P53 MUTATION CORRELATES WITH CISPLATIN SENSITIVITY IN HEAD AND NECK SQUAMOUS CELL CARCINOMA LINES

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Abstract: *Background.* A critical factor for successful organ preservation treatment in head and neck cancer may be selecting tumors that respond to chemotherapy and radiation. Previous results in patients indicated that tumors that overexpressed p53 were more sensitive to chemotherapy than those that did not overexpress p53.

Methods. To determine the relationship of p53 mutations to sensitivity to cisplatin in vitro, 23 head and neck squamous cell carcinoma (HNSCC) cell lines were analyzed for cisplatin sensitivity, p53 expression, and p53 mutation status.

Results. Mutations of the p53 gene were identified in 13 of 23 of the cell lines tested. Mutation of the p53 gene was significantly associated with high levels of expression of the p53 protein. The average ID_{50} (drug dose required to inhibit 50% of cell growth) for cell lines with mutant p53 was 6.8 μM, whereas the average ID_{50} for cell lines with wild-type p53 was 13.7 μM.

Conclusions. These in vitro data support a role for mutation of the p53 tumor suppressor gene as a marker for response to cisplatin in HNSCC. © 2003 Wiley Periodicals, Inc. *Head Neck* **25:** 654–661, 2003

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Effective treatment of advanced head and neck cancer remains one of the most difficult challenges in head and neck oncology. Tumor cell resistance limits the effective use of chemotherapy and radiation regimens for organ preservation and improved quality of life. To accomplish successful organ sparing and allow prompt surgical salvage of those patients for whom organ-sparing

treatment is unlikely to work, it is necessary to identify the tumors most likely to respond. Clinical studies have shown that patients whose tumors achieve a complete response to chemotherapy have a better prognosis than those with an incomplete response.[1] However, some patients selected for organ preservation have poor response to chemotherapy and require late salvage surgery. In these patients, a delay in effective treatment may contribute to the poor overall prognosis. Furthermore, the selection of a highly resistant and aggressive tumor cell population after unsuccessful organ-preservation approaches remains a clinical dilemma with an exceedingly poor outcome. Therefore, the identification of accurate predictive markers for chemotherapy response may optimize treatment by selection of tumors most likely to respond to organ preservation regimens.

The p53 gene, located on chromosome 17p, encodes a 393-amino acid phosphoprotein that induces G1/S cell cycle arrest in response to DNA damage.[1,2] This p53-dependent G1/S checkpoint allows the cell to repair damaged DNA before DNA synthesis. In addition to extending the DNA repair time, p53 also participates in DNA repair processes.[3] If DNA repair fails or the damage to the DNA is too extensive, p53 may induce apoptosis.[4,5] The p53 gene is frequently mutated in head and neck cancer.[6] Mutations and other genetic alterations may abrogate wild-type p53 function. Our group previously reported that overexpression of p53 predicted successful organ preservation in patients with advanced laryngeal cancer treated with chemotherapy plus radiation.[6] The full multivariate model for predicting larynx preservation in patients treated with induction chemotherapy plus radiation revealed that T (tumor) class ($p = .0070$; risk ratio = 7.1; CI = 1.7–29.5), p53 overexpression ($p = 0.0281$, risk ratio = 3.4; CI = 1.2–10.3), and elevated proliferating cell nuclear antigen index ($p = .0101$; risk ratio = 4.2; CI = 1.5–14.9) were independent predictors of successful organ preservation.[7] These data strongly suggest that p53-regulated pathways may be one of the critical components of tumor cell response to chemotherapy and radiation.[6] Another line of evidence to support the role of wild-type p53 in cisplatin resistance in head and neck tumors comes from p53 mutation analysis of treatment failures on the chemotherapy arm of the Department of Veterans Affairs Laryngeal Cancer Group study. Most (14 of 18, 78%) nonresponding tumors lacked p53 mutations, suggesting that wild-type p53 is important in resistance to cisplatin-based chemotherapy (Bradford CR and Prince M, unpublished results). Several other studies present conflicting data on the relationship between p53 mutation and chemosensitivity.[8–11] Further investigation of the role of p53 mutation in chemosensitivity is needed to explore the opportunity of using it as a predictive biomarker for chemotherapy response.

Other pathways including the cell death cascades are likely also involved in tumor cell response to chemotherapy. The Bcl-2 protein family members are particularly important candidates in this regard.[12,13] Recent data indicate that low expression of the apoptosis-blocking protein, Bcl-x_L, in tumor specimens of patients enrolled on the chemotherapy arm of the Department of Veterans Affairs Laryngeal Cancer Group Study is an excellent predictor of response to chemotherapy.[14] Recent data from our group and others indicates that cyclin D1 is likely to be involved in chemotherapy response as well.[15] There are other components of the cell cycle control pathway, including mdm2, p16, p14ARF, ATM, and others, that may be play a crucial role, but they have not been fully evaluated to date by our group.

To confirm the results obtained previously in patient tumor specimens, we investigated in vitro cisplatin sensitivity and p53 gene mutation and expression status in 23 established head and neck squamous cell carcinoma (HNSCC) cell lines. Our hypothesis was that the mutations of the p53 gene would correlate with cisplatin sensitivity in these cell lines. Investigations are ongoing with regard to evaluation of cell death promoting and blocking proteins in the Bcl-2 family in these cell lines. Herein we report that HNSCC cell lines harboring p53 mutations, as a group, have an increased sensitivity to cisplatin in vitro. Furthermore, most cell lines with mutant p53 overexpress the p53 protein. These data further substantiate the role of p53 in determining the sensitivity of head and neck tumor cells to cisplatin.

MATERIALS AND METHODS

Cell Culture. Twenty-three human head and neck squamous cell carcinoma cell lines were established from primary head and neck squamous carcinoma specimens in the laboratory of Dr. Thomas Carey. Informed consent was obtained from all patients, and the University of Michigan Institutional Review Board reviewed and approved the study. This study includes 19 laryn-

geal carcinoma cell lines and 4 oral cancer cell lines. Cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing glutamine, nonessential amino acids, and 10% fetal bovine serum in a humidified atmosphere of 5% CO_2 at 37°C.

Chemosensitivity Assay (MTT Method). Exponentially growing University of Michigan squamous cell carcinoma (UM-SCC) established cell lines were cultured, washed, counted, and plated at 10,000 cells per well in duplicate wells of 96-well plates and incubated in DMEM at 37°C overnight. The next morning, cells were incubated with cisplatin (Sigma, St. Louis, MO) at increasing concentrations (0, 5, 10, 20, 40, 80 μ*M*) for 2 hours, washed twice with media, then incubated in 300 μL DMEM for 6 days. The media was removed from the wells, and 110 μL DMEM containing 10 μL MTT labeling reagent was added to each well. After a 4-hour incubation, 100 μL of solubilization buffer was added, and the plates were incubated at 37°C overnight. The absorbance (OD) of each well was determined with a spectrophotometer at a wavelength of 590 nm. Absorbance at 590 nm is directly proportional to cell survival. The absorbance was plotted on a semi-logarithmic graph of absorbance (y-axis) against drug concentration (x-axis). The 50% inhibitory doses (ID_{50}s) were identified as the concentration of cisplatin required to achieve 50% growth inhibition (ie, 50% reduction in absorbance relative to no cisplatin control). We defined $ID_{50} > 10$ μ*M* as resistant and $ID_{50} < 10$ μ*M* as sensitive to cisplatin.

Protein Extraction. Logarithmically growing cells were harvested at 60% to 90% confluence. Protein extracts were prepared by lysing cells in the flask in a solution of phosphate-buffered saline (BioWhittaker; Walkersville, MD) containing 1 mL NP-40 (Sigma; St. Louis, MO), 1 m*M* PMSF (Sigma), and 1 tablet of a cocktail of protease inhibitors (Boehringer Mannheim, Germany) per 100 mL of PBS. The protein extracts were quantitated using a colorimetric assay (Bradford Reagent; BioRad; Hercules, CA).

Western Blotting. For Western blotting, 50 to 80 μg of protein were resolved at 125 V for 90 minutes on 12% Tris-glycine sodium dodecyl sulfate polyacrylamide gels (SDS-PAGE) under denaturing conditions. Transfer at 30 V overnight immobilized the resolved proteins on PVDF membranes (Millipore; Bedford, MA). The membranes were probed with primary antibodies (mouse anti-p53 monoclonal antibody Ab-6 at 2 μg/mL [Oncogene Research Products; Boston, MA], β-actin at 1 μg/mL [Roche Molecular Biochemicals; Indianapolis, IN], tubulin at 0.2 μg/mL [Roche Molecular Biochemicals]), or GAPDH at 1:100,000 (Chemicon International, Temecula, CA) and sheep anti-mouse IgG (Amersham, Piscataway, NJ) as secondary antibody. The blots were developed using the ECL system (Amersham). All experiments were performed in duplicate, and protein expression was graded negative (no band observed), weakly positive, moderately positive, or strongly positive relative to the intensity of the β-actin, tubulin, or GAPDH bands.

Genomic DNA Isolation. One to 3 million cells were suspended in 600 μL of DNA ZOL (GibcoBRL, Grand Island, NY). The cell suspension was extracted in an equal volume of phenol-chloroform-isoamyl alcohol (25:24:1; Sigma). DNA was precipitated with 2 volumes of 100% cold ethanol, washed with 70% ethanol, air dried, and redissolved in double distilled water.

Single-Strand Conformational Polymorphism (SSCP) Analysis. Exons 4 through 8 of the p53 gene were analyzed for mutations by SSCP technique as previously described,[16] and all suspected mutations (band shifts) were confirmed by DNA sequencing. Exons 4 through 8 were amplified by polymerase chain reaction (PCR) using the primers described previously.[11] The PCR mixture (total 10 μL) contained 100 ng of genomic DNA, 10 m*M* Tris hydrochloride, 50 m*M* potassium chloride, 1.5 m*M* magnesium chloride, 50 μ*M* of each deoxyribonucleoside triphosphate, 1 μCi of [^{32}P]-deoxycytidine triphosphate, 0.2 μ*M* primer, and 0.2 U of Taq polymerase (Promega; Madison, WI). The PCR mixture was heated to 94°C for 5 minutes, and 30 thermal cycles were repeated. Each cycle included 40 seconds of denaturation at 94°C, 40 seconds of primer annealing at 58°C, and 50 seconds of extension and synthesis at 72°C. After 30 cycles of PCR, an additional 7 minutes of extension at 72°C was followed by chilling on ice. Dilution of 5 μL of PCR product in 45 μL loading buffer (10 m*M* sodium hydroxide, 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol) was followed by heated denaturation at 95°C for 5 minutes immediately chilled on ice. From the mixture, 5 μL of the mixture was immediately loaded onto 0.5× mutation detection enhanced

(MDE) polyacrylamide gel (BioWhittaker Molecular Applications, Rockland, ME) and run at constant power of 2 W for 18 to 24 hours. The gel was vacuum dried at 80°C for 1 hour and exposed to film (Kodak, X-Omat) at room temperature for 1 to 2 days.

DNA Sequencing. For PCR samples that demonstrated band shifts by SSCP analysis, a PCR reaction (in a total volume of 20 μL) was performed that was similar to that described previously for SSCP except that it lacked [^{32}P]-deoxycytidine triphosphate. The PCR-amplified DNA fragments were ligated with 10 ng of pCR 4-TOPO vector of the TA cloning kit as per the manufacturer's instructions (Invitrogen, Carlsbad, CA). TOP10 competent *Escherichia coli* were transformed with the ligated DNA and plated onto ampicillin LB agar plates. After overnight incubation at 37°C, selected individual colonies were grown in LB medium. Plasmid DNA was prepared with mini-prep Plasmid DNA purification Kit (Promega) and sequenced by Applied Biosystems DNA sequencers according to manufacture's protocols at the Core Facility at the University of Michigan Comprehensive Cancer Center. All p53 mutations were confirmed in independent PCR reactions to ensure that the mutation was not an artifact of PCR.

p53 GeneChip Assay. Sequence analysis of the p53 gene was confirmed in several cell lines by use of the p53 GeneChip assay (Affymetrix, Inc., Santa Clara, CA) per the manufacturer's protocol. The p53 GeneChip assay permits the analysis of the entire coding region of the human p53 tumor suppressor gene (exons 2–11), including both sense and antisense strands in a single hybridization reaction. The assay identifies missense mutations and single-base deletions while detecting mutant p53 in a background of wild-type p53.

RESULTS

p53 Mutation in UMSCC Cell Lines. p53 mutations in exons 4 through 8 were identified in 13 of 23 (56.5%) cell lines using SSCP analysis and direct sequencing. Results of SSCP and subsequent sequencing of p53 exons with bands with altered mobility are shown in Table 1. A representative SSCP experiment is shown in Figure 1. Two cell lines had mutations in exon 4, four cell lines had mutations in exon 5, one cell line had a mutation in exon 6, three cell lines had mutations in exon 7, and three cell lines had mutations in exon 8. The mutations identified included eight transversions, three transitions, one termination, and one two-codon deletion. p53 GeneChip assay was performed in 19 cell lines. In 17 of 19 cell lines, the results obtained by SSCP and direct sequencing were consistent with those obtained by p53 GeneChip assay. Two exon 10 mutations were identified by p53 GeneChip assay that were not tested for by SSCP of exons 4 through 8. In UM-SCC-12, SSCP/sequencing and p53 GeneChip assay identify the same mutation, but the mutation is below threshold for the GeneChip assay. This observation may suggest a mixed population of mostly wild-type p53 cells and a small number of p53 mutant cells. In two cell lines, SSCP detects a p53 mutation that is not confirmed by the p53 GeneChip assay.

p53 Expression in UMSCC Cell Lines. Mutation of the p53 gene was significantly associated with p53 protein overexpression ($p = .036$, X^2). Western blot analysis demonstrated that 9 of 13 (69%) cell lines with p53 mutations showed considerably elevated levels of p53 protein, whereas only 2 of 10 (20%) cell lines with wild-type p53 had p53 overexpression (Figure 2). One of these had a nonsense mutation resulting in an early stop codon in exon 4 (UM-SCC-12). The second had a two-codon deletion in exon 4 (UM-SCC-20). The other two cell lines with mutant p53 that did not express the p53 protein had transversions in exons 5 and 6, respectively (UM-SCC-23 and 68). Interestingly, both of these cell lines were wild type by p53 GeneChip assay. Of the 10 cell lines with wild-type p53, 5 did not express p53 at levels detectable by Western blot, 3 had very low levels of p53 expression, 1 showed moderate expression, and 1 had strong expression of the p53 protein.

Chemosensitivity Assay in UMSCC Cell Lines. On the average, those HNSCC cell lines with mutant p53 are more sensitive to cisplatin than those with wild-type p53. The average ID_{50} for the 13 cell lines with mutant p53 is 6.8 μM. In contrast, in the 10 cell lines with wild-type p53, the average ID_{50} is 13.7 μM ($p = .048$, Student's t test). Most (10 of 13 or 77%) cell lines with mutant p53 were sensitive to cisplatin ($ID_{50} < 10$), whereas only 4 of 10 (40%) cell lines with wild-type p53 were sensitive, although this difference did not reach statistical significance ($p = .102$, Fisher's exact test).

Cell lines were also analyzed for chemosensitivity according to level of p53 protein expression. Cell lines with weak or no expression of the p53

Table 1. Head and neck cell lines characterized by Cisplatin sensitivity, p53 mutation, and expression analysis.

Cell lines	SSCP*	Sequencing results			Agrees w/p53GeneChip result	p53†	Mean ID_{50} (μM)‡
		Codon	Type	aa Change			
UM-SCC-5	e5 m/w	157 gtc⇒ttc	Transversion	V⇒F	Yes§	3	3.8
UM-SCC-10B	e7 m/m	245 ggc⇒tgc	Transversion	G⇒C	Yes	3	23.7
UM-SCC-11B	e7 m/m	242 tgc⇒tcc	Transversion	C⇒S	Yes	3	12.0
UM-SCC-12	e4 m/w	104 cag⇒tag	Termination	Q⇒stop	Yes‖	0	8.2
UM-SCC-13	e5 m/m	163 tac⇒tgc	Transition	Y⇒C	No data	2	4.8
UM-SCC-14A	e8 m/m	280 aga⇒agt	Transversion	R⇒S	Yes	3	3.2
UM-SCC-20	e4 m/w	71–72	2-codon deletion		No data	0	2.7
UM-SCC-23	e5 m/m	176 tgc⇒ttc	Transversion	C⇒F	No¶	0	3.2
UM-SCC-36	e5 m/m	158 cgc⇒ccc	Transversion	R⇒P	Yes	3	4.5
UM-SCC-46	e8 m/w	278 cct⇒gct	Transversion	P⇒A	Yes§	3	3.0
UM-SCC-57	e8 m/m	273 cgt⇒ctt	Transversion	R⇒L	Yes	3	5.4
UM-SCC-68	e7 m/m	248 cgg⇒tgg	Transition	R⇒W	No¶	1	4.3
UM-SCC-81B	e6 m/m	193 cat⇒cgt	Transition	H⇒R	Yes	3	10.2
Average ID_{50} mutant p53							6.8
UM-SCC-1	w				Yes	0	14.0
UM-SCC-6	w				Yes	0	36.7
UM-SCC-17B	w				Yes	0	4.0
UM-SCC-25	w				No data	0	18.7
UM-SCC-47	e4 m/m		polymorphism		Yes	1	9.3
UM-SCC-54	e4 m/m		polymorphism		No data	3	3.4
UM-SCC-72	w				Yes	0	17.6
UM-SCC-74A	w				Yes	1	11.3
UM-SCC-74B	w				Yes	1	4.8
UM-SCC-81A	w				Yes	2	17.3
Average ID_{50} wild-type p53							13.7

**Individual head and neck cancer cell lines were tested by single-strand conformational polymorphism analysis for p53 mutations followed by DNA sequencing of all bands with altered mobilities. The p53 exons with mutant bands are shown. The m/m designation indicated no wild-type bands were present, whereas the m/w indicates both wild-type and mutant bands were present.*

†p53 protein expression is graded according to intensity of bands on Western blot relative to control proteins. Grading scale is as follows: (0) no expression, (1) weak expression, (2) moderate expression, (3) strong expression.

‡The mean ID_{50} for cisplatin, measured by MTT assay (see Materials and Methods) is shown for each cell line. Experiments were performed in triplicate, and the mean value is recorded.

§GeneChip reports a second mutation for UM-SCC-5 and UM-SCC-46 in exon 10 (which was not evaluated by SSCP). For UM-SCC-5, a mutation was identified at codon 342 (cga⇒tga, R⇒stop). In UM-SCC-46 a mutation was identified at codon 339 (gag⇒tag, E⇒stop).

‖The GeneChip reports this mutation, but with a score that is below the threshold level.

¶The p53 GeneChip reports no mutations above threshold for UM-SCC-23 or UM-SCC-68, whereas direct sequencing identified mutations at codon 176 and 248, respectively.

protein had a mean ID_{50} of 11.2 μ*M,* whereas cell lines with elevated expression of the p53 protein had a mean ID_{50} of 8.3 μ*M*. This difference, however, was not statistically significant ($p = .4171$, Student's t test). Similarly, there was no clear association between the proportion of cell lines with p53 overexpression and $ID_{50} < 10$ or > 10 ($p = 1.000$, Fisher's exact test).

DISCUSSION

Previous studies have suggested that p53 mutation is associated with tumor cell resistance to chemotherapy and radiation resistance.[17–24] However, our previous report indicated that overexpression of p53 protein in the tumor cells was strongly associated with chemotherapy response and larynx preservation in a cohort of patients treated with chemotherapy plus radiation in the VA trial.[6] Because p53 overexpression is associated with p53 mutation, this observation challenged the concept that tumor cells with p53 mutation are resistant to chemotherapy. In fact, our subsequent analysis of p53 status in the tumor specimens from this trial has confirmed that overexpression and mutation are closely related.[16] These observations stimulated an investigation into the relationship between cisplatin resistance and p53 status in a panel of well-characterized head and neck tumor cell lines established in our laboratory. Specifically, we investigated cisplatin sensitivity using the MTT cell survival assay, as well as determining the p53 expression and mutation status of each cell line. The rationale for choosing the MTT cell survival assay over clono-

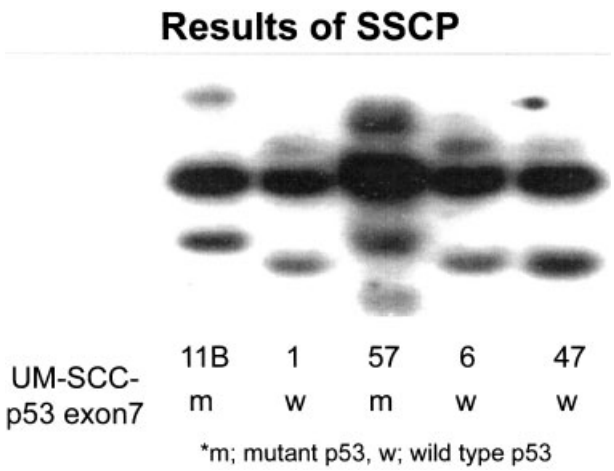

FIGURE 1. Results of single-strand conformational polymorphism analysis of exon 7 in five representative cell lines. UM-SCC-11B and UM-SCC-57 (lanes 1 and 3) show bands with altered mobility relative to the wild-type bands shown in lanes 2, 4, and 5 (UM-SCC-1, UM-SCC-6, and UM-SCC-47). All sequencing results are confirmed in an independent PCR reaction.

genic cell survival assay is the lack of clonogenicity of most of the HNSCC cell lines.[25]

The results of this study demonstrate that p53 mutation status is predictive of tumor cell response to cisplatin. Specifically, tumor cell lines with mutant p53, as a group, were significantly more sensitive to cisplatin than were those with wild-type p53. Furthermore, mutation of p53 was, in most cases, associated with overexpression of the p53 protein. Previous results obtained in patients with laryngeal cancer enrolled in the Department of Veterans Affairs Laryngeal Cancer Group revealed that tumor cells that overexpressed p53 were more sensitive to cisplatin and 5-fluorouracil than those that did not.[6] Analysis of mutations in tumors derived from this patient population is ongoing.[16] Results from this large study of pretreatment biopsy specimens will more definitively identify the predictive value of p53 mutations and other proteins in predicting the likelihood of response to chemotherapy. This study, however, corroborates the notion that p53 status correlates with response to cisplatin in head and neck carcinoma cell lines. When p53 is wild type and functional, we hypothesize that the cells are able to undergo cell cycle arrest at G1/S after exposure to cisplatin and repair cisplatin adducts. Clearly, however, p53 is not the only critical biomarker for cisplatin response. The fact that p53 mutation leads to cisplatin sensitivity in HNSCC may be more reflective of other changes occurring in concert with p53 mutation rather that the mutation status of p53 alone. In fact, normal cells containing wild-type p53 treated with cisplatin are easily killed. Why then would cisplatin resistance occur in tumor cells that retain wild-type p53? Because apoptosis pathways are likely to be involved in tumor cell response to chemotherapy, we think at least part of the explanation is that the tumors that are resistant to cisplatin may overexpress antiapoptotic proteins. In fact, we consistently find high levels of expression of the antiapoptotic proteins Bcl-2 and Bcl-x_L.[14] Bcl-x_L is overexpressed in ~75% of the specimens we examined, and Bcl-2 is overexpressed in ~15%.[14] This previous work also implicates the Bcl-x_L protein in the regulation of laryngeal cancer response to therapy.[14] We observed that low expression of the apoptosis-blocking protein, Bcl-x_L, in cancer specimens correlated with response to induction chemotherapy in patients enrolled in the Department of Veterans Affairs Laryngeal Cancer Group Study. Our hypothesis is that the combination of high levels of Bcl-x_L and wild-type p53 in HNSCC cell lines can result in cisplatin resistance. In preliminary laboratory

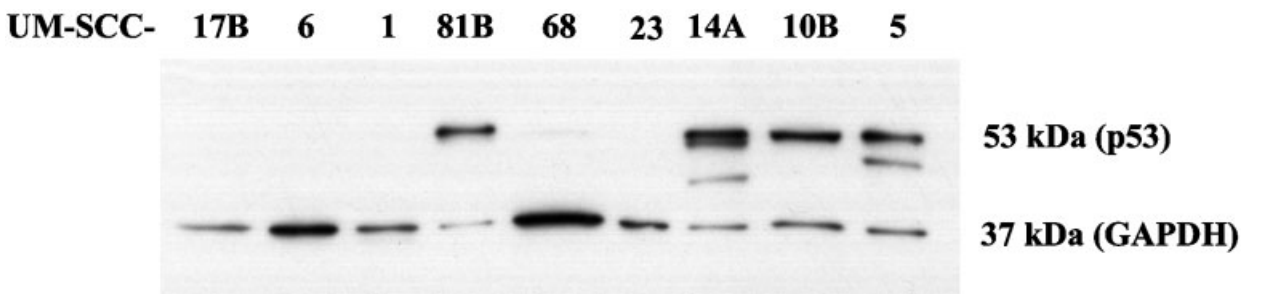

FIGURE 2. Results of Western blot for p53 protein expression relative to control GAPDH expression in nine representative cell lines. Four cell lines show no expression of p53 protein (UM-SCC-17B, UM-SCC-6, UM-SCC-1, and UM-SCC-23). One cell line has weak p53 protein expression (UM-SCC-68), and the remainder have strong p53 protein expression (UM-SCC-81B, UM-SCC-14A, UM-SCC-10B, and UM-SCC-5).

experiments conducted to date, two cell lines with this genetic makeup (ie, wild-type p53 and high Bcl-x_L) are, in fact, highly resistant to cisplatin chemotherapy (mean ID_{50} of 36.7 and 18.7 μM). Presumably, these cells do not undergo apoptosis because of high levels of Bcl-x_L but can arrest at G1/S to repair cisplatin adducts. Thus, we hypothesize that in normal cells with wild-type p53, damage induced by agents such as cisplatin leads to upregulation of p53 and upregulation of proapoptotic proteins such as Bax, Bad, and others that then lead to induction of programmed cell death. In contrast, in the squamous cell carcinomas with wild-type p53, high levels of expression of Bcl-x_L and Bcl-2 block the programmed cell death pathway allowing the cells to survive. In fact, we consistently find high levels of Bcl-x_L expression in our resistant cell lines (Oliver C, Bradford CR, Carey TE, et al, unpublished data). However, because p53 is wild type in these tumor cells, it can upregulate expression of proteins associated with cell cycle arrest and repair. Although we do not yet have complete data on the induction of these proteins, our unpublished flow cytometry results (Narayan A, Carey TE, Bradford CR) do show cell cycle arrest in the cisplatin-resistant tumor cells with wild-type p53 but not in the sensitive cell lines with mutant p53. Thus, we conclude that the combination of wild-type p53 and high Bcl-x_L/Bcl-2 expression represent one combination likely to exhibit resistance to cisplatin.

Both expression and mutation data give information that is indicative of the likely functional nature of the p53 protein. In the context of wild-type p53, there is typically little or no detectable p53 protein because of its short half-life, whereas most tumor cells harboring a mutated p53 gene do overexpress the p53 protein. However, a p53 gene mutation that results in an early stop codon is unlikely to yield functional p53 protein when the other copy of the p53 gene is lost or inactivated. Furthermore, expression of the p53 protein in the setting of wild-type p53 gene status may indicate an alteration in the pathway that affects p53 function. For example, UM-SCC-47, derived from a tongue carcinoma, is known to harbor numerous copies of HPV 16 (Carey TE, Bradford CR, unpublished results). The E6 oncoprotein is known to inactivate the p53 tumor suppressor gene.[26–29] This cell line has wild-type p53 and weakly expresses the p53 protein but is sensitive to cisplatin with an ID_{50} of 9.3 μM. One explanation of this observation could be that the E6 oncoprotein inactivates the wild-type p53 protein and thereby confers cisplatin sensitivity to this cell line.

The p53 GeneChip assay allows rapid assessment of large numbers of specimens for p53 mutations by use of microarray technology. The technique involves a single PCR amplification followed by fragmentation and fluorescent labeling of the PCR product with the oligonucleotide probe array. The array contains oligonucleotide probes with the wild-type p53 sequence in addition to the sequences of the most commonly occurring p53 mutations. The correlation of mutation data from p53 GeneChip assay and SSCP followed by direct sequencing was 89.5% in this study. This concordance rate is similar to the rate of 81% reported by Ahrendt et al.[30]

Additional markers for treatment response merit more detailed study and are ongoing in our own and in other investigator's laboratories. In particular, overexpression of cyclin D1 correlates with sensitivity to cisplatin in this panel of cell lines.[15] Preliminary studies are underway using cDNA microarrays to compare gene expression differences in panels of cisplatin-sensitive and cisplatin-resistant cell lines. Studies of this nature will no doubt explain additional components of pathways critical for chemotherapy and response in head and neck cancers. An understanding of these pathways will no doubt enhance our ability not only to predict and select patients for cisplatin-based regimens but also to develop strategies to overcome resistance.

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