Review

Multidrug resistance in non-small-cell lung cancer

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Summary

Resistance to cytotoxic drugs is an important cause of treatment failure. The causes are complex and may be determined by a combination of the tumour characteristics, such as the proportion of resting cells, adequacy of blood supply, and specific cellular mechanisms, as in the multidrug resistance phenotype. In lung cancer four types of multidrug resistance have been defined on the basis of the cellular drug targets involved, i.e., classical multidrug resistance (MDR), non-P-glycoprotein MDR (also called MRP), atypical MDR (mediated through altered expression of topoisomerases II) and lung resistance-related protein. In lung cancer the role of the different forms of multidrug resistance is complex and only partially understood.

Key words: lung resistance-related protein, multidrug resistance, multidrug resistance protein, non-small-cell lung cancer, P-glycoprotein, topoisomerases

Introduction

The development of lung cancer cell lines has allowed significant advances to be made in the biology of lung cancer, including the way in which these tumors become resistant to cytotoxic drugs. The current prognosis for patients with unresectable lung cancer is very poor, and only minor improvements in treatment have occurred in the past decade. A better understanding of drug resistance in lung cancer cells is clearly needed to improve survival in this very large group of patients.

Clearly there are multiple mechanisms of drug resistance in lung cancer cells, reflecting the heterogeneity of tumor subpopulations by the time of clinical presentation. In some tumors such as small-cell lung cancer (SCLC) this resistance is acquired during treatment while in others such as non-SCLC (NSCLC) it is inherent.

Types of multidrug resistance

In cell culture systems, multidrug resistance is a phenotype exhibited by tumor cells which, although exposed to only a single natural product type drug, display cross-resistance to an array of structurally unrelated compounds. In general these compounds are lipophilic, they range in molecular weight from 300 to 900 Da, and they appear to enter cells by passive diffusion.

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Classical multidrug resistance

Pgp is a transmembrane glycoprotein that is physiologically present in cells of the adrenal cortex, biliary canaliculi, endothelium of the blood-brain and blood-testicle barriers, placenta, gastrointestinal epithelium, proximal renal tubuli, and some bone marrow stem cells [1, 2]. In many of these organs Pgp is thought to act as a detoxifying agent by pumping toxins or xenobiotics, including anticancer agents, out of cells. A disparate group of antitumor drugs derived from natural products, e.g., vincristine, etoposide, doxorubicin, actinomycin D, are susceptible to this efflux process.

Many methods of determining the levels of Pgp in clinical samples and cell lines have been developed and include mainly measurement of MDR1 mRNA and direct assessment of the protein using immunological reagents. In addition Pgp has been shown to be capable of extruding a number of lipophilic fluorescent dyes such as rhodamine123, obtaining in such a way information about Pgp function by using flow cytometry or fluorescent microscopy to measure cellular accumulation or...
efflux. Presently there is still no consensus regarding the optimal method for detecting and quantifying levels of Pgp in human tumor samples.

Overexpression of Pgp seems to have only a minor role in clinical lung cancer resistance. Goldstein et al. [3] reported that messenger RNA levels were only detectable in five of 40 NSCLC cell lines and in 7 of 19 lung cancer tissues and that levels of expression were low. Ninety-one samples of NSCLC and corresponding normal bronchial tissues, obtained in patients submitted to radical surgery and without previous exposure to cytotoxic drugs, were investigated for the expression of Pgp using C-219 monoclonal antibody and an immunohistochemical technique. In normal bronchial tissue the immunostaining was confined to the luminal surface of the epithelium. Fifteen out of eighty-six NSCLC had more than 1/4 of examined cells positive for Pgp, but the heterogeneity of the expression ranged from rare scattered cells to a positive pattern for nearly all cells considered, without any relationship with pathologic and clinical prognostic variables [4]. Shin et al. [5] also found that multidrug resistance DNA and RNA were only detectable in three of 23 untreated NSCLCs.

Oka et al. [6] evaluated 87 lung cancer surgical tissue samples for the levels of MDR1 mRNA determined by Northern blotting and compared these findings with MDR1 positive cell lines. Fifteen percent (13 of 87) of the tumors were positive for the MDR1 gene, but the level was low in all samples except in one adenocarcinoma which expressed a high level of MDR1. The gene expression in these tumors did not correlate with any pathologic factor such as histologic type, pathologic stage or tumor size and its expression in NSCLC was not associated with tumor progression and drug resistance.

Taken together these results indicate that multidrug resistance may have only a minor role in drug resistance in lung cancer.

Several drugs, such as calcium-channel blockers and cyclosporine, can efficiently reverse the MDR phenotype in vitro. These compounds act mainly through inhibition of the drug-pumping by Pgp. As a result, they enhance the intracellular concentration of cytotoxic drugs. Clinical studies of such agents given in combination with chemotherapy have been performed in patients with hematologic cancers or solid tumors. Many of these studies attempted to achieve plasma concentrations of these agents that matched the concentrations that were effective in vitro. Moreover, at these doses many reversing agents have been too toxic; consequently, a number of studies actually used suboptimal concentrations.

Despite occasional responses in patients with hematologic malignancies and despite the positive results of a small, randomized study of the addition of verapamil to chemotherapy in untreated NSCLC [7], three large randomized trials failed to show any benefit of agents that reverse the MDR phenotype in terms of the response to chemotherapy or the length of survival. One of these trials was performed in untreated patients with SCLC and failed to demonstrate any benefit of adding verapamil to chemotherapy [8].

Multidrug resistance protein

A number of cell lines, such as the human SCLC cell line H69AR, display multidrug resistance but do not overexpress Pgp. In these cells a previously unidentified 190 kDa integral membrane phosphoglycoprotein encoded by a 6.5-kilobase mRNA, designated MRP, was isolated. Like Pgp, transfection of drug-sensitive cells with a full-length MRP cDNA is sufficient to confer multidrug resistance [9] to doxorubicin, vincristine, etoposide and colchicine.

Recently Narasaki et al. investigated MRP mRNA expression and drug sensitivity in lung cancer cell lines and the modulating effects of verapamil were also tested. Nine cell lines showed various levels of MRP gene expression but not the MDR1 gene. The levels were higher in NSCLC than in SCLC. Clear correlations between the MRP gene level and the sensitivity to etoposide and doxorubicin were observed except for one cell line which highly expressed DNA topoisomerase II [10].

Investigations of the clinical relevance of MRP in lung cancer are still in an early phase. MRP appears to be expressed at a basal level in normal lung tissue and, consequently, this point emphasizes the importance of measuring MRP expression in normal cells as a baseline and comparison for detection in malignant cells [11].

The levels of expression of the MRP gene, quantified by Northern blot analysis, in comparison with those of the MDR1 gene have been determined in 104 NSCLC specimens. Thirty-three (33%) of the one hundred four NSCLC expressed the MRP gene at various levels. Sixty-one patients received chemotherapy with MRP-related drugs (vindesine or etoposide). Twenty-three patients (38%) with tumors expressing high or moderate levels of MRP showed significantly worse prognoses than those with non- or low-MRP-expressing tumors [12].

Altered topoisomerases II expression (atypical multidrug resistance)

Another type of resistance is mediated by alterations in a single cellular target, the nuclear enzyme topoisomerase II (topo II). Drugs such as epipodophyllotoxins (etoposide and teniposide) and anthracyclines exert their cytotoxicity by stabilizing cleaved complexes of DNA and topo II. Resistance may then occur when fewer cleaved complexes are formed. This type of resistance has previously been associated with either a decrease in the levels of topo II or a mutation that alters the interaction of the enzyme with drug or DNA.

DNA topoisomerases are ubiquitous enzymes, which change DNA conformation, during important steps of DNA metabolism, such as DNA synthesis, transcription, chromosomal segregation and recombination, which
requires uncoiling of DNA. There are two major types of topoisomerases, topo I and II, functionally distinct because topo I cuts one strand of DNA, whereas topo II cuts both strands of DNA at one time, and requires ATP for its function. Recently two isoforms of topo II, called α and β, of 170 and 180 kDa, respectively have been discovered in mammalian cells [13]. Despite the high degree of homology, the two topo II isoforms are differentially regulated, have distinct properties and probably different functions. Topo II-α and to a lesser extent topo I levels are sensitive to changes in cell proliferation state, being high during rapid cell proliferation and low when cells have reached a plateau of growth, whereas topo II-β expression is rather stable during different phases of the cell cycle.

Drugs commonly used in the treatment of SCLC and NSCLC are potent topo II inhibitors (e.g., anthracyclines and epipodophyllotoxins). It is likely that the two topo II isoforms have different sensitivities to cytotoxic drugs. Moreover, water soluble derivatives of camptothecin, a potent topo I inhibitor, have been shown to be very active in the treatment of lung cancer. DNA topo inhibitors interfere with the religation step of enzyme activity, thus a stable cleavable complex is formed between the enzyme, DNA and the drug, which prevents the resealing of strand breaks. The formation of these DNA strand breaks eventually leads to cell death.

Although the expression and activity of topo enzymes has been extensively investigated in cell lines, few reports described the expression of topo genes in human tissues and malignancies and its implication with therapy. Tumor samples of 60 patients who underwent surgery for early-stage NSCLC were investigated for topo genes expression by Rnase protection assay and immunohistochemistry (Table 1). The expression of topo II-α gene was either undetectable or very low in normal tissue, while most NSCLC expressed readily quantifiable levels of this gene. No alteration of the topo II-α gene was found by Southern blotting in the NSCLC samples. In contrast to topo II-α, topo II-β was expressed in most normal as well as in tumor tissue samples, at a similar level. In tumor cells there was a positive association between the expression of topo II-α and Ki67, a marker of cell proliferation, assessed by immunohistochemistry, but not with topo II-β or topo I. Clinical characteristics of the patients and their survival did not appear to be correlated to the level of expression of any of the topo genes, although a trend towards a shorter survival was observed in patients whose tumors expressed relatively high topo II-α mRNA levels [15]. These data suggest different functions of the two topo II genes and different regulation in normal lung and lung tumor tissue.

An allelic topoisoMerase II rearrangement is present in NCI-H69 SCLC cells, which are relatively resistant to topoisoMerase II inhibitors. This rearrangement results in an abnormal transcript, which may code for an altered topoisoMerase II molecule with decreased sensitivity to topoisoMerase II inhibitors.

In addition to the quantitative and qualitative alterations in topo II that have been implicated in drug resistance, a third mechanism that involves an aberrant subcellular localization of the enzyme has been described.

Investigations of the role of topo II in drug resistance in lung cancer have been carried out predominantly in cultured lung tumor cell lines. Using SCLC and NSCLC cell lines Kasahara et al. [15] and Giaccone et al. [16] measured topo II-α mRNA or protein levels and activity, as well as drug sensitivity. In both studies, correlations were found between topo II-α content and sensitivity to etoposide and doxorubicin for some cell line subsets.

A study examined the expression of multiple resistance factors in 94 untreated NSCLC samples. Expression of glutathione-S-transferase and Pgp was found in 62% and 45%, respectively, whereas topoisoMerase II levels were decreased in 47%. In most tumors, at least two of these factors were identified. In vitro resistance to doxorubicin occurred in 40%, 56%, 90% and 100% of tumors with no, one, two, three affected factors, respectively. The high rate of resistance in the 21% of tumors unaffected by these three mechanisms emphasizes the need for further research into drug resistance in patients with lung cancer.

**Lung resistance-related protein**

A recently cloned gene, the LRP gene, appears to be associated with a multidrug-resistant phenotype [17]. This gene codes for a 110 kDa protein termed the lung resistance-related protein (FRP) that is overexpressed in several non-Pgp MDR cell lines of different histogenetic origin. Rather than an active pumping out of cytotoxic drug by a surface membrane pump, this protein may be primarily located on internal membranes and responsible for the uptake of drugs in cytoplasmic vesicles. The

### Table 1. DNA topo II-α and topo II-β expression by Rnase protection assay in normal bronchial and NSCLC tissue samples.

<table>
<thead>
<tr>
<th>Expression Level</th>
<th>Range</th>
<th>Median</th>
<th>Expression Level</th>
<th>Range</th>
<th>Median</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal lung</td>
<td></td>
<td></td>
<td>topo II-α</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluate</td>
<td>22</td>
<td>0.1</td>
<td>0.03</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Quantifiable</td>
<td>0 (0%)</td>
<td>0.08</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Undetectable</td>
<td>15 (68%)</td>
<td>-</td>
<td>0.08</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Detectable not quantifiable</td>
<td>7 (32%)</td>
<td>-</td>
<td>0.08</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>NSCLC</td>
<td></td>
<td></td>
<td>topo II-β</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Evaluate</td>
<td>57</td>
<td>0.1</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Quantifiable</td>
<td>48 (84%)</td>
<td>30</td>
<td>0.02</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Undetectable</td>
<td>7 (12%)</td>
<td>-</td>
<td>0.08</td>
<td>0.02</td>
<td>0.08</td>
</tr>
<tr>
<td>Detectable not quantifiable</td>
<td>2 (4%)</td>
<td>-</td>
<td>0.08</td>
<td>0.02</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* Relative to gene expression level of NCI-H1284 cell line, arbitrarily set as expression level = 1.
sequestered drug could not cause DNA strand breaks and would be slowly extruded from the cell by exocytosis. Reversal of MDR parallels a decrease in LRP expression.

In a panel of 61 cancer cell lines which have not been subjected to laboratory drug selection, LRP was a superior predictor for in vitro resistance to MDR-related drugs when compared to Pgp and MRP, and LRP's predictive value extended to MDR unrelated drugs, such as platinum compounds. LRP has been shown to be a strong predictor of poor response to chemotherapy and prognosis in acute myeloid leukemia and in ovarian carcinoma patients. Based on a 57% and 88% amino-acid homology with major vault proteins from Dictyostelium discoideum and Rattus norvegicus respectively, LRP has been identified as the human major vault protein. LRP is broadly distributed in normal and malignant cells but in tumor types its frequency is variable, fairly reflecting the chemosensitivity of different cancers [18]. In a preliminary study 36 NSCLC samples were studied for LRP expression and, in addition, 17 lung cancer samples (10 NSCLC and 7 SCLC) derived from patients treated with chemotherapy were analyzed in order to investigate the relationship between LRP or MRP expression and the response to chemotherapy. LRP expression was significantly higher in NSCLC samples than in SCLC samples, and all SCLC samples displayed very low LRP expression. In NSCLC patients LRP expression was not a prognostic factor for survival and at the initial analysis LRP expression levels did not predict the response to chemotherapy [19]. In another study LRP expression detected by immunohistochemistry was assessed in 87 cases of untreated NSCLC. LPR expression was detected in 45% of patients and a significant correlation between its expression and tumor resistance to doxorubicin was found ($P = 0.03$, Fisher's exact test) [20].

Conclusions

The resistance-related proteins P-glycoproteins, glutathione-dependent enzymes, topoisomerase II, metallothioneins, O-6-alkyl guanine-DNA alkyltransferase, thymidylate synthase, dihydrofolate reductase and heat shock proteins have been found in lung carcinomas, but these alone cannot explain the drug-resistant phenotype. Cell-cycle-related proteins, angiogenic factors, proto-oncogenes, and tumor suppressor genes also interplay a role in the resistant lung cancer phenotype. In addition physical and kinetic factors are relevant: quiescent cells are generally resistant to antimitabolites, including topoisomerase II inhibitors. Resistance may simply reflect a change in proliferation rate.

References


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