Investigation of the Role of p53 in Chemotherapy Resistance of Lung Cancer Cell Lines

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Abstract. Background: p53 is a tumour suppressor gene, which is mutated in more than half of all tumours. Most chemotherapeutic drugs cause DNA damage, which is sensed by p53; the cell can then try to repair the damage or induce cell suicide. If the p53 machinery is defective, effective chemotherapy is made more difficult. Materials and Methods: Wild-type p53 was transfected into lung cancer cell lines with different p53 status. The transfected cells were tested for changes in sensitivity to a range of chemotherapeutic agents. Results: We observed only modest changes in the sensitivity to the chemotherapeutic agents adriamycin, taxol and carboplatin in the transfected cell lines. p53 protein was detected in a transfected clone of the cell line H1299, whose parent cells are p53 null. However, the protein did not accumulate after DNA damage, suggesting that this cell line utilises alternative pathways for responding to stress, and no longer has a functional p53 pathway. Conclusion: The results suggest that introduction of wild-type p53 alone is not sufficient to substantially alter the sensitivity of a cell line to a given chemotherapeutic agent.

Many agents used in the treatment of cancer cause DNA damage that is sensed by the tumour suppressor protein p53, which triggers repair of the damage or induces apoptosis. In case of a mutation or deletion in the p53 gene the efficiency of the chemotherapy is compromised. Mutations of p53 are present in more than half of all tumours. However, the role that p53 plays in dictating a cell’s response to chemotherapy remains unclear. On one hand, the p53 protein can mediate apoptosis in response to DNA damage caused by chemotherapy, thereby helping the cell to die. On the other hand, by inducing cell cycle arrest and favouring DNA repair, it has the potential to increase resistance by allowing cells to live after DNA has been damaged by chemotherapeutic agents (1). Loss of p53 function has been found to enhance cellular resistance to chemotherapeutic agents (2). The role of p53 in multiple drug resistance (MDR) may be due to its transcription of some MDR genes. Inactivation of p53 has been associated with upregulation of Mdr-1 (3, 4). MRP overexpression has also been linked to aberrant p53 expression in some cancers (5, 6). In contrast, lack of p53 function has been shown to increase the sensitivity of cells to chemotherapy (7). In the present study, to investigate the role of p53 in drug resistance, a p53 plasmid was stably transfected into three lung cancer cell lines with differing p53 status – A549 (wild-type p53), DLKP-SQ (mutant p53) and H1299 (p53 null). These cell lines were tested for the presence of wild-type p53 protein and any related changes in sensitivity to the chemotherapeutic agents adriamycin, taxol and carboplatin.

Materials and Methods

Cell culture. All cell-lines were cultured as adherent monolayers at 37°C: A549 and DLKP-SQ in Dulbecco’s modified Eagle’s medium: Hams F12 (1:1) supplemented with 5% (v/v) foetal bovine serum; NCI-H1299 in RPMI 1640 medium supplemented with 1% sodium pyruvate and 5% (v/v) foetal bovine serum.

Transfection of wild-type p53. A wild-type p53 plasmid from ATCC (VA, USA) was sub-cloned into a pcDNA3.1 vector. This was transfected into the cell lines using lipofectin reagent (Invitrogen, CA, USA). A control transfection of the empty vector was also carried out. The plasmid contained a zeocin resistance marker and successfully transfected cells were selected using a concentration of zeocin toxic to untransfected cells.

Toxicity testing. Toxicity was assessed in 96-well plates with acid phosphatase activity as the end point (8). Cells were set up in growth medium at 1x10³ per well and allowed to incubate overnight at 5% CO₂ and 37°C. Cytotoxic drug dilutions were prepared freshly at 2x final concentration and an equivolume added to each well. The plates were incubated for a further 6 or 7 days until confluency was approached in the control cells before assessment of cell survival. The concentration of drug causing 50% kill (IC₅₀ of the drug) was determined from a plot of % survival versus cytotoxic drug concentration.

Key Words: p53, lung cancer, chemotherapy resistance.
Western blotting. Western blotting for detection of p53 was performed on cell lysates that were centrifuged at 1000 rpm to remove nuclear material. Protein determination was made using the Biorad reagent (Biorad, CA, USA) according to manufacturers instructions. Samples were separated on a 10% SDS gel (9) with 20 mg protein loaded per well. After Western blotting (10), blots with the primary antibody (Calbiochem, CA, USA) were incubated overnight at 4°C. Secondary antibody conjugated to horse-radish peroxidase (Sigma, Poole, UK) were detected was by enhanced chemiluminescence (ECL, Amersham, UK). Positive controls were used for each antibody.

Results

Sensitivity to chemotherapeutic drugs. The mixed populations of the transfected cell lines were tested for any changes in sensitivity to the chemotherapeutic agents taxol, adriamycin and carboplatin. The empty vector (EV) control transfected cell lines were also included in this analysis. Some changes in sensitivity were observed in both EV-transfected and p53-transfected cells as shown in Table 1. A549 p53-transfected cells were found to be more resistant to the agents than parent and control-transfected cells. This was most evident in adriamycin treatment where the p53-transfected cells had an IC50 of 25.5 ng/ml in comparison to the parent IC50 of 15.4 ng/ml. H1299-p53-transfected cells displayed an increased sensitivity to adriamycin, however a similar increase was observed in control-transfected cells. A slight increase in resistance to taxol and carboplatin was shown in p53-transfected H1299 cells, compared to both parent and control-transfected cells. DLKP-SQ cells transfected with p53 showed a modest increase in sensitivity to taxol compared to parent and control-transfected cells. This increased resistance was not observed with adriamycin or carboplatin.

Expression of p53 protein. The mixed population of p53-transfected H1299 cells was cloned out in order to obtain pure cell lines expressing the exogenous protein. Ten clonal populations were screened for p53 protein expression. Only one of these clones, clone 8, demonstrated the presence of p53 protein (Figure 1). The p53 protein expressed in H1299-p53 clone 8 was found to accumulate after DNA damage caused by the chemotherapeutic agent adriamycin. Figure 2 shows this cell line H1299-p53 clone 8 before (−) and after (+) adriamycin treatment. The clones were tested for changes in sensitivity associated with p53 protein expression (Figure 3). This p53-expressing clone showed an increase in sensitivity to taxol. Other p53-transfected clones, which did not express detectable p53 protein, showed some changes in sensitivity to the three drugs. H1299-p53 clone 6 expresses p53 mRNA but does not express any protein and showed increased sensitivity to taxol and carboplatin and increased resistance to adriamycin (Figure 3).

Table 1. IC50s of mixed population transfection. The concentration of drug causing 50% kill (IC50 of the drug) was determined from a plot of % survival versus cytotoxic drug concentration. Results are expressed as IC50±SD, n=3.

<table>
<thead>
<tr>
<th></th>
<th>Adriamycin (ng/ml)</th>
<th>Taxol (ng/ml)</th>
<th>Carboplatin (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549-parent</td>
<td>15.4±4.2</td>
<td>1.1±0.2</td>
<td>6.7±0.4</td>
</tr>
<tr>
<td>A549-ev mp</td>
<td>13.6±3.1</td>
<td>1.7±0.5</td>
<td>12.4±3.8</td>
</tr>
<tr>
<td>A549-p53 mp</td>
<td>25.5±1.4</td>
<td>2±0.2</td>
<td>15.5±0.7</td>
</tr>
<tr>
<td>H1299-parent</td>
<td>31±0.8</td>
<td>4.3±0.1</td>
<td>5.4±0.2</td>
</tr>
<tr>
<td>H1299-ev mp</td>
<td>13.2±1.1</td>
<td>4.2±0.6</td>
<td>4.1±0.2</td>
</tr>
<tr>
<td>H1299-p53 mp</td>
<td>14.6±2.6</td>
<td>2.8±0.4</td>
<td>3.1±0.3</td>
</tr>
<tr>
<td>SQ parent</td>
<td>10.9±0.8</td>
<td>2.2±0.2</td>
<td>2.3±0.2</td>
</tr>
<tr>
<td>SQ – ev mp</td>
<td>15.7±1.4</td>
<td>2.2±0.1</td>
<td>3.3±0.2</td>
</tr>
<tr>
<td>SQ – p53 mp</td>
<td>10.3±0.7</td>
<td>1.6±0.2</td>
<td>2.2±0.3</td>
</tr>
</tbody>
</table>

ev: empty vector control transfection; p53: p53 transfection; mp: mixed population.
Discussion

The development of acquired drug resistance in tumours is believed to be based on drug-induced mutational changes in the cells. Mutations in tumour suppressor genes have been shown to be important in sensitivity changes to certain chemotherapy drugs. One well-characterised tumour suppressor gene is \( p53 \). It has been shown that it is mutated in more than half of all tumours of various types. However, the role of \( p53 \) in the development of multidrug resistance in cancer is complex and not yet fully understood. \( p53 \) protein acts as a sensor for DNA damage caused by a drug or any other mutational influence. On one hand, its presence might facilitate chemotherapy by sensing DNA damage and consequently inducing apoptosis of the cell. On the other hand, it might arrest the cell cycle allowing time for the cell to partially repair the damage then allowing mutated cells to continue growing. Within lung cancer, mutation of the \( p53 \) gene is present in about 70% of SCLC and 50% of NSCLC. In NSCLC, mutations are more frequently found in squamous cell carcinomas than in adenocarcinomas (11).

Many studies have shown that loss of \( p53 \) function confers drug resistance on cells. Loss of \( p53 \) has been associated with poor clinical prognosis in cancer treatment. Wild-type \( p53 \) was stably expressed in a p53-null cell line (SaOS-2) in order to examine the relationship between \( p53 \) expression and sensitivity to cisplatin (12). Both \( p53 \)-mediated drug resistance and \( p53 \)-mediated drug sensitivity were observed in the same model under different growth conditions, implying that growth-related pathways may be involved in \( p53 \)-mediated gene regulation. There has also been interest in development of apoptosis-inducing drugs such as PRIMA-1 that target mutant \( p53 \)-expressing cells specifically (13, 14).

In the present study a number of lung cancer cell lines with different \( p53 \) status were transfected with wild-type \( p53 \) in order to determine the role of \( p53 \) in the development of drug resistance. Many groups have observed the difficulty of expressing wild-type \( p53 \) in a cell model that does not express it endogenously (15-17). \( p53 \) protein was detected in the mixed population of transfected H1299, yet only one clone was determined to express \( p53 \). However, the \( p53 \) protein expressed in this clone did not appear to be functional. This was determined by inducing DNA damage and observing that no accumulation of the protein occurred. All of the clones isolated from the H1299-\( p53 \) transfection were tested for changes in sensitivity to adriamycin, taxol and carboplatin. Although some significant changes were detected in the sensitivity of the clones to the drugs tested, these have to be attributed to the transfection since no \( p53 \) protein was detected in most of these clones. The \( p53 \) protein expressed in H1299-\( p53 \) does not seem to function as normal wild-type \( p53 \). Wild-type \( p53 \) is characterised by low levels of the protein in untreated cells and higher levels of protein after treatment. Mutant \( p53 \) will be present at high levels in the cells regardless of DNA damaging treatment. In H1299-\( p53 \), \( p53 \) is present at quite a low level untreated and does not accumulate after treatment. It is unlikely that the protein is mutant \( p53 \) (since the plasmid contained cDNA for wild-type \( p53 \)), so the probable reason is that the cell has switched to other mechanisms for dealing...
with DNA damage, since there is no endogenous p53 protein in H1299.

In summary, the results presented indicate that p53 status alone does not determine a cell line response to chemotherapeutic agents. Others have reported that tumours with inactivated p53 are more likely to be drug resistant since functional p53 is necessary to induce apoptosis (2, 18). Also mutant p53 has been associated with upregulation of Mdr-1 (4), which would increase resistance. It has been shown here that introduction of wild-type p53 into mutant and p53 null cell lines was not sufficient to restore the p53 response to the cell lines. In contrast others have shown chemosensitivity upon introduction of wild-type p53 into cancer cells (19, 20). There are several studies indicating that inactivation of p53 is associated with an increase in sensitivity, rather than the other way around (7, 21). The results presented here suggest that wild-type p53 alone is not sufficient to significantly alter resistance to chemotherapeutic agents.

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References