed four wells to be irradiated at a time, with adjacent μ g/ml aprotinin]. The lysates were collected by microcentrifugation at 4°C, wells receiving negligible amount (<2%) of the dose. Cytotoxic BY CISPLATIN AND X-RAYS
postincubated at 37°C in drug-free medium for 4–72 h. The cells were then
washed with PBS and lysed for 10 min on ice with 1 ml of lysis buffer [50 mM BY CISPLATIN AND X-RAYS
postincubated at 37°C in drug-free medium for 4–72 h. The cells were then
washed with PBS and lysed for 10 min on ice with 1 ml of lysis buffer [50 mM
Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium az BY CISPLATIN AND X-RAYS
postincubated at 37°C in drug-free medium for 4–72 h. The cells were then
washed with PBS and lysed for 10 min on ice with 1 ml of lysis buffer [50 mm
Tris-HCl (pH 8.0), 150 mm NaCl, 0.02% sodium az postincubated at 37°C in drug-free medium for 4–72 h. The cells were then washed with PBS and lysed for 10 min on ice with 1 ml of lysis buffer [50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 1% NP40, 0.1% SDS, p53) or 40 (for p21^{Waf1/Cip1}) μ g of total cell protein was electrophoresed on a Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 1% NP40, 0.1% SDS,
Tris-HCl (pH 8.0), 150 mM NaCl, 0.02% sodium azide, 1% NP40, 0.1% SDS,
0.5% sodium deoxycholate, 100 μ g/ml phenylmethylsulfonyl fluoride, and 1
 μ 10.5% sodium deoxycholate, 100 μ g/ml phenylmethylsulfonyl fluoride, and 1
 μ g/ml aprotinin]. The lysates were collected by microcentrifugation at 4°C,

and the protein was determined by the standard Lowry procedure. μ g/ml aprotinin]. The lysates were collected by microcentrifugation at 4°C, and the protein was determined by the standard Lowry procedure. Five (for p53) or 40 (for p21^{Waf1/Cip1}) μ g of total cell protein was elec and the protein was determined by the standard Lowry procedure. Five (for p53) or 40 (for p21^{Waf1/Cip1}) μ g of total cell protein was electrophoresed on a 10% (p53) or 15% (p21^{Waf1/Cip1}) SDS-polyacrylamide gel, blot p53) or 40 (for p21^{Waf1/Cip1}) μ g of total cell protein was electrophoresed on a 10% (p53) or 15% (p21^{Waf1/Cip1}) SDS-polyacrylamide gel, blotted on a supported nitrocellulose 0.2 μ m membrane (Bio-Rad, Hercules, C 10% (p53) or 15% (p21^{wari/Cip1}) SDS-polyacrylamide gel, blotted on a supported nitrocellulose 0.2 μ m membrane (Bio-Rad, Hercules, CA), and blocked overnight in TBS-20 buffer [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, and overnight in TBS-20 buffer [10 mm Tris-HCl (pH 8.0), 150 mm NaCl, and 0.05% Tween 20] containing 3% nonfat milk powder and 0.2% BSA. The membranes were probed for 2 h with either the DO-1 (for mutant and wild-type p53; Onc 0.05% Tween 20] containing 3% nonfat milk powder and 0.2% BSA. The membranes were probed for 2 h with either the DO-1 (for mutant and wild-type p53; Oncogene Science, Cambridge, MA) or the sdi1 (for $p21^{Waf1/Cip1}$; PharMin membranes were probed for 2 h with either the DO-1 (for mutant and wild-type
p53; Oncogene Science, Cambridge, MA) or the sdi1 (for p21^{Waf1/Cip1}; PharM-
ingen, San Diego, CA) antibody. The antibody reaction was visualize ingen, San Diego, CA) antibody. The antibody reaction was visualized by the Amersham chemiluminescence procedure using a sheep antimouse horseradish peroxidase as the second antibody (Amersham Corp., Arlington Heights, IL)

RESULTS

samples were electrophoresed for 18 h at 4.5 W of constant power. Gels were and 51.4 for continuous exposure. In contrast, 2780CP cells were only dried and visualized by autoradiography. The SSCP variants were identified, **Cytotoxicity and Biochemical Pharmacology of Cisplatin in**
Cytotoxicity and Biochemical Pharmacology of Cisplatin in
Cytotoxicity and Biochemical Pharmacology of Cisplatin in
Cytotoxicity and Biochemical Pharmacology RESULTS
 Cytotoxicity and Biochemical Pharmacology of Cisplatin in
 Ovarian Tumor Models. Differences between parental A2780 and

variant 2780CP cells were readily apparent at the level of growth time RESULTS
Cytotoxicity and Biochemical Pharmacology of Cisplatin in
Ovariant 2780CP cells were readily apparent at the level of growth time
and GSH content: the variant 2780CP line displayed a 37% longer cell **Cytotoxicity and Biochemical Pharmacology of Cisplatin in**
 Ovarian Tumor Models. Differences between parental A2780 and

variant 2780CP cells were readily apparent at the level of growth time

and GSH content: the vari Cytotoxicity and Biochemical Pharmacology of Cisplatin in
Ovarian Tumor Models. Differences between parental A2780 and
variant 2780CP cells were readily apparent at the level of growth time
and GSH content: the variant 278 **Ovarian Tumor Models.** Differences between parental A2780 and variant 2780CP cells were readily apparent at the level of growth time and GSH content: the variant 2780CP line displayed a 37% longer cell doubling time and Wariant 2780CP cells were readily apparent at the level of growth time
and GSH content: the variant 2780CP line displayed a 37% longer cell
doubling time and a more than 2-fold greater intracellular GSH
concentration (Tab and GSH content: the variant 2780CP line displayed a 37% longer cell
doubling time and a more than 2-fold greater intracellular GSH
concentration (Table 1). From an antitumor viewpoint, A2780 cells
were highly sensitive t doubling time and a more than 2-fold greater intracellular GSH concentration (Table 1). From an antitumor viewpoint, A2780 cells were highly sensitive to both X-rays (IC₅₀, 1.5 Gy) and cisplatin (IC₅₀, 0.22 or 3.9 μ concentration (Table 1). From an antitumor viewpoint, A2780 cells
were highly sensitive to both X-rays (IC₅₀, 1.5 Gy) and cisplatin
(IC₅₀, 0.22 or 3.9 μ M for continuous or 2-h drug exposure, respec-
tively). In com were highly sensitive to both X-rays $(IC_{50}$, 1.5 Gy) and cisplatin $(IC_{50}$, 0.22 or 3.9 μ M for continuous or 2-h drug exposure, respectively). In comparison, 2780CP cells were substantially less sensitive to cisplat (IC_{50} , 0.22 or 3.9 μ M for continuous or 2-h drug exposure, respectively). In comparison, 2780CP cells were substantially less sensitive to cisplatin, resulting in resistance factors of 28.6 for a 2-h exposure and 51 tively). In comparison, 2780CP cells were substantially less sensitive to cisplatin, resulting in resistance factors of 28.6 for a 2-h exposure and 51.4 for continuous exposure. In contrast, 2780CP cells were only 3-fold intery). In comparison, 2700Cr cents were substantiantly less sensitive
to cisplatin, resulting in resistance factors of 28.6 for a 2-h exposure
and 51.4 for continuous exposure. In contrast, 2780CP cells were only
3-fold and 51.4 for continuous exposure. In contrast, 2780CP cells were only 3-fold cross-resistant to X-rays. Differences between the two cell lines were also observed at the biochemical pharmacological level. Following a 2-h 1 3-fold cross-resistant to X-rays. Differences between the two cell lines
were also observed at the biochemical pharmacological level. Follow-
ing a 2-h 100 μ M cisplatin exposure, the 2780CP tumor cell line
accumulated Note that class-resistant to A-tays. Directness octive that two certains were also observed at the biochemical pharmacological level. Following a 2-h 100 μM cisplatin exposure, the 2780CP tumor cell line accumulated about ing a 2-h 100 μ M cisplatin exposure, the 2780CP tumor cell line
accumulated about 60% less cisplatin and formed approximately 50%
less DNA adducts compared to levels found in sensitive cells (Table
1). Repair of DNA add accumulated about 60% less cisplatin and formed approximately 50% less DNA adducts compared to levels found in sensitive cells (Table 1). Repair of DNA adducts, on the other hand, was 2-fold greater in 2780CP cells compare less DNA adducts compared to levels found in sensitive cells (Table 1). Repair of DNA adducts, on the other hand, was 2-fold greater in 2780CP cells compared to parental A2780 cells. From these data, it was possible to cal Sossible to calculate AUC (adduct *versus* time curve) as a state of exposure of cells to the cytotoxic DNA lesion. The AU Table 1 Cytotoxicity and biochemical pharmacology of cisplatin against ovarian tumor cells^a

	A2780	2780CP
Doubling time (h)	19.0	26.0
Glutathione (nmol/10 ⁶ cells)	7.6	17.0
X-rays $IC_{50} (Gy)^b$	1.5	5.0
Radiation resistance factor ^c	1.0	3.3
Cisplatin $IC_{50} (\mu M)^b$		
Continuous exposure	0.22	11.3
2-h exposure	3.9	112
Cisplatin resistance factor c		
Continuous exposure	1.0	51.4
2-h exposure	1.0	28.6
2-h drug uptake $(100 \mu \text{m} \text{ cisplatin})$ (ng of platinum/mg protein)	83.0	30.6
DNA adducts at 2 h (100 μ M cisplatin) (ng of platinum/mg DNA)	48.5	23.9
% DNA repair in 8 h	7.8	15.5
AUC of DNA adducts	421	200
(ng of platinum \times h/mg DNA) ^a		
DNA damage tolerance (ng of platinum \times h/mg DNA) ^e	16.4	224

Let a are presented as means of two or three independent experiments.

Data are presented as means of two or three independent experiments.

IC_{SO} were determined 5 days after exposure to X-rays or cisplatin (5-day conti bala are presenced as ineass of the log were determined 5 days after
or 2-h pulse exposure.
CResistance factor is defined as IC
d AUC was determined by the trap
drug exposure to 8 h postexposure.
DNA damage tolerance is de ^P IC₅₀ were determined 5 days after exposure to X-rays or cisplatin (5-day continuous
2-h pulse exposure).
^C Resistance factor is defined as IC₅₀ versus 2780CP/IC₅₀ versus A2780.
 d^2 AUC was determined by the

or 2-n pulse exposure).
 G_{AGU} cas determined by the trapezoidal rule from
 G_{AUC} as determined by the trapezoidal rule from
 G_{HUC} exposure to 8 h postexposure.
 G_{SO} of cisplatin obtained using 2-h d

DIFFERENTIAL $p53$ INDUCTION BY CIS
for these lesions in A2780 cells was approximately 2-fold greater than Tabi
that found in the 2780CP line. At the IC₅₀ cisplatin concentration, the DIFFERENTIAL *p53* INDUCTION BY C
for these lesions in A2780 cells was approximately 2-fold greater than
that found in the 2780CP line. At the IC₅₀ cisplatin concentration, the
AUC provides an indication of DNA damage t DIFFERENTIAL $_{p53}$ INDUCTION BY (
for these lesions in A2780 cells was approximately 2-fold greater than
that found in the 2780CP line. At the IC₅₀ cisplatin concentration, the
AUC provides an indication of DNA damage between the selections in A2780 cells was approximately 2-fold greater than
that found in the 2780CP line. At the IC_{50} cisplatin concentration, the
AUC provides an indication of DNA damage tolerance, which is
defined a for these lesions in A2780 cells was approximately 2-fold greater than
that found in the 2780CP line. At the IC_{50} cisplatin concentration, the
AUC provides an indication of DNA damage tolerance, which is
defined as a l that found in the 2780CP line. At the IC₅₀ cisplatin concentration, the AUC provides an indication of DNA damage tolerance, which is defined as a level of exposure to DNA lesions that will kill 50% of exposed cells (37) AUC provides an indication of DNA damage tolerance, which is
defined as a level of exposure to DNA lesions that will kill 50% of
exposed cells (37). The results indicate that 2780CP cells can tolerate
almost a 14 times gr defined as a level of exposure to DNA lesions that will kill 50% of
exposed cells (37). The results indicate that 2780CP cells can tolerate
almost a 14 times greater level of exposure to DNA lesions than can
parental cells exposed cells (37). The results indicate that 2780CP cells can tolerate almost a 14 times greater level of exposure to DNA lesions than can parental cells (Table 1). Thus, the mechanism of resistance of 2780CP cells to cis almost a 14 times greater level of exposure to DNA lesions than parental cells (Table 1). Thus, the mechanism of resistance of 2780 cells to cisplatin is multifactorial, which includes increased Glevels, reduced drug accum **SECP and DNA Sequencing Analysis for p53 Status. The correlation of the correlation**, which includes increased GSH creased DNA repair, and increased DNA adduct formation, positions are correlated DNA Sequencing Analysis cells to cisplatin is multifactorial, which includes increased GSH allevels, reduced drug accumulation, reduced DNA adduct formation, increased DNA repair, and increased DNA damage tolerance.
SSCP and DNA Sequencing Analy

levels, reduced drug accumulation, reduced DNA adduct formation,
increased DNA repair, and increased DNA damage tolerance.
SSCP and DNA Sequencing Analysis for p53 Status. The cor-
relation between p53 mutation in tumors increased DNA repair, and increased DNA damage tolerance.

SSCP and DNA Sequencing Analysis for $p53$ Status. The correlation between $p53$ mutation in tumors and poor prognosis is well

documented (23). To determine whet **SSCP and DNA Sequencing Analysis for** $p53$ **Status.** The correlation between $p53$ mutation in tumors and poor prognosis is well documented (23). To determine whether resistance of 2780CP cells to cisplatin was associated relation between $p53$ mutation in tumors and poor prognosis is well
documented (23). To determine whether resistance of 2780CP cells to
cisplatin was associated with a mutant $p53$ status, exons 5–9 of the
 $p53$ gene wer documented (23). To determine whether resistance of 2780CP cells to
cisplatin was associated with a mutant $p53$ status, exons 5–9 of the
 $p53$ gene were examined by SSCP analysis. The DNA from 2780CP
cells did indeed sho cisplatin was associated with a mutant $p53$ status, exons 5–9 of the $p53$ gene were examined by SSCP analysis. The DNA from 2780CP cells did indeed show an exon 5 SSCP variant. Sequencing of the PCR product from the exo p53 gene were examined by SSCP analysis. The DNA from 2780CP p53 gene were examined by SSCP analysis. The DNA from 2780CP product from the exon 5 SSCP variant. Sequencing of the PCR product from the exon 5 variant indicat cells did indeed show an exon 5 SSCP variant. Sequencing of the PCR
product from the exon 5 variant indicated a transverse mutation in
codon 172 (GTT to TTT), which results in substitution of valine with
phenylalanine in No SSCP variants were identified in DNA from the A2780 cell line. **Effect of X-Rays on Cell Cycle Kinetics.** Mutation in the p53 metallion is present in 2780CP cells.
 Effect of X-Rays on Cell Cycle Kinetics. Mutation in the p53

The may lead to a loss in protein function and thereby

phenylalanine in the p53 protein (data not shown). These data demonstrate unequivocally that a $p53$ mutation is present in 2780CP cells.
No SSCP variants were identified in DNA from the A2780 cell line.
Effect of X-Rays constrate unequivocally that a $p53$ mutation is present in 2780CP cells.
No SSCP variants were identified in DNA from the A2780 cell line.
Effect of X-Rays on Cell Cycle Kinetics. Mutation in the $p53$
gene may lead to SIGNS CP variants were identified in DNA from the A2780 cell line.
 Effect of X-Rays on Cell Cycle Kinetics. Mutation in the $p53$

gene may lead to a loss in protein function and thereby contribute to

cisplatin resist **Effect of X-Rays on Cell Cycle Kinetics.** Mutation in the $p53$
gene may lead to a loss in protein function and thereby contribute to
cisplatin resistance in 2780CP cells. Thus, p53 function was investi-
gated using the gene may lead to a loss in protein function and thereby contribute to
cisplatin resistance in 2780CP cells. Thus, p53 function was investi-
gated using the ability of X-rays to activate the G_1 checkpoint in
p53-profici extract that are in 2780CP cells. Thus, p53 function was investigated using the ability of X-rays to activate the G_1 checkpoint in p53-proficient cells only (12, 13). Exponentially growing cells were Fig. 2. C exposed gated using the ability of X-rays to activate the G_1 checkpoint in
p53-proficient cells only (12, 13). Exponentially growing cells were
exposed to 12.6 Gy and incubated for 24 h with nocodazole, a mitotic 2780
inhi p53-proficient cells only (12, 13). Exponentially growing cells were exposed to 12.6 Gy and incubated for 24 h with nocodazole, a mitotic inhibitor that arrests cells in the G_2 -M phase and prevents them from contributi exposed to 12.6 Gy and incubated for 24 h with nocodazole, a mitotic $\frac{2780C}{0 (Lam)}$
inhibitor that arrests cells in the G₂-M phase and prevents them from and the
contributing to the G₁ population. The flow cytometry inhibitor that arrests cells in the G_2 -M phase and prevents them from
contributing to the G_1 population. The flow cytometry data demon-
strate that untreated A2780 and 2780CP control cells had similar cell
cycle dis exposure of A2780 or 2780CP cells to X-rays, followed by a 24-h
protein and the contributing to the G_1 population. The flow cytometry data demon-
strate that untreated A2780 and 2780CP control cells had similar cell
cy strate that untreated A2780 and 2780CP control cells had similar cell
cycle distribution profiles (Fig. 1; Table 2). As expected, exposure to
nocodazole alone caused cells to arrest in the G_2 -M phase. In contrast,
expo

Fig. 1. Arrest of ovarian tumor cells in G_1 by X-rays. Cells in exponential growth phase
were exposed to 12.6 Gy and then incubated for 24 h in the presence of the mitotic
inhibitor nocodazole (0.4 μ g/ml), which pre Fig. 1. Arrest of ovarian tumor cells in G_1 by X-rays. Cells in exponential growth phase muttan
were exposed to 12.6 Gy and then incubated for 24 h in the presence of the mitotic tant p.
inhibitor nocodazole (0.4 μ g cytometry.

Nocodazole
 $X-rays + nocodazole$

² Attached cells in an exponential-growth phase w

postincubated at 37°C with or without the mitotic inh

24 h, the cells were analyzed by flow cytometry.

A2780 Fig. 2. Coordinate increases in p53 and p21^{Waf1/Cip1} by X-rays in the A2780 and 2780CP ovarian tumor models. Cells in exponential growth phase were exposed to either 0 (*Lanes 1* and 5), 3.1 (*Lanes 2* and 6), 6 0 (*Lanes 1* and 5), 3.1 (*Lanes 2* and 6), 6.3 (*Lanes 3* and 7), or 12.6 Gy (*Lanes 4* and 8) and then reincubated. After 4 h, cells were harvested, and protein was extracted and examined for p53 and p21^{WarI/Cip1} leve

and then reincubated. After 4 h, cells were harvested, and protein was extracted and examined for p53 and $p21^{Wat1/Cip1}$ levels by the Western technique.
arrest, with a concomitant decrease in numbers of cells in the S pha arrest, with a concomitant decrease in numbers of cells in the S phase.
According to these data, both sensitive and resistant cells have wild-
type p53 function.
To determine whether the activation of the G_1 checkpoint

arrest, with a concomitant decrease in numbers of cells in the S phase.
According to these data, both sensitive and resistant cells have wild-
type p53 function.
To determine whether the activation of the G_1 checkpoint According to these data, both sensitive and resistant cells have wild-
type p53 function.
To determine whether the activation of the G_1 checkpoint was
mediated through p53 and the p53-regulated $p21^{Waf/Cip1}$ gene, A2780 According to these data, both sensitive and resistant cents have wild
type p53 function.
To determine whether the activation of the G_1 checkpoint was
mediated through p53 and the p53-regulated $p21^{Waf1/Cip1}$ gene, A2780 To determine whether the activation of the G_1 checkpoint was
mediated through p53 and the p53-regulated $p21^{WafI/CipI}$ gene, A2780
and 2780CP cells were exposed to X-rays (3.1, 6.3, or 12.6 Gy), and
levels of p53 and p2 To determine whether the activation of the G_1 checkpoint was
mediated through p53 and the p53-regulated $p21^{Waf1/Cip1}$ gene, A2780
and 2780CP cells were exposed to X-rays (3.1, 6.3, or 12.6 Gy), and
levels of p53 and p2 and 2780CP cells were exposed to X-rays (3.1, 6.3, or 12.6 Gy), and
levels of p53 and p21^{Waf1/Cip1} were examined 4 h later. The immu-
noblots, shown in Fig. 2, indicate that X-rays induced p53 in both
tumor models. Leve and 2780CP cens were exposed to X-rays (3.1, 0.3, or 12.0 Gy), and
levels of p53 and p21^{Waf1/Cip1} were examined 4 h later. The immu-
noblots, shown in Fig. 2, indicate that X-rays induced p53 in both
tumor models. Level noblots, shown in Fig. 2, indice that X-rays induced p53 in both tumor models. Levels of p21^{Waf1/Cip1} were also increased in both cell
lines by X-rays in a dose-dependent manner. These results demon-
strate that X-rays monois, shown in Fig. 2, include that A-rays induced pose in both cell
tumor models. Levels of p21^{Waf1/Cip1} were also increased in both cell
lines by X-rays in a dose-dependent manner. These results demon-
strate that X innes by X-rays in a dose-dependent manner. These results demonstrate that X-rays induce p53, and also $p21^{Waf1/Cip1}$, in both parental and cisplatin-resistant 2780CP cells to similar extents. Thus, the regulation of p53 a Extrate that X-rays
and cisplatin-resi
regulation of p53
ing DNA damage
2780CP cells.
Yeast Functio and cisplatin-resistant 2780CP cells to similar extents. Thus, the regulation of p53 and p53-dependent transactivation function following DNA damage by X-rays appears to be normal in A2780 and 2780CP cells. **Yeast Functio**

colonies. Repair by inactivating mutant $p53$, on the other hand,
produces red colonies. Similarly, temperature-sensitive $p53$ mutants
generate pink colonies. The Li-Fraumeni cell lines gave colonies of
the appropriate c ing DNA damage by X-rays appears to be normal in A2780 and 2780CP cells.
 Yeast Functional Assay for p53. The ability to transactivate $p21^{WafI/CipI}$ in A2780 and 2780CP cells and induce G_1 arrest suggests that p53 is 2780CP cells.

Yeast Functional Assay for p53. The ability to transactivate
 $p21^{Waf1/Cip1}$ in A2780 and 2780CP cells and induce G_1 arrest suggests

that p53 is functionally competent in the two models. To confirm this, **Procence is example 10** Assay for p53. The ability to transactival $p21^{waf1/Cip1}$ in A2780 and 2780CP cells and induce G_1 arrest suggest that p53 is functionally competent in the two models. To confirm this we examined $P21^{Waf1/CipT}$ in A2780 and 2780CP cells and induce G_1 arrest suggests that p53 is functionally competent in the two models. To confirm this, we examined the functional integrity of p53 in yeast (32, 33). Homologous rec that p53 is functionally competent in the two models. To confirm this, we examined the functional integrity of p53 in yeast (32, 33). Homologous recombination in yeast between the reverse transcription-PCR product spannin we examined the functional integrity of p53 in yeast (32, 33). Homologous recombination in yeast between the reverse transcription-
PCR product spanning exons $4-10$ of $p53$ from tumor cells and the
gapped $p53$ plasmid restores $p53$ sequence and activates gene expres-
sion. Repair o PCR product spanning exons $4-10$ of $p53$ from tumor cells and the gapped $p53$ plasmid restores $p53$ sequence and activates gene expression. Repair of the gap by wild-type $p53$ results in white yeast colonies. Repair gapped $p53$ plasmid restores $p53$ sequence and activates gene expression. Repair of the gap by wild-type $p53$ results in white yeast colonies. Repair by inactivating mutant $p53$, on the other hand, produces red coloni sion. Repair of the gap by wild-type $p53$ results in white yeast colonies. Repair by inactivating mutant $p53$, on the other hand, produces red colonies. Similarly, temperature-sensitive $p53$ mutants generate pink colon colonies. Repair by inactivating mutant $p53$, on the other hand,
produces red colonies. Similarly, temperature-sensitive $p53$ mutants
generate pink colonies. The Li-Fraumeni cell lines gave colonies of
the appropriate c produces red colonies. Similarly, temperature-sensitive $p53$ mutants
produces red colonies. The Li-Fraumeni cell lines gave colonies of
the appropriate color and distribution that were consistent with the
presence of wil produces feat colonies. Unimality, dimperature-sensitive p_{53} indicates
generate pink colonies. The Li-Fraumeni cell lines gave colonies of
the appropriate color and distribution that were consistent with the
presence the appropriate color and distribution that were consistent with the presence of wild-type $p53$ (A15–010 cells), heterozygous inactive mutant $p53$ (A14–000), or heterozygous temperature-sensitive mutant $p53$ (A14–000; presence of wild-type $p53$ (A15-010 cells), heterozygous inactive
mutant $p53$ (A14-000), or heterozygous temperature-sensitive mu-
tant $p53$ (A14-000), Table 3). With A2780 cells, the yeast functional
assay revealed ab

DIFFERENTIAL p53 INDUCTION
Table 3 Analysis of p53 function using the homologous recombination assay in yeasf⁴

Table 3 Analysis of p53 function using the homologous recombination assay in yeast ^a						
Cell line		Number of colonies		q, wild type		
	White	Red	Pink			
A15-010	295	12		96		
A14-000	260	259	0	50		
A1-000	70		117	36		
A2780	119		0	96		
2780CP	228		346	40		

A1-000 70 6 117 36 tial
A2780 119 5 0 96 p21
2780CP 228 0 346 40 p21
 ${}^{\text{4}}$ p53 mRNA from tumor cells was reverse transcribed, amplified by PCR, and
cotransformed into yeast with a gapped p53 expression vector. The yea cotransformed into yeast with a gapped $p53$ expression vector. The yeast was then grown at 35°C for 2-3 days and colonies counted. Data are presented as a mean of two independent experiments.

Expanding COVE 1989.

Fig. 3. Temporal increases in p53 by cisplatin in ovarian tumor cell lines. Cells in exponential growth phase were exposed to 20 μ M cisplatin for 2 h and then incubated for up to 72 h without th **Time (h)**

Fig. 3. Temporal increases in p53 by cisplatin in ovarian tumor cell lines. Cells in

extronential growth phase were exposed to 20 μ M cisplatin for 2 h and then incubated for

up to 72 h without the drug. C Fig. 3. Temporal increases in p53 by cisplatin in ovarian tumor cell lines.
exponential growth phase were exposed to $20 \mu\text{m}$ cisplatin for 2 h and then incu
up to 72 h without the drug. Cells were harvested at selecte

extracted and examined for p53 levels by the Western technique. \overline{A} , immunoblot analysis of p53. *B*, levels of p53 estimated from immunoblots by laser densitometry.
product of mRNA from 2780CP cells yielded colonies have a heterozygous temperature-sensitive mutation in their $p53$. product of mRNA from 2780CP cells yielded colonies that were about 40% white and 60% pink. These results indicate that resistant cells have a heterozygous temperature-sensitive mutation in their p53.
Effect of Cisplatin Effect of mRNA from 2780CP cells yielded colonies that were about $\%$ white and 60% pink. These results indicate that resistant cells ve a heterozygous temperature-sensitive mutation in their *p53*.
Effect of Cisplatin

product of mRNA from 2780CP cells yielded colonies that were about 40% white and 60% pink. These results indicate that resistant cells have a heterozygous temperature-sensitive mutation in their $p53$. **Effect of Cisplati** 40% white and 60% pink. These results indicate that resistant cells
have a heterozygous temperature-sensitive mutation in their $p53$.
Effect of Cisplatin on p53 and p21^{Waf1/Clp1} Levels. To examine
whether the differe have a heterozygous temperature-sensitive mutation in their $p53$.
 Effect of Cisplatin on p53 and p21^{War1/Cip1} Levels. To examine

whether the differential cytotoxic response of A2780 and 2780CP

cells to cisplatin c **Effect of Cisplatin on p53 and p21**^{Waft/Clp1} Levels. To examine whether the differential cytotoxic response of A2780 and 2780CP cells to cisplatin correlated with differences in p53 induction, cells were exposed to a c whether the differential cytotoxic response of A2780 and 2780CP cells to cisplatin correlated with differences in p53 induction, cells were exposed to a cisplatin concentration (20μ m; 2 h) that is clinically relevant (cells to cisplatin correlated with differences in p53 induction, cells
were exposed to a cisplatin concentration (20 μ M; 2 h) that is clini-
cally relevant (38), and cellular extracts subjected to Western analysis.
In bens to enghalar concluded with directness in β 55 induction, censeled the vertex exposed to a cisplatin concentration (20 μ m; 2 h) that is clinically relevant (38), and cellular extracts subjected to Western analysi cally relevant (38), and cellular extracts subjected to Western analysis.
In parental A2780 cells, induction of p53 was readily apparent (Fig.
3): levels increased rapidly, peaked at 24 h posttreatment, and then
decayed a In parental A2780 cells, induction of p53 was readily apparent (Fig. 3): levels increased rapidly, peaked at 24 h posttreatment, and then decayed approximately 35–40% by 72 h. Induction of p53 in A2780 cells was dependent 3): levels increased rapidly, peaked at 24 h posttreatment, and then decayed approximately 35–40% by 72 h. Induction of p53 in A2780 cells was dependent on cisplatin concentration, with increased levels being observed aft decayed approximately 35–40% by 72 h. Induction of p53 in A2780 cells was dependent on cisplatin concentration, with increased levels being observed after 24 h at drug concentrations as low as 1μ M (Fig. 4). In contrast cells was dependent on cisplatin concentration, with increased levels
being observed after 24 h at drug concentrations as low as 1 μ M (Fig.
4). In contrast, treatment of 2780CP cells with cisplatin produced
minimal inc cells was dependent on cisplatin concentration, with increased levels
being observed after 24 h at drug concentrations as low as $1 \mu M$ (Fig.
4). In contrast, treatment of 2780CP cells with cisplatin produced
minimal incr In contrast, treatment of 2780CP cells with cisplatin produced
nimal increases in p53 levels (Figs. 3 and 4). These data sugges
it the pathway involved in p53 induction by cisplatin is defective in
80CP cells.
Because inc 4). In contrast, treatment or 2780CP cells with cisplatin produced
minimal increases in p53 levels (Figs. 3 and 4). These data suggest
that the pathway involved in p53 induction by cisplatin is defective in
2780CP cells.

DISCUSSION

The mechanism of cisplatin resistance is characterized as multifac-
The mechanism of cisplatin resistance is characterized as multifac-
tial, with reduced cisplatin uptake, reduced DNA adduct formation **DISCUSSION**
The mechanism of cisplatin resistance is characterized as multifac-
torial, with reduced cisplatin uptake, reduced DNA adduct formation,
increased DNA adduct repair, and increased intracellular GSH being **DISCUSSION**
The mechanism of cisplatin resistance is characterized as multifac-
torial, with reduced cisplatin uptake, reduced DNA adduct formation,
increased DNA adduct repair, and increased intracellular GSH being
cited **CHENGE THE MORE CHENGE THE MORE CHENGE THE MORE CHENGE THE most often (3-6, 27, 39, 40). In the present investigation, the** The mechanism of cisplatin resistance is characterized as multifactorial, with reduced cisplatin uptake, reduced DNA adduct formation, increased DNA adduct repair, and increased intracellular GSH being cited the most often torial, with reduced cisplatin uptake, reduced DNA adduct formation, increased DNA adduct repair, and increased intracellular GSH being cited the most often (3–6, 27, 39, 40). In the present investigation, the cisplatin-re to a 2-fold reduction in exposure of resistant cellular GSH being
increased DNA adduct repair, and increased intracellular GSH being
cited the most often (3–6, 27, 39, 40). In the present investigation, the
cisplatin-resis cited the most often $(3-6, 27, 39, 40)$. In the present investigation, the cisplatin-resistant 2780CP model was no different and displayed these mechanisms also. We found that these mechanisms collectively lead to a 2-fo cisplatin-resistant 2780CP model was no different and displayed these mechanisms also. We found that these mechanisms collectively lead to a 2-fold reduction in exposure of resistant cells to the cytotoxic lesion, as evide sure, however, is not consistent with the 29- to 51-fold reduction, as evidenced by the difference in AUC of DNA adducts between sensitive and resistant cells. This difference in adduct exposure, however, is not consistent to a 2-fold reduction in exposure of resistant cells to the cytotoxic
lesion, as evidenced by the difference in AUC of DNA adducts
between sensitive and resistant cells. This difference in adduct expo-
sure, however, is no lesion, as evidenced by the difference in AUC of DNA adducts
between sensitive and resistant cells. This difference in adduct expo-
sure, however, is not consistent with the 29- to 51-fold resistance of
2780CP cells to cis between sensitive and resistant cells. This difference in adduct exposure, however, is not consistent with the 29- to 51-fold resistance of 2780CP cells to cisplatin, thereby suggesting that other mechanisms of cisplatin r sure, however, is not consistent with the 29- to 51-fold resistance of 2780CP cells to cisplatin, thereby suggesting that other mechanisms of cisplatin resistance may be more important in this model. In accord with this vi can to here it, is not echoically what the 25 to 31 fold festivative of
2780CP cells to cisplatin, thereby suggesting that other mechanisms
of cisplatin resistance may be more important in this model. In accord
with this v 270001 Units to Unpham, unrorpy suggeoung that once incordination of cisplatin resistance may be more important in this model. In accord with this view, we have noted a major biochemical pharmacological difference between with this view, we have noted a major biochemical pharmacological
difference between the sensitive and resistant cell lines: 2780CP cells
can tolerate about 14-fold greater DNA damage than parental A2780
cells. The 2-fold difference between the sensitive and resistant cell lines: 2780CP cells
can tolerate about 14-fold greater DNA damage than parental A2780
cells. The 2-fold reduction in AUC of DNA adducts and the 14-fold
increase in DNA da can tolerate about 14-fold greater DNA damage than parental A2780 cells. The 2-fold reduction in AUC of DNA adducts and the 14-fold increase in DNA damage tolerance combine to give a theoretical resistance factor of 28. I cells. The 2-fold reduction in AUC of DNA adducts and the 14-fold increase in DNA damage tolerance combine to give a theoretical resistance factor of 28. Interestingly, this value, which is derived from results using a 2-

CONCENTATION (μ **M)**
Fig. 4. Concentration-dependent increases in p53 by cisplatin in ovarian tumor cell
lines. Cells in exponential growth phase were exposed to 0–20 μ *m* cisplatin for 2 h,
washed, and then incubate **COTIGETIM ALIOTT (FIFT)**
Fig. 4. Concentration-dependent increases in p53 by cisplatin in ovarian tumor cell
lines. Cells in exponential growth phase were exposed to 0-20 μ m cisplatin for 2 h,
washed, and then incubat etry.

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A2780) and resistant (R; 2780CP) ovarian tumor models. Cells in exponential growth phase were exposed to a clinically relevant concentration of cisplatin (20 μ M) for 2 h, **Lane...1** 2 3 4 age

Fig. 5. Coordinate induction of p53 and p21^{Waf1/Cip1} by cisplatin in the sensitive (S;

A2780) and resistant (R; 2780CP) ovarian tumor models. Cells in exponential growth the

phase were exposed to Fig. 5. Coordinate induction of p53 and p21^{wart/ctp1} by cisplatin in the sensitive (S;
A2780) and resistant (R; 2780CP) ovarian tumor models. Cells in exponential growth
phase were exposed to a clinically relevant conce From the server exposed to a clinically related
washed, and then incubated in drug-free me
washed, and then incubated in drug-free me
protein was extracted and examined for p5
technique. A, immunoblot analysis of p53 as
f washed, and then incubated in drug-free medium. Cells were harvested 24 h later, and
protein was extracted and examined for p53 and p21^{Waf1/Cip1} levels by the Western T(
technique. A, immunoblot analysis of p53 and p21

Experiment and solution and state in the chinese A, immunoblot analysis of p53 and p21^{Warit/Cip1}. B, levels of p53 estimated can
from immunoblots by laser densitometry.
2-h exposure protocol. The results indicate that D from immunoblots by laser densitometry.

2-h exposure protocol. The results indicate that DNA damage toler-

ance is a major mechanism of resistance in 2780CP cells. This

conclusion is consistent with that reported recent 2-h exposure protocol. The results indicate that DNA damage tolerance is a major mechanism of resistance in 2780CP cells. This conclusion is consistent with that reported recently with a panel of human ovarian tumor models In a exposure protocol. The results indicate that DNA damage toler-

Inclusion is consistent with that reported recently with a panel of

man ovarian tumor models (37).

In an attempt to determine the underlying basis for ance is a major mechanism of resistance in 2780CP cells. This regulation is consistent with that reported recently with a panel of In human ovarian tumor models (37). cispl
In an attempt to determine the underlying basis

human ovarian tumor models (37).

In an attempt to determine the underlying basis for the substantial

increase in DNA damage tolerance in 2780CP cells, we have focused

pour attention on $p53$. The rationale for this was In an attempt to determine the underlying basis for the substantial a noncrease in DNA damage tolerance in 2780CP cells, we have focused p53 our attention on $p53$. The rationale for this was based on reports that Our mut increase in DNA damage tolerance in 2780CP cells, we have focused
our attention on $p53$. The rationale for this was based on reports that
mutation in $p53$ can deregulate cell cycle control and apoptosis (13, 1
19) and c our attention on $p53$. The rationale for this was based on reports that
mutation in $p53$ can deregulate cell cycle control and apoptosis (13, lat
19) and contribute to cisplatin resistance (20). Consistent with these in mutation in $p53$ can deregulate cell cycle control and apoptosis (13, lation in the signaling pathway for p53 induction is a significant factor
19) and contribute to cisplatin resistance (20). Consistent with these in th whereas resistant 2780CP cells do indeed harbor a heterozygous A2780 and 2780CP models led to induction of p53, coordinate inwhereas resistant 2780CP cells do indeed harbor a heterozygous strone mutation at codon 172 of $p53$ gene. However, contrary to expectation, inducene mutation in resistant cells did not disrupt p53 function. This was clea matation at educit 172 of post generation, constantly to expectation, indet
gene mutation in resistant cells did not disrupt p53 function. This was
clearly evident from data demonstrating that X-ray exposure of both
A2780 clearly evident from data demonstrating that X-ray exposure of bot A2780 and 2780CP models led to induction of p53, coordinate in creases in p21^{Waf1/Cip1}, and activation of the G_1 -checkpoint, which are all characteri A2780 and 2780CP models led to induction of p53, coordinate in-
creases in p21^{Waf1/Cip1}, and activation of the G₁-checkpoint, which
are all characteristics associated with p53-proficient cells exposed to
DNA-damaging creases in p21^{Waf1/Cip1}, and activation of the G_1 -checkpoint, which ance.
are all characteristics associated with p53-proficient cells exposed to
DNA-damaging agents (12, 36, 41–45). However, the temperature-
sensiti are all characteristics associated with p53-proficient cells exposed to
DNA-damaging agents (12, 36, 41–45). However, the temperature-
sensitive nature of mutant p53 in 2780CP cells suggests that the
protein has partial a DNA-damaging agents (12, 36, 41-45). However, the temperature-
sensitive nature of mutant p53 in 2780CP cells suggests that the
protein has partial activity, and this could explain its lack of a
dominant-negative effect o sensitive nature of mutant p53 in 2780CP cells suggests that the protein has partial activity, and this could explain its lack of a
protein has partial activity, and this could explain its lack of a
dominant-negative effe protein has partial activity, and this could explain its lack of a dominant-negative effect on wild-type p53. It is interesting to note that, although our present investigations and those of Fan *et al.* (20) suggest that dominant-negative effect on wild-type p53. It is interesting to note
that, although our present investigations and those of Fan *et al.* (20) c c
suggest that loss of p53 function can lead to cisplatin resistance, other
r that, although our present investigations and those of Fan *et al.* (20) suggest that loss of p53 function can lead to cisplatin resistance, other reports provide evidence to the contrary. Thus, inactivation of wild-
type suggest that loss of p53 function can lead to cisplatin resistance, other

increased cisplatin sensitivity. Thus, inactivation of wild-

terization of a cis-diamminedichloroplatinum(II)-resistant luman ovarian cancer cell

BY CISPLATIN AND X-RAYS
factors or events may work in concert with p53 dysfunction to dictate
the final cytotoxic response to cisplatin. EX CISPLATIN AND X-RAYS
factors or events may work in concert with p
the final cytotoxic response to cisplatin.
Another major difference noted betwee

CISPLATIN AND X-RAYS

Another major difference noted between sensitive and resistant

Another major difference noted between sensitive and resistant

Ils was their ability to respond to cisplatin-induced DNA damage BY CISPLATIN AND X-RAYS
factors or events may work in concert with p53 dysfunction to dictate
the final cytotoxic response to cisplatin.
Another major difference noted between sensitive and resistant
cells was their abilit factors or events may work in concert with p53 dysfunction to dictate
the final cytotoxic response to cisplatin.
Another major difference noted between sensitive and resistant
cells was their ability to respond to cisplati the final cytotoxic response to cisplatin.

Another major difference noted between sensitive and resistant

cells was their ability to respond to cisplatin-induced DNA damage

through p53. Cisplatin could readily induce p5 Another major difference noted between sensitive and resistant
cells was their ability to respond to cisplatin-induced DNA damage
through p53. Cisplatin could readily induce p53 in A2780 cells but not
in resistant cells. T Thouse mayor directive noted servicent sensitive and resistant
cells was their ability to respond to cisplatin-induced DNA damage
through p53. Cisplatin could readily induce p53 in A2780 cells but not
in resistant cells. T through p53. Cisplatin could readily induce p53 in A2780 cells but not
in resistant cells. This indicates that the signal transduction pathway
involved in p53 induction was defective in 2780CP cells. Because
X-rays were ca charge the complete control of the signal transduction pathway
in resistant cells. This indicates that the signal transduction pathway
involved in p53 induction was defective in 2780CP cells. Because
X-rays were capable of involved in p53 induction was defective in 2780CP cells. Because X-rays were capable of inducing p53 in both sensitive and resistant tumor models, it is reasonable to conclude that induction of p53 by cisplatin and X-rays X-rays were capable of inducing p53 in both sensitive and resistant
tumor models, it is reasonable to conclude that induction of p53 by
cisplatin and X-rays must occur through distinctly different signal
transduction path tumor models, it is reasonable to conclude that induction of p53 by cisplatin and X-rays must occur through distinctly different signal transduction pathways. Analogous conclusions have been reached recently by Artuso *et* cisplatin and X-rays must occur through distinctly different signal
transduction pathways. Analogous conclusions have been reached
recently by Artuso *et al.* (48) and Zhang *et al.* (49) from their studies
with ataxia te transduction pathways. Analogous conclusions have been reached recently by Artuso *et al.* (48) and Zhang *et al.* (49) from their studies with ataxia telangiectasia cells, in which a converse effect was reported. These a recently by Artuso *et al.* (48) and Zhang *et al.* (49) from their studies with ataxia telangiectasia cells, in which a converse effect was reported. These authors found that $p53$ induction was poor following exposure t light. ported. These authors found that $p53$ induction was poor following
exposure to ionizing radiation but was comparable to that in normal
cells when exposed to methylmethane sulfonate, cisplatin, or UV
light.
Once p53 is in

Fig. 5. Coordinate induction of p53 and p21^{Wart/Cipl} by cisplatin in the sensitive (5). Index (5) and pair $\frac{1}{1}$

Fig. 5. Coordinate induction of p53 and p21 war. ($\frac{1}{1}$). This is the sensitive discontract o exposure to ionizing radiation but was comparable to that in normal
cells when exposed to methylmethane sulfonate, cisplatin, or UV
light.
Once p53 is induced, it can transactivate a number of genes,
including $p21^{Waf1/Cip1$ Light.

Once p53 is induced, it can transactivate a number of genes,

including $p21^{Waf1/Cip1}$ (13). Indeed, coordinate increases in

p21^{Waf1/Cip1} were observed in both A2780 and 2780CP cells by

X-rays, but only in A278 Once p53 is induced, it can transactivate a number of genesincluding $p21^{Waf1/Cip1}$ (13). Indeed, coordinate increases in $p21^{Waf1/Cip1}$ were observed in both A2780 and 2780CP cells b X-rays, but only in A2780 cells by cisp including $p21^{Waf1/Cip1}$ (13). Indeed, coordinate increases in $p21^{Waf1/Cip1}$ were observed in both A2780 and 2780CP cells by X-rays, but only in A2780 cells by cisplatin. This indicates that p53 induction is of paramount i p21^{Waf1/Cip1} were observed in both A2780 and 2780CP cells by
X-rays, but only in A2780 cells by cisplatin. This indicates that p53
induction is of paramount importance for p53-mediated transactiva-
tion functions. Moreo Examples of p53 by in both A2780 cells by cisplatin. This indicates that p53 induction is of paramount importance for p53-mediated transactivation functions. Moreover, induction of p53 appears to be mandatory also for p53for tags, out omg in 112760 cens by supplaum. This indicates and position functions of paramount importance for p53-mediated transactiva-
tion functions. Moreover, induction of p53 appears to be mandatory
also for p53-medi tion functions. Moreover, induction of p53 appears to be mandatory also for p53-mediated apoptotic functions (41). Thus, similar inductions of p53 by X-rays in both A2780 and 2780CP cells may account for the low cross-res also for p53-mediated apoptotic functions (41). Thus, similar induc-
tions of p53 by X-rays in both A2780 and 2780CP cells may account
for the low cross-resistance of 2780CP cells to this ionizing radiation,
whereas a lack also to p_{22} -included apoptotic functions (+1). Thus, similar inductions of p53 by X-rays in both A2780 and 2780CP cells may account for the low cross-resistance of 2780CP cells to this ionizing radiation, whereas a la signals of postage of 2780CP cells to this ionizing radiation,
for the low cross-resistance of 2780CP cells to this ionizing radiation,
whereas a lack of p53 induction by cisplatin in the 2780CP line is
strongly implicated whereas a lack of p53 induction by cisplatin in the 2780CP line is
strongly implicated in the resistance of this model to the platinum
agent. It is highly likely that in the absence of p53-mediated apoptotic
signals, plati strongly implicated in the resistance of this model to the platinum
agent. It is highly likely that in the absence of p53-mediated apoptotic
signals, platinum-induced DNA lesions accumulate and account for
the observed in rate of p53 in the sessure of any house. The two planner agent. It is highly likely that in the absence of p53-mediated apoptotic signals, platinum-induced DNA lesions accumulate and account for the observed increase in 27 signals, platinum-induced DNA lesions accumulate and account for
the observed increase in 2780CP cells of DNA damage tolerance to
cisplatin; this would be consistent with currently held concepts on the
role of $p53$ in dr the observed increase in 2780CP cells of DNA damage tolerance to cisplatin; this would be consistent with currently held concepts on the role of $p53$ in drug-induced cell death (10, 12, 19). Induction of $p53$ can involve up-regulation of transcriptional, translational, or postis whether the lack of p53 induction in resistant cells by cisplatin is due to a pathway-specific signaling defect in any of these or other p53

ance is a major mechanism of resistance in 2780CP cells. This regulatory mechanisms.

conclusion is consistent with that reported recently with a panel of

In summary, DNA damage tolerance is the major mechanism of

human In summary, DNA damage tolerance is the major mechanism of regulatory mechanisms.
In summary, DNA damage tolerance is the major mechanism of
cisplatin resistance in ovarian 2780CP tumor cells. These cells possess
a normal and a mutated p53 allele, but the mutation does not affect
 In summary, DNA damage tolerance is the major mechanism of
cisplatin resistance in ovarian 2780CP tumor cells. These cells possess
a normal and a mutated $p53$ allele, but the mutation does not affect
p53 functions and, t cisplatin resistance in ovarian 2780CP tumor cells. These cells possess
a normal and a mutated $p53$ allele, but the mutation does not affect
p53 functions and, therefore, is not directly responsible for resistance.
Our d a normal and a mutated $p53$ allele, but the mutation does not affect p53 functions and, therefore, is not directly responsible for resistance.
Our data, however, are consistent with the premise that down-regulation in th a normalism and therefore, is not directly responsible for resistance.
Our data, however, are consistent with the premise that down-regulation in the signaling pathway for p53 induction is a significant factor
in the resis Our data, however, are consistent with the premise that down-regulation in the signaling pathway for p53 induction is a significant factor

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