Quantitation of the Change in GADD153 Messenger RNA Level as a Molecular Marker of Tumor Response in Head and Neck Cancer


Abstract

Cells injured by exposure to cisplatin (cDDP) undergo a cellular injury response that shares characteristics with responses produced by many other injurious agents. We sought to determine whether the increase of the "growth arrest and DNA damage-inducible" gene, GADD153, could be used to assess the extent of the cellular injury response in model systems and in patients with head and neck cancer after treatment with cDDP. The mRNA levels of GADD153, a gene highly transcriptionally activated by cDDP damage, were increased in a transient, concentration-dependent manner by cDDP when human UMSCC10b head and neck carcinoma cells were treated with cDDP both in vitro and when grown as tumor xenografts in nude mice. There was a good correlation between the change in level of GADD153 mRNA and UMSCC10b cell kill by cDDP in vitro (r = 0.98). The magnitude of the increase was proportionally reduced in UMSCC10b sublines that were 3- or 6-fold resistant to cDDP. GADD153 mRNA levels were measured in biopsies obtained before and 24 h after treatment with cDDP from 32 patients with stage III/IV head and neck cancer. There was a relationship between the increase in GADD153 mRNA levels and the response rate. Seven of the 32 patients had no response and no increase in GADD153 mRNA level. Among the eight patients who attained a partial response, the increase in GADD153 message ranged from 0.7–2.5-fold. In contrast, 17 of 32 patients had a complete response, and this was accompanied by a 2–9-fold induction of GADD153. The mean increase in the complete responders (3.8 ± 2.2-fold) differed significantly from that for the partial responders (1.6 ± 0.9) and nonresponders (0.8 ± 0.5; P <0.05); the difference between the partial responders and nonresponders was also significant (P <0.05). An increase of GADD153 mRNA of 1.75-fold or higher predicted a complete response, with a sensitivity of 94% and a specificity of 87%. We conclude that the magnitude of the increase in GADD153 mRNA is a promising candidate for service as an intermediate marker of head and neck tumor response to cDDP. The fact that the change in GADD153 mRNA reflects the actual extent of injury sustained by the tumor makes it particularly attractive as a potential marker. One strength of this approach is that it can provide a measure of the effectiveness of therapy as early as 24–48 h after the first dose of treatment.

Introduction

Cells cope with injury by changing their pattern of gene expression, and some of these changes occur in common in different types of cells. Transcription of the proto-oncogenes c-Fos and c-Jun, for example, is rapidly induced by exposure to a wide variety of exogenous stimuli, including cytotoxic agents (1–3). The expression of these "early-response genes" results in the rapid induction of many other genes, primarily regulated at the level of transcription (4, 5). GADD153 is one of these genes that has been implicated in the cellular response to stress. Initially isolated as a gene that is induced rapidly by alkylating agents and UV light (6) and was isolated from hamster (7) and human cells (8), GADD153 has been found to be responsive to other forms of stress and injury (9), including a broad spectrum of genotoxic agents and metabolic insults (10–15). Subsequent work has indicated that the activation of the GADD153 promoter occurred, at least in part, as a direct result of DNA damage (16). The mechanism responsible for the activation of GADD153 expression after DNA damage is still unclear, but current data suggest that the magnitude of the increase is proportional to the extent of cellular injury with maximal GADD153 promoter activity occurring under circumstances of severe toxicity to the cell (10, 16–19).

The accurate quantitation of the extent of tumor injury in patients as a result of treatment is often a problem. Normally, the effectiveness of chemotherapy or radiation therapy cannot be determined for at least several weeks and often longer, and this is an obstacle to improving the management of cancer patients, particularly when alternative therapeutic modalities are available. Quantitation of molecular events occurring in response to injury in the tumor in vivo after cytotoxic injury may permit more rapid assessment of the likelihood of response (18, 20). The fact that GADD153 is induced by DNA and cellular damage in a dose-dependent manner (17, 18, 20, 21) makes the change in the level of GADD153 mRNA an interesting candidate to monitor the extent of the injury in tumors and to serve as an intermediate marker of response. We have investigated the...
use of GADD153 mRNA measurement for this purpose in head and neck cancer. This tumor type was selected for study because of the ease of obtaining two serial tumor biopsies and the fact that several alternative therapeutic modalities are available to those patients who respond poorly to initial chemotherapy.

Materials and Methods

Cell Lines. The UMSCC10b cell line was derived from a human head and neck squamous cell carcinoma (22). Cells were cultured at 37°C under 5% CO2 in 150-cm2 flasks (Corning, Corning, NY) with RPMI 1640 (Mediatech Inc., Herndon, VA) containing 10% fetal bovine serum (Gemini Bioproducts Inc., Calabasas, CA), 2 mM L-glutamine, and 100 units/ml penicillin G and 100 μg/ml streptomycin sulfate. Cells were subcultured after reaching confluence (~5 × 10^6/150 cm^2) by trypsinization and replating at a density of ~10^5 cells/150 cm^2.

Selection for cDDP Resistance. UMSCC10b cells were selected for cDDP resistance by chronic exposure to cDDP, which was kindly supplied by Bristol-Myers-Squibb (Princeton, NY). The first three selections were performed at a concentration of 0.5 μM, which allowed ~10^7 cells to grow to confluence in a 150-cm^2 flask within 1 week. For every following set of three selections, the cDDP concentration was increased by ~20% (23, 24).

Sensitivity to cDDP in Vitro. The sensitivity of the UMSCC10b cell line and its cDDP-resistant variants to cDDP was determined by clonogenic assay using a 1-h cDDP exposure. A single-cell suspension was plated into 60-mm tissue culture dishes (Corning) at 300 cells/dish in fresh medium. After incubation for 24 h at 37°C, cells had attached to the plates and cDDP was added to the cells and incubated for 1 h, after which the cells were washed twice with PBS and 5 ml of fresh medium was added. Cultures were incubated for an additional 13–15 days, after which they were fixed with methanol, stained with Giemsa, and clusters of >50 cells were scored as colonies. cDDP-selected cells were cultured in drug-free media for 2 weeks before cDDP sensitivity was determined by clonogenic assay.

Xenografts. UMSCC10b cells were injected s.c. over the shoulder of athymic (BALB/c nu/nu) female mice, 3–4 weeks of age (Harlan Sprague Dawley, Indianapolis, IN). The xenografts were allowed to grow until they reached ~1 mm^3 in volume, at which time cDDP treatment was initiated.

Expression of GADD153 in Vivo. To determine the time course of the change in the GADD153 mRNA level, UMSCC10b cells were incubated with 100 μM cDDP for 1 h, and total RNA was extracted at 0, 1, 2, 6, 12, 24, 48, 72, and 96 h after treatment. To analyze the effect of cDDP concentration on the expression of GADD153, UMSCC10b cells were incubated for 1 h with various cDDP concentrations (0, 0.1, 1, 10, 100, and 1000 μM), and RNA was extracted 24 h after cDDP exposure.

3 The abbreviations used are: cDDP, cisplatin; NR, no response; PR, partial response; CR, complete response; RT-PCR, reverse transcription-PCR.
solution, placed in a SW40Ti rotor, and centrifuged at 32,000 rpm for 16 h at 20°C. The pellet was resuspended in 300 μl of sodium acetate (pH 7); ethanol (100%; 600 μl) was added and precipitated overnight at −70°C. The precipitate was resuspended in 50 μl of diethylpyrocarbonate-water.

**PCR Quantitation.** GADD153 message levels were quantified using a modification of the technique reported by Horikoshi et al. (29). Total RNA acquired from the biopsies or xenografts was reverse-transcribed using random hexamers to produce cDNA (28, 30, 31). Serial dilutions of the cDNA were made, and 5 μl of each dilution were placed in a sterile 0.5-ml Eppendorf tube. Taq mix (10 μl) containing 2.5 μl of Taq buffer [100 mM of Tris hydrochloride (pH 8.3) and 500 mM potassium chloride; Perkin-Elmer Corp., Norwalk, CT], 0.5 μl of 10 mM dNTPs, 1.5 μl or 2.0 μl of a 25 mM magnesium chloride buffer, and 5.48 μl or 4.88 μl of sterile PCR water, respectively, were added. In addition, 10 μl of primer mix were added containing: 1 μl of each of the sense and antisense primers (12.5 μM), 6 μl of sterile PCR water, 1.87 μl of total volume of 1 × Taq buffer and 32P-labeled dCTP (ratio 1 × buffer 23 P-dCTP, 15:1), and 0.126 μl of AmpliTaq DNA polymerase enzyme. Mineral oil was added to each tube. The tubes were placed in the thermocycler and amplified. Each set of PCR reactions contained at least one positive and one negative control (cDNA was substituted by sterile water). Each PCR reaction was performed in triplicate or quadruplicate. PCR products were separated on a 8 M (6%) acrylamide gel and analyzed using a Molecular Imaging System (Bio-Rad, Hercules, CA). For each RNA sample to be analyzed, a series of PCR reactions was performed to generate a graph of the amount of PCR product as a function of input cDNA for both GADD153 and β-actin. These data were fit to a regression line, and the result was expressed as the ratio of the slope of the regression line for GADD153 to that for β-actin. The primers listed below were synthesized by the Molecular Core Facility of the UCSD Cancer Center.

- **GADD153:** CATACATCACCACAC (sense); TGACCACTCTTGTTC (antisense);
- **β-ACTIN:** GAGCGGGAAATCGTGCTGACATT (sense); GATGGAGTTGAAGGTAGTTTCGTG (antisense);

**Statistics.** One-way ANOVA (Scheffe’s procedure) was used to evaluate the significance of differences in the magnitude of the change in GADD153 level between patients who achieved either a CR, PR, or NR.

**Results**

**Assay Validation.** The PCR technique offers the possibility to quantify mRNA after conversion to cDNA and, therefore, can be a measurement of the expression of specific genes in tumor cells. Fig. 1 shows a relative determination of GADD153 expression in UMSCC10b cells exposed to cDDP (10 μM) for 1 h. The key feature is the amount of GADD153 and β-actin product as a function of the input cDNA over a 20-fold range for both genes. For template amounts up to 2 nl and 5 nl, the increase in β-actin and GADD153 products were linear, respectively. At higher levels, the PCR products reached a plateau. Because the cellular transcript level of β-actin, a housekeeping gene, is constant under cDDP damaging conditions (32), we used β-actin as a normalization standard, determining the expression of GADD153 to that of β-actin by calculating the ratio of the slopes of the linear portion of GADD153 and β-actin curves. In Fig. 1, the slope for GADD153 was 8.3 × 10³, and that of β-actin was 1426 × 10³, resulting in a slope ratio of 0.006. This number is an empirical ratio and can be considered as an accurate measure of the relative expression of the target gene within the sample. Comparing the relative expression of the target gene in one sample with that in another sample will...
provide information about the relative expression of the target gene in the two samples. To further validate the use of RT-PCR, the coefficient of variation of the slope ratio (GADD153:β-actin) in four different cDNAs obtained from different batches of the UMSCC10b cell line was 16.6%, indicating that the PCR technique can determine relative small changes in the level of RNA messages (data not shown).

Expression of GADD153 in Human Head and Neck Carcinoma Cells in Vitro. In anticipation of the fact that the clinical use of GADD153 mRNA measurement will be facilitated if needle aspiration biopsies are sufficient for obtaining tumor tissue, the highly sensitive technique of RT-PCR, rather than the less sensitive technique of Northern blot analysis, was used throughout these studies for quantitation of mRNA. The magnitude of the change in the GADD153 mRNA level as a function of cDDP concentration was determined by treating UMSCC10b cells with 0.1, 1, 10, or 100 μM cDDP for 1 h and quantifying message level in untreated and treated cells harvested at 24 h by RT-PCR. Fig. 2 shows that there was a concentration-dependent increase in GADD153 mRNA levels that reached 6-fold at the highest cDDP concentration tested. Fig. 3 shows the time course of increase in GADD153 message level after a 1-h exposure to 100 μM cDDP. An increase in the GADD153 mRNA level was evident at 12 h after cDDP exposure, and the peak occurred at 24 h, followed by a decline to basal levels by 96 h.

Relationship between Extent of Cellular Injury and Change in the GADD153 mRNA Level. If the magnitude of the increase in GADD153 mRNA reflects the extent of tumor cell injury, then one would expect a good correlation between clonogenic survival and fold increase in the GADD153 mRNA level after treatment of UMSCC10b cells with increasing concentrations of cDDP. Fig. 4 shows that this was, in fact, the case; over a 2-log range of tumor cell kill, the correlation coefficient was 0.98. Further corroboration that the magnitude of the change in the GADD153 mRNA level reflects the extent of tumor cell damage was obtained using sublines of UMSCC10b selected for cDDP resistance. The parental cells, the 3-fold-resistant subline UMSCC10b-Pt/S6 (23, 24), and the 6-fold-resistant subline UMSCC10b-Pt/S15 (23, 24) were exposed to 2 μM, 7 μM, and 12 μM cDDP for 1 h. These levels of exposure correspond to the IC_{10}, IC_{50}, and IC_{70} for the parental cell line, respectively. Fig. 5 (left) shows that the magnitude of the increase in the GADD153 mRNA level in the 3-fold-resistant variant was lower than in the parental cells. In the 6-fold cDDP-resistant variant (Fig. 5, left), the expression of GADD153 did not increase above its basal level at all after exposure to these cDDP concentrations. These results confirm that the increase in GADD153 depends on the extent of injury caused by the drug. A cDDP concentration that was highly toxic to the sensitive parental cells and resulted
in a high level of GADD153 expression failed to produce any increase in the GADD153 message level in resistant cells in which there was little toxicity. To assure that the resistant variants had not lost their capacity to activate the GADD153 gene, and to validate the change in the GADD153 message level as a molecular marker of tumor injury in resistant as well as sensitive cells, the effect of cDDP applied at equitoxic concentrations was compared between the parental cells and the two resistant sublines. As shown in Fig. 5 (right), no difference between the cell lines in the change in expression of GADD153 was detected at the three different levels of cytotoxicity tested (IC10, IC50, IC70), indicating that the extent of cellular injury is a determinant of the level of GADD153 mRNA expression.

Expression of GADD153 in Xenografts. Nude mice were inoculated s.c. with human UMSCC10b tumor cells and then treated with cDDP (0, 10, 15, 30, or 50 mg/kg cDDP i.p.) when the tumors reached 1 ml in volume. The effect of cDDP dose on the level of GADD153 mRNA is demonstrated in the left graph, and the effect of time posttreatment is demonstrated in the right graph. Note that the scale of the ordinate differs for the two graphs.

Expression of GADD153 in Human Tumor Biopsies. If quantitation of message levels is to be used successfully to assess the extent of tumor injury, then the coefficient of variation between samples taken from the same tumor must be small enough that the changes detected in any one biopsy are reasonably representative of changes occurring throughout the tumor. A needle biopsy samples only a limited portion of a tumor nodule, and regional variation in histology, drug delivery, and cellular response is to be expected. The challenge is to demonstrate that, despite these potential sources of variance, the induction of the genes of interest can be quantitated with a sufficiently small coefficient of variation to have predictive value. We analyzed the level of GADD153 mRNA in three to five biopsies obtained from different portions of the same tumor mass in four cancer patients 24 h after administration of cytotoxic therapy, and did the same thing with an UMSCC10b xenograft.

Fig. 5 Magnitude of increase in GADD153 mRNA level in cDDP-sensitive and -resistant variants of UMSCC10b cells after exposure to equimolar concentrations of cDDP (left) and equitoxic concentrations of cDDP (right). RNA was extracted 24 h after a 1 h exposure to CDDP. Error bars, ± SD. M, parent line; □, 3-fold resistant; △, 6-fold resistant.

Fig. 6 Change in GADD153 mRNA levels in UMSCC10b xenografts growing in nude mice. The effect of cDDP dose on the level of GADD153 mRNA is demonstrated in the left graph, and the effect of time posttreatment is demonstrated in the right graph.
GADD153 is a gene that is strongly transcriptionally activated by cDDP (17, 31), as well as by other types of cellular stress (11–13, 34), including other classes of chemotherapeutic drugs (10, 35). Among the genes, the message levels of which are known to increase after cellular injury, GADD153 is of particular interest as a potential marker because of the magnitude of its induction. The results reported here further document the linkage between the extent of cellular injury as quantitated by clonogenic survival and change in mRNA level. Not only was there an excellent correlation between clonogenic survival and fold increase in GADD153 mRNA in the parental UMSCC10b cells, but the UMSCC10b-Pt/S6 and UMSCC10b-Pt/S15 sublines, which sustain less lethal injury than the parental cells at a given concentration of cDDP, had proportionately smaller increases in GADD153 message. When the cells lines were compared at concentrations of cDDP that produced the same clonogenic survival, the magnitude of GADD153 message increase was the same for all three lines. This linkage seems to extend to the in vivo situation, as well. The magnitude of the change in the GADD153 mRNA level in the xenografts increased with increasing cDDP dose over a range in which UMSCC10b tumors are known to be responsive.

Little is known about the role or function of GADD153 in the cellular injury response. Several DNA-damage inducible genes have been shown to be regulated transcriptionally via the phorbol ester-responsive element within the promoter region (1, 36). Both the collagenase and c-Jun genes contain AP-1 sites that are critical for both UV- and 12-O-tetradecanoylphorbol-13-acetate-induced expression (1). The GADD153 gene is unique in that it is not responsive to 12-O-tetradecanoylphorbol-13-acetate despite the presence of an AP-1 binding site, distinguishing itself from other response genes such as the early-response gene c-Jun and heat shock family genes. However, GADD153 can undergo inducible phosphorylation on two adjacent serine residues by a specific stress-activated MAP kinase (37), which enhances the ability of GADD153 to function as a transcriptional activator (36). Our observations indicated that GADD153 is functionally located downstream of a hypothetical injury detection site, as shown by the induction kinetics of GADD153 (Fig. 3), but upstream of the cell cycle control and cell growth events [Fig. 4, (32, 36)]. The latter suggests that GADD153 may serve as a link between early and late events in the response to cellular damage. This hypothetical chain of events is further based on findings by Wang et al. (15), who established the GADD153 protein as a stress-inducible tran-

---

**Table 1** Heterogeneity between biopsies

<table>
<thead>
<tr>
<th>Patients</th>
<th>Biopsy 1</th>
<th>Biopsy 2</th>
<th>Biopsy 3</th>
<th>Biopsy 4</th>
<th>Biopsy 5</th>
<th>Mean ± SD</th>
<th>(Variance %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0047</td>
<td>0.0068</td>
<td>0.0045</td>
<td>0.009</td>
<td>0.006</td>
<td>0.0062 ± 0.0018</td>
<td>(29.0%)</td>
</tr>
<tr>
<td>B</td>
<td>0.0015</td>
<td>0.0012</td>
<td>0.0020</td>
<td>0.004</td>
<td>0.0015</td>
<td>0.0015 ± 0.0004</td>
<td>(25.8%)</td>
</tr>
<tr>
<td>C</td>
<td>0.0030</td>
<td>0.0022</td>
<td>0.0039</td>
<td>0.004</td>
<td>0.0028</td>
<td>0.0028 ± 0.0008</td>
<td>(28.6%)</td>
</tr>
<tr>
<td>D</td>
<td>0.0003</td>
<td>0.0002</td>
<td>0.0004</td>
<td>0.0003</td>
<td>0.0003</td>
<td>0.0003 ± 0.0001</td>
<td>(27.2%)</td>
</tr>
<tr>
<td>Xenograft</td>
<td>0.0074</td>
<td>0.0078</td>
<td>0.0063</td>
<td>0.0049</td>
<td>0.0053</td>
<td>0.0064 ± 0.0012</td>
<td>(19.9%)</td>
</tr>
</tbody>
</table>

* A–D, expression of GADD153 in human tumor, determined in three to five different biopsies of the same tumor 24 h after treatment.

* Xenograft, expression of GADD153 in a human (UMSCC10b squamous carcinoma) xenograft growing in nude mice 24 h after treatment with cDDP (50 mg/kg).

---

Discussion

Elucidation of the molecular processes involved in the cellular injury response is yielding opportunities for the identification of novel markers that reflect the extent of injury produced in tumors by treatment with chemotherapeutic agents (33). Work by this and other laboratories has identified GADD153 as an important player in the cellular injury response and potentially a useful marker for response (9, 10, 14, 17, 18, 20). The work reported here documents that changes in GADD153 mRNA are quantitatively linked to the extent of tumor cell kill in vitro and to the dose of drug when the same cells are grown as xenografts in vivo. Most important, we demonstrate that change in the GADD153 mRNA level measured at 24 h is linked to the clinical response in patients with advanced head and neck carcinomas.
Table 2: Expression of GADD153 in tumor tissue of head and neck patients before and after cDDP treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Response</th>
<th>Before treatment (24 h)</th>
<th>After treatment (24 h)</th>
<th>Induction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CR</td>
<td>0.01</td>
<td>0.019</td>
<td>1.9</td>
</tr>
<tr>
<td>2</td>
<td>CR</td>
<td>0.0028</td>
<td>0.0051</td>
<td>1.85</td>
</tr>
<tr>
<td>3</td>
<td>CR</td>
<td>0.0063</td>
<td>0.0123</td>
<td>1.95</td>
</tr>
<tr>
<td>4</td>
<td>CR</td>
<td>0.00032</td>
<td>0.00114</td>
<td>3.53</td>
</tr>
<tr>
<td>5</td>
<td>CR</td>
<td>0.0105</td>
<td>0.0182</td>
<td>1.73</td>
</tr>
<tr>
<td>6</td>
<td>CR</td>
<td>0.041</td>
<td>0.143</td>
<td>3.47</td>
</tr>
<tr>
<td>7</td>
<td>CR</td>
<td>0.051</td>
<td>0.376</td>
<td>7.36</td>
</tr>
<tr>
<td>8</td>
<td>CR</td>
<td>0.00028</td>
<td>0.00126</td>
<td>4.59</td>
</tr>
<tr>
<td>9</td>
<td>CR</td>
<td>0.0235</td>
<td>0.0622</td>
<td>2.45</td>
</tr>
<tr>
<td>10</td>
<td>CR</td>
<td>0.0159</td>
<td>0.0761</td>
<td>4.80</td>
</tr>
<tr>
<td>11</td>
<td>CR</td>
<td>0.0027</td>
<td>0.0144</td>
<td>5.28</td>
</tr>
<tr>
<td>12</td>
<td>CR</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0003</td>
<td>1.75&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>13</td>
<td>CR</td>
<td>0.0145</td>
<td>0.104</td>
<td>7.15</td>
</tr>
<tr>
<td>14</td>
<td>CR</td>
<td>0.00201</td>
<td>0.00724</td>
<td>3.59</td>
</tr>
<tr>
<td>15</td>
<td>CR</td>
<td>0.00095</td>
<td>0.00895</td>
<td>9.22</td>
</tr>
<tr>
<td>16</td>
<td>CR</td>
<td>0.00612</td>
<td>0.01364</td>
<td>2.23</td>
</tr>
<tr>
<td>17</td>
<td>CR</td>
<td>0.01</td>
<td>0.0302</td>
<td>3.0</td>
</tr>
<tr>
<td>18</td>
<td>PR</td>
<td>0.00222</td>
<td>0.00242</td>
<td>1.09</td>
</tr>
<tr>
<td>19</td>
<td>PR</td>
<td>0.00661</td>
<td>0.00919</td>
<td>1.39&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>PR</td>
<td>0.0084</td>
<td>0.0105</td>
<td>1.25</td>
</tr>
<tr>
<td>21</td>
<td>PR</td>
<td>0.0069</td>
<td>0.0047</td>
<td>0.68</td>
</tr>
<tr>
<td>22</td>
<td>PR</td>
<td>0.039</td>
<td>0.046</td>
<td>1.2</td>
</tr>
<tr>
<td>23</td>
<td>PR</td>
<td>0.00215</td>
<td>0.00513</td>
<td>2.39&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>24</td>
<td>PR</td>
<td>0.00347</td>
<td>0.00398</td>
<td>1.15</td>
</tr>
<tr>
<td>25</td>
<td>PR</td>
<td>0.0228</td>
<td>0.0828</td>
<td>3.63&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>26</td>
<td>NR</td>
<td>0.00307</td>
<td>0.00277</td>
<td>0.9</td>
</tr>
<tr>
<td>27</td>
<td>NR</td>
<td>0.00126</td>
<td>0.00157</td>
<td>1.24&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>28</td>
<td>NR</td>
<td>0.001</td>
<td>0.0013</td>
<td>1.3</td>
</tr>
<tr>
<td>29</td>
<td>NR</td>
<td>0.047</td>
<td>0.0104</td>
<td>0.22</td>
</tr>
<tr>
<td>30</td>
<td>NR</td>
<td>0.204</td>
<td>0.0266</td>
<td>0.13</td>
</tr>
<tr>
<td>31</td>
<td>NR</td>
<td>0.1447</td>
<td>0.1515</td>
<td>1.05&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>32</td>
<td>NR</td>
<td>0.0073</td>
<td>0.0066</td>
<td>0.91</td>
</tr>
</tbody>
</table>

<sup>a</sup> In addition to cDDP, patients received radiation (1.8–2 Gy) within 24 h after cDDP treatment.
<sup>b</sup> Biopsy obtained in cycle 3.
<sup>c</sup> ND, not determined.
<sup>d</sup> GADD153 expression was not detectable. The detection limit for GADD153 in this sample was 0.05, resulting in a GADD153:β-ACTIN ratio of 0.00017.

**Fig. 7** Change in GADD153 mRNA levels in patients attaining either a CR, PR, or NR to cDDP-based treatment. The increase in the GADD153 message level was significantly higher in the complete responders compared with either the partial responders or nonresponders (P < 0.05 and P < 0.005, respectively, determined by one-way ANOVA, Scheffe’s procedure).

Practically, to use GADD153 as a marker for the extent of the cellular injury response in human tumor biopsies, the within-tumor heterogeneity must be small enough that a single biopsy would be sufficient to provide a reliable indication of the response of the whole tumor nodule. Our results establish that it is possible to make GADD153 mRNA measurements on the very small amounts of tissue typically obtained from a needle aspiration or cutting biopsy, and that the within-tumor coefficient of variance seems to be acceptably small, ranging from 26–29% in the patient tumors examined, indicating that the expression of GADD153 in a single tumor biopsy is sufficiently representative of the whole tumor that major changes in mRNA level can be detected. This is in agreement with results reported by two other investigators. Horikoshi et al. (29) demonstrated that thymidylate synthase mRNA levels in three different parts of the same tumor varied by only 21%. In a second study, in which bcl-2 expression was measured immunohistochemically, heterogeneous staining of the bcl-2 protein in paraffin sections of a subgroup of head and neck tumors (stages II–IV) with weak bcl-2 expression was found to be <25% (38), indicating that locally obtained molecular information can be representative for the whole tumor. Irrespective of whether a single biopsy or a paraffin section can be used as a representative sample of a rather heterogeneous tumor, our data indicated that the measurement of the GADD153 message level has clinical relevance.

Another issue of great concern with this approach is the fact that an aspiration biopsy inevitably contains some normal tissue elements, as well as tumor cells. The actual fraction of tumor cells in the sample cannot practically be determined without sacrificing most of the sample, and it is not known how GADD153 induction differs in the normal and malignant elements of the biopsy. However, analyzing the cDDP-DNA adduct formation and increase in GADD153 protein level in both tumor and stromal cells in a xenograft model, we recently estimated that ~25-fold more GADD153 protein per cDDP-DNA adduct was formed in tumor cells than in stromal cells (39). The latter indicates that the contribution of nontumor cells to the level of GADD153 expression within biopsies is minimal, at least in cases where >30% of the cells biopsied are tumor cells. Although more information is needed to resolve this issue, the good correlation between the measured increase in GADD153 mRNA and clinical response in this study argues that operationally useful information on the extent of tumor injury can be obtained.

As was expected from our in vitro and in vivo xenograft experiments, GADD153 mRNA was detectable in all tumor samples obtained from patients. The important observation that
emerged from analysis of this group of 32 patients was that the level of GADD153 mRNA after treatment increased in patients who went on to attain a CR, but not in patients who failed to respond. Thus, it seems that the linkage between actual cellular injury and increase in the GADD153 message level that was evident in the in vitro and xenograft studies of UMSCC10b, and in similar types of studies that we have previously reported on a human melanoma and ovarian carcinoma (17, 31), does translate to the clinical setting in the case of head and neck cancers treated with cDDP. It is of some concern that the magnitude of the increase in message level averaged only 3.8-fold in the patients who went on to attain a CR and that there was some overlap in the fold increase between complete and partial responders and partial and nonresponders. Additional clinical studies will be required to determine whether greater separation between responders and nonresponders can be obtained in other tumor types and with other chemotherapeutic regimens. It seems quite likely that it will be possible to identify other genes for which the magnitude of the change in mRNA is even greater than for GADD153 and that assessment of the extent of tumor injury can be refined by combining information obtained from several genes, the induction of which reflects different elements of the cellular injury response.

It was the goal of the clinical study reported here to determine the feasibility of making GADD153 measurements in the clinical setting. The fact that the magnitude of the increase in the GADD153 message in patients with far-advanced head and neck carcinoma differed as a function of whether or not the patient had a clinical response or not and that an increase of GADD153 mRNA of 1.75-fold or greater predicted a CR with a sensitivity of 94% and a specificity of 87%, indicates that the measurement of a molecular marker of cellular injury does provide prognostic information. However, there are a number of limitations to this approach, and its validation will eventually require direct comparison with other indicators of tumor response in the same group of patients. Operationally, facile clinical use of this approach requires that it be possible to make the GADD153 mRNA measurement on very small tissue samples, that the within-tumor variance be relatively small, and that the magnitude of the increase in mRNA level be large enough relative to the within-tumor variance to detect clinically significant degrees of tumor injury.

Despite the limitations mentioned above, this study has confirmed the validity of this novel approach to determine the response of tumors to treatment by analyzing the increase of GADD153 mRNA levels after cDDP treatment. It provides the basis for similar studies examining a diverse group of gene products, including other cellular injury-response gene products involved in cell cycle control and apoptosis.

References


Quantitation of the Change in *GADD153* Messenger RNA Level as a Molecular Marker of Tumor Response in Head and Neck Cancer

Gerrit Los, Khalid Benbatoul, Dennis P. Gately, et al.

*Clin Cancer Res* 1999;5:1610-1618.

**Updated version**
Access the most recent version of this article at:
http://clincancerres.aacrjournals.org/content/5/7/1610

**Cited articles**
This article cites 33 articles, 14 of which you can access for free at:
http://clincancerres.aacrjournals.org/content/5/7/1610.full.html#ref-list-1

**Citing articles**
This article has been cited by 3 HighWire-hosted articles. Access the articles at:
/content/5/7/1610.full.html#related-urls

**E-mail alerts**
Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.