Expression of Proteins Associated With Multidrug Resistance to Chemotherapy in Lung Cancer

Alfredo Paredes Lario,[a] Carlos Blanco García,[b] Miguel Echenique Elizondo,[c] and Carmen Lobo[d]

[a]Servicio de Oncología, Hospital Donostia, San Sebastián, Guipúzcoa, Spain
[b]Servicio de Radioterapia, Hospital Donostia, San Sebastián, Guipúzcoa, Spain
[c]Departamento de Cirugía, Facultad de Medicina, Universidad del País Vasco, San Sebastián, Guipúzcoa, Spain
[d]Servicio de Patología, Hospital Donostia, San Sebastián, Guipúzcoa, Spain

OBJECTIVE: Membrane transporters are proteins that play a crucial role in resistance to chemotherapy. The aim of this study was to assess the influence of membrane transporter protein expression on chemotherapeutic response.

MATERIAL AND METHODS: One hundred and forty seven samples of tumor tissue were collected from 143 patients; 35 samples were obtained by bronchoscopy and 112 were surgical specimens. A total of 101 samples from 99 patients were adequate for study. Cryopreserved samples were subjected to immunohistochemical analysis to detect 3 proteins associated with multidrug resistance: P-glycoprotein (Pgp), multidrug-resistance–associated protein 1 (MRP1), and lung resistance protein (LRP).

RESULTS: In 16 cases none of the proteins were expressed. A single protein was expressed in 32 (3 Pgp, 11 MRP1, and 18 LRP); 2 in 34 cases (24 Pgp and LRP; 5 MRP1 and Pgp; 5 MRP1 and LRP); and all 3 in 17 cases. No significant relationship was found between age and the expression of Pgp (*P*=.74), MRP1 (*P*=.95), or LRP (*P*=.26). Nor were there significant differences in number (*P*=.72) or type of coexpressed proteins (*P*=.39) by sex, by tumor stage (number, *P*=.55; type, *P*=.21), or by tumor grade (number, *P*=.59; type, *P*=.51). There was a highly significant trend toward coexpression of Pgp and LRP (*P*<.01) but not of Pgp and MRP1 (*P*=.18) or MRP1 and LRP (*P*=.26). MRP1 was expressed less often in adenocarcinoma. LRP was expressed less often in squamous cell carcinoma than in adenocarcinoma and undifferentiated large cell carcinoma. Coexpression of Pgp, MRP1, and LRP was observed most often in squamous cell carcinoma.

CONCLUSIONS: Proteins associated with multidrug resistance are commonly expressed in lung cancer. Of the 3 proteins studied, LRP was the one most often found. Coexpression of more than 1 of the proteins was found in a considerable percentage of patients. Pgp was mainly found to be coexpressed with LRP. Pgp expression and the number of coexpressed proteins seemed to have a negative impact on response to chemotherapy.

Key words: *Multidrug-resistance proteins. Lung cancer. Chemotherapy.*

Correspondence: Dr. M. Echenique Elizondo.
Facultad de Medicina. UD San Sebastián. Universidad del País Vasco.
P.° Dr. Begiristain, 105. 20014 San Sebastián. Guipúzcoa. España.
E-mail: gepecelm@sc.ehu.es

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Expresión de proteínas relacionadas con resistencia a múltiples fármacos y resistencia a la quimioterapia en el cáncer de pulmón

OBJETIVO: Las proteínas transportadoras de membrana desempeñan un papel esencial en la resistencia a la quimioterapia. El objetivo del estudio ha sido intentar valorar la influencia de su expresión en la respuesta a la quimioterapia.

MATERIAL Y MÉTODOS: Se recogieron 147 muestras tumorales procedentes de 143 pacientes. De ellas, 35 eran broncoscópicas y 112 quirúrgicas. Resultaron válidas para el estudio 101, correspondientes a 99 pacientes. Las muestras tumorales criocongeladas se sometieron a análisis inmunohistoquímico para la detección de las 3 proteínas relacionadas con resistencia a múltiples fármacos (MDR-proteínas): Pgp, Mrp1 y Lrp.

RESULTADOS: No expresaban ninguna proteína 16 casos. Se encontró expresión de una sola proteína en 32 casos (3 Pgp, 11 Mrp1 y 18 Lrp); de 2 proteínas en 34 casos (24 Pgp + Lrp; 5 Mrp1 + Pgp; 5 Mrp1 + Lrp), y de las 3 proteínas en 17. No encontramos relación significativa entre la edad y la expresión de Pgp (p = 0,74), Mrp1 (p = 0,95) o Lrp (p = 0,26). No observamos diferencias significativas entre sexos por el número (p = 0,72) ni por el tipo (p = 0,39) de proteínas expresadas de forma simultánea. Tampoco detectamos diferencias significativas entre estadios tumorales por el número (p = 0,55) ni por el tipo (p = 0,21) de MDR-proteínas. No encontramos diferencias significativas entre los diferentes grados histológicos ni por el número (p = 0,59) ni por el tipo (p = 0,51) de MDR-proteínas expresadas simultáneamente. La tendencia de Pgp y Lrp a expresarse asociadas resultó muy significativa (p < 0,01), pero no fue así en el caso de la asociación de Pgp y Mrp1 (p = 0,18) o Mrp1 y Lrp (p = 0,26). Los adenocarcinomas expresaron menos la Mrp1. Los carcinomas escamosos expresaron menos Lrp que los adenocarcinomas y carcinomas indiferenciados de células grandes. Los carcinomas escamosos fueron los que con más frecuencia expresaron Pgp, Mrp1 y Lrp de forma simultánea.

CONCLUSIONES: El cáncer de pulmón expresa con frecuencia MDR-proteínas. De las 3 estudiadas (Pgp, Mrp1 y Lrp), la más frecuentemente observada fue Lrp. En una proporción importante de pacientes se halló expresión simultánea de más de una MDR-proteína. Pgp se expresó fundamentalmente asociada a Lrp. La expresión de Pgp y el número de proteínas expresadas simultáneamente parecieron afectar de forma negativa a la respuesta a la quimioterapia.

Palabras clave: *MDR-proteínas. Cáncer de pulmón. Quimioterapia.*

Introduction

Resistance to chemotherapy may be innate or acquired irrespective of the mechanisms involved.[1] Two tumor characteristics seem to determine resistance: tumor kinetics[2] and, in close association, the appearance of spontaneous mutations.[3,4] When chemotherapy is administered, many tumors, lung neoplasms among them, follow the rules set out by Skipper and Simpson-Herren[4] and Goldie and Coldman.[3] Most patients with non-small cell lung cancer do not initially respond to chemotherapy[5]; that is to say, they display intrinsic, or innate, resistance.

No definition of a resistant tumor has been clearly established for clinical purposes. Some authors consider the criteria of the World Health Organization (WHO) to be appropriate for assessing response to chemotherapy.[6] According to those criteria sensitive tumors display complete response and all others are resistant or partially resistant. For the moment no single mechanism that would confer resistance to all known drugs has been identified,[7,8] and it is therefore highly likely that resistance is caused by multiple factors.

Reduced intracellular drug accumulation is one of the most common mechanisms by which resistance to antineoplastic agents develops. The drug might be expelled through the cell membrane,[9,10] or it might be sequestered by cytoplasmic vesicles.[6] Alternatively, transport between the nucleus and the cytoplasm might be affected, or the intracellular metabolism of the drug might be altered.[11]

The study of proteins associated with multidrug resistance started in 1973 when Dano[12] discovered the active expulsion of daunomycin from resistant tumor cells; interest in these proteins has continued.[13] Multidrug-resistance–associated proteins (MRPs) are located in the plasma membrane, as is P-glycoprotein (Pgp), whereas lung resistance protein (LRP) is found in cytoplasmic vaults.[13-15] Vaults, which have been described relatively recently, are ribonucleoprotein particles of complex composition and structure.[16,17] They are nearly identical in species that are as phylogenetically distinct as amoebas and humans, suggesting that they play an essential role in eukaryotic cells.

The aim of this study was to assess the expression of Pgp, MRP1, and LRP in lung tumor tissue using immunohistochemical analysis and to study the possible correlation of that expression with chemotherapeutic response.

TABLE 1
Clinical Characteristics and Pathology Findings for 99 Patients With Lung Cancer*

Age, mean (range), y	64 (36-83)
Sex	
M	85
F	14
Tumor stage	
I	45 (IA, 14; IB, 31)
II	23 (IIA, 0; IIB, 23)
III	22 (IIIA, 15; IIIB, 7)
IV	9
Histology	
Non-small cell lung cancer	93
Squamous cell carcinoma	49 (M, 47; F, 2)
Adenocarcinoma	37 (M, 28; F, 9)
Undifferentiated large cell carcinoma	7 (M, 6; F, 1)
Small cell lung carcinoma	3 (M, 3; F, 0)
Other	3 (M, 1†; F, 2‡)
Grade	
Well differentiated	20
Moderately differentiated	35
Poorly differentiated	43
Undetermined	1

*M indicates male; F, female.
†Carcinoma with neuroendocrine differentiation, carcinoid tumor.
‡Carcinoma, unspecified.

Material and Methods

This study was performed with tumor tissue samples from patients diagnosed with and/or treated for lung cancer at Hospital Donostia, San Sebastián, Spain between April 1995 and July 2997 (Table 1).

Collection and Preservation of Samples

Surgical and bronchoscopic samples were obtained. Two surgical samples of the tumor were collected, placed in cryotubes, and stored in a portable container with liquid nitrogen until processing. A single bronchoscopic sample was collected, and transport and storage were the same as for the surgical samples.

One hundred forty-seven tumor samples were collected from 143 patients (35 samples from bronchoscopy and 112 from surgery). In the cases of 4 patients for whom both bronchoscopic and surgical samples were available, only the surgical samples were processed. Forty-six were considered unsuitable for analysis: 27 were bronchoscopic biopsies, considered inadequate due to absence of tumor tissue, and 19 were surgical samples (15 due to absence of tumor tissue, 3 because of prior chemotherapy, and 1 because the primary tumor was not pulmonary). One hundred one samples from 99 patients were considered valid for the study, which was finally carried out on 99 samples from 99 patients. Six of the 99 samples were from bronchoscopy and 93 from surgery.

Immunohistochemical Analysis of Tumor Samples and Reagents Used

The cryopreserved tumor samples underwent immunohistochemical analysis to detect the 3 proteins associated with multidrug resistance: Pgp, MRP1, and LRP. The streptavidin–biotin technique was carried out using a commercial kit (LSAB, Dako, Barcelona, Spain). Noncommercial monoclonal antibodies from the Free University of Amsterdam were also used.

The following monoclonal antibodies were used: MRPr1 and MRPm6, which recognize MRP1; LRP-56, which recognizes LRP; and JSB-1, which recognizes Pgp. The LSAB visualization system and diaminobenzidine kits (Dako) were used with acetone, bovine serum albumin, and phosphate buffered saline.

Study Variables

Dependent variable: *expression of proteins associated with multidrug resistance*. The percentages of cells labeled with each monoclonal antibody were recorded, and a result was considered positive if at least 10% of cells in the solution expressed the protein. Protein expression and the associations between expression and clinical and pathologic variables were studied from 2 perspectives:

1. Individual, or independent, expression: We analyzed the expression of each protein separately, without taking into

consideration whether or not it was coexpressed with other proteins.

2. Coexpression: We analyzed the coexpression of the proteins from 2 standpoints: *a)* positive versus negative coexpression, regardless of the number or type of proteins coexpressed; *b)* number of proteins coexpressed, regardless of type (none, 1, 2, or 3); and *c)* number and type of coexpressed proteins (none, Pgp, MRP1, LRP, Pgp and LRP, MRP1 and Pgp, MRP1 and LRP, and all 3 at once).

Independent variables:

– Age
– Sex
– Histology. The hematoxylin–eosin-stained tissue samples were examined under a microscope and classified according to the WHO criteria as follows: squamous cell carcinoma, adenocarcinoma, undifferentiated large cell carcinoma, undifferentiated small cell carcinoma, and other
– Histologic grade. The histologic grade of the samples was determined by hematoxylin–eosin staining and examination under a microscope. The most undifferentiated portion of the sample was used to determine the grade according to the following groups: well-differentiated, moderately differentiated, and poorly differentiated.
– Tumor stage. The 1997 TNM staging system of the International Union Against Cancer was used. The clinical stage was analyzed in cases that were not treated surgically and the pathology classification was used in the remaining cases

Clinical and pathologic variables (independent variables): *a)* pathology report (histologic type and grade of differentiation), and *b)* review of patient charts, from which the following information was extracted: age, sex, tumor stage, disease-free interval, survival, and response to chemotherapy. Telephone calls were made to determine survival in several cases. In other cases the official death registry in the Basque Country was consulted.

Results

The mean (SD) age of the 99 patients was 64 (10.2) years. The series was made up of 85 men with a mean age of 64 (9.7) years and 14 women with a mean age of 61 (13) years. Ninety-three patients had non-small cell tumors (49, squamous cell carcinoma; 37, adenocarcinoma; and 7, undifferentiated large cell carcinoma), 3 had undifferentiated small cell carcinoma, and 3 had other types (1 carcinoma with neuroendocrine differentiation, 1 carcinoma with no further specification, and 1 carcinoid tumor). Twenty cases were well differentiated, 35 moderately differentiated, and 43 poorly differentiated. Histologic grade could not be established in 1 case. With regard to tumor stages, 45 cases were in stage I (IA, 14; IB, 31); 23 were in stage II (IIA, 0; IIB, 23); 22 were in stage III (IIIA, 15; IIIB: 7), and 9 were in stage IV.

The expression of the studied proteins is depicted in the figure. The percentages of individual expression of each were as follows: Pgp was not expressed in 50 cases (50.5%) and expressed in 49 (49.5%); MRP1 was not expressed in 61 cases (61.6%) and expressed in 38 (38.4%); and LRP was not expressed in 35 cases (35.4%) and expressed in 64 (64.6%).

Of the 38 patients with MRP1 expression, 30 were positive for both monoclonal antibodies used and 8 were positive for only 1 (4 for each monoclonal antibody). Eighty-three of the 99 patients expressed some protein associated with multidrug resistance and 16 expressed none (Table 2). Distribution by histologic type is shown in Table 3.

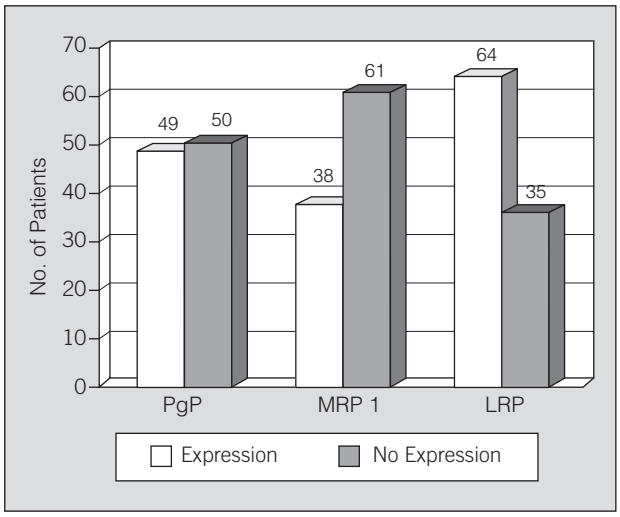

Figure 1. Expression of proteins associated with multidrug resistance in the series of 99 patients. Pgp indicates P-glycoprotein; MRP1, multidrug-resistance–associated protein 1; LRP, lung resistance protein.

The analysis of the tendency for coexpression yielded the following results: *a)* in 3 of the 49 cases with Pgp expression (6%), Pgp was the only protein identified and in 46 (93%) it was coexpressed (with LRP in 24, with MRP1 in 5, and with both simultaneously in 17); *b)* in 11 of the 38 cases with MRP1 expression (29%), MRP1 was the only protein identified and in 27 (71%) it was coexpressed (with Pgp in 5 cases, with LRP in 5, and with both in 17); and *c)* in 18 of the 64 cases with LRP expression (28%), LRP was the only protein identified and in 46 (72%) it was coexpressed (with Pgp in 24 cases, with MRP1 in 5 cases, and with both in 17). There was a highly significant tendency for Pgp and LRP coexpression ($P<.01$) but not for Pgp and MRP1 ($P=.18$) or MRP1 and LRP coexpression ($P=.26$).

TABLE 2
Number and Type of Coexpressed Proteins Associated With Multidrug Resistance*

Expression	No. of Patients
Negative	16
Positive	83
1 protein	32
Pgp	3
MRP1	111
LRP	18
2 coexpressed proteins	34
Pgp+LRP	24
MRP1+Pgp	5
MRP1+LRP	5
3 coexpressed proteins (Pgp+MRP1+LRP)	17
Total	99

*Pgp indicates P-glycoprotein; MRP1, multidrug-resistance–associated protein 1; LRP, lung resistance protein.

TABLE 3
Type and Number of Expressed Proteins Associated With Multidrug Resistance: Histology*

Proteins	Histology					Total
	Squamous Cell Carcicoma	Adenocarcinoma	Large Cell Carcicoma	Small Cell Carcicoma	Other	
None	5 (10.2%)	7 (18.9%)	0 (0.0%)	2 (66.7%)	2 (66.7%)	16 (16.2%)
Pgp	2 (4.1%)	1 (2.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.0%)
MRP1	8 (16.3%)	0 (0.0%)	1 (14.3%)	1 (33.3%)	1 (33.3%)	11 (11.1%)
LRP	6 (12.2%)	10 (27.0%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	18 (18.2%)
Pgp+RP	5 (10.2%)	17 (45.9%)	2 (28.6%)	0 (0.0%)	0 (0.0%)	24 (24.2%)
MRP1+Pgp	5 (10.2%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (5.1%)
MRP1+LRP	4 (8.2%)	0 (0.0%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	5 (5.1%)
Pgp+MRP1+LRP	14 (28.6%)	2 (5.4%)	1 (14.3%)	0 (0.0%)	0 (0.0%)	17 (17.2%)
Total	49 (100.0%)	37 (100.0%)	7 (100.0%)	3 (100.0%)	3 (100.0%)	99 (100.0%)

*Data are shown as number of patients (%). Pgp indicates P-glycoprotein; MRP1, multidrug-resistance–associated protein 1; LRP, lung resistance protein.

TABLE 4
Patients Valid for Assessment of Response to Chemotherapy: Histology, Baseline Condition, Expression of Proteins Associated With Multidrug Resistance (MDR), and Response*

Case	Histology	Stage	MDR-Proteins			Response
			Pgp	MRP1	LRP	
1	Adenocarcinoma	IIIB	–	–	–	CR
2	Neuroendocrine differentiation	MR	–	–	–	PR
3	Small cell lung carcinoma	IV	–	–	–	PR
4	Squamous cell carcinoma	MR	–	–	–	PR
5	Squamous cell carcinoma	IIIA	–	+	+	PR
6	Large cell lung carcinoma	IIIB	–	+	+	PR
7	Adenocarcinoma†	MR	+	–	+	S
8	Adenocarcinoma	LRR	+	–	+	PR
9	Adenocarcinoma	MR	+	–	+	PR
10	Adenocarcinoma	IV	+	–	+	PR
11	Squamous cell carcinoma†	MR	+	+	–	F
12	Large cell lung carcinoma	IV	–	–	+	F
13	Small cell lung carcinoma	IIIA	–	+	–	PR
14	Squamous cell carcinoma	IIIA	–	+	–	PR
15	Squamous cell carcinoma	LRR	+	+	+	S
16	Squamous cell carcinoma	LRR	+	+	+	F
17	Squamous cell carcinoma†	MR	+	+	+	F

*Pgp indicates P-glycoprotein; MRP1, multidrug-resistance–associated protein 1; LRP, lung resistance protein; CR, complete response; MR, metastatic recurrence; PR, partial response; S, stable disease; LRR, local/regional recurrence; F, failure.
†Patients who received adjuvant chemotherapy after surgery, during recurrence.

Of the 27 patients who received chemotherapy, response could be assessed in 17 (Table 4). Poor response was significantly associated with Pgp expression and the number of coexpressed proteins (Table 5). Eight of the 9 patients for whom expression of Pgp was not observed responded to treatment, as opposed to 3 of the 8 who were positive for Pgp expression. All 4 patients who expressed no protein associated with multidrug resistance responded to treatment, whereas none of the 3 patients with coexpression of all 3 proteins responded. Also detected was a nonsignificant trend toward poor response in patients with expression of LRP.

TABLE 5
Significant Pgp Expression and Response to Chemotherapy *

Pgp	Response to Chemotherapy		Total
	RC+PR	S+P	
Negative	8 (72.7%)	1 (16.7%)	9 (52.9%)
Positive	3 (27.3%)	5 (83.3%)	8 (47.1%)
Total	11 (100.0%)	6 (100.0%)	17 (100.0%)

*Pgp indicates P-glycoprotein; S, indicates stable; P, in progression; CR, complete response; PR, partial response. Significance was set at $P<.05$.

Discussion

Lung cancer, a highly prevalent disease with high mortality, represents a significant health problem.[18] The development of a cell phenotype that is resistant to chemotherapy has been the subject of intense study in recent years,[19] leading to the identification of various cellular mechanisms to explain the resistance and design ways to overcome it. Some of the treatment strategies proposed are in clinical trials.

Most of the mechanisms that are associated with lower concentrations of chemotherapeutic agents or their intracellular redistribution have been related to membrane transport proteins such as Pgp and MRP1 or vault proteins such as LRP. To date, conclusive evidence that the expression of these proteins is implicated in resistance to chemotherapy for lung cancer has been unavailable, even though such resistance is common in clinical practice. Whether resistance is intrinsic or acquired, it affects several antineoplastic agents, suggesting that it is probably the result of multiple factors.

Our study's first objective was to determine the expression of 3 proteins associated with multidrug resistance in lung cancer, and their presence was confirmed in a large number of the patients studied. LRP was the most commonly expressed protein (in 64% of the patients), followed by Pgp (49%) and MRP1 (38%). These results

are consistent with data in the literature. The earliest work on these proteins,[1,4] with the exception of that of Radosevich et al[20] in 1989, detected low expression of Pgp and it was accepted for some years that such expression was absent in lung cancer and that this protein therefore did not affect resistance to chemotherapy. Later studies, mainly based on immunohistochemistry, reported rates of expression ranging from 35% to 52% of cases (reviewed in reference 21). In frozen samples, Beer et al[22] and Scagliotti et al[23] found elevated expression in 35% and 41%, respectively, of their patients with non-small cell lung cancer. Consistent with those reports, we found expression of Pgp in 49% of our patients, and the percentage rose to 51% when only patients with non-small cell carcinoma were considered. That percentage is slightly higher than the rates in the previously cited studies, confirming that there is considerable expression of this protein in these tumors.

The range of results published for MRP1 expression is wider (between 38% and 88% of cases[24] but none of the studies that included a large number of patients used frozen material. An important aspect of the present study that distinguishes it from most of those in the literature is that the expression of 3 proteins associated with multidrug resistance was studied in the same patients. The hypothesized existence of and interaction between different mechanisms of resistance in vivo justify the importance of that design. When the sample is studied from that point of view, we find that 83% of the patients expressed a relevant protein; of those, 32% expressed only 1, 34% expressed 2, and 17% expressed all 3. This is to say, 51% of the patients expressed more than 1 protein.

Few publications examine the simultaneous expression of proteins associated with multidrug resistance in lung cancer. Zhou et al[23] studied 30 patients with non-small cell lung cancer, finding that around 40% expressed Pgp, MRP1, and LRP simultaneously, a percentage higher than the 17% we observed.

Associations between Pgp, MRP1, and LRP expression and the various histologic subtypes of non-small cell carcinoma are not usually found, but some authors have described the preferential expression of Pgp and MRP1 in squamous cell carcinoma in comparison with adenocarcinoma. We found significant differences in LRP and MRP1 expression according to histologic type. The most noteworthy differences were in MRP1 expression, which was much less in adenocarcinoma (5.4%) than in undifferentiated large cell (42.9%) or squamous cell carcinoma (63.3%). That finding of lower MRP1 expression is consistent with reports in the literature. LRP, on the other hand, was expressed less in squamous cell carcinoma than in adenocarcinoma or undifferentiated large cell carcinoma (59.2%, 78.4%, and 85.7%, respectively).

For the most common histologic types, similar percentages of patients expressed 1 or more proteins: 90% of patients with squamous cell carcinoma, 81% of those with adenocarcinoma, and 100% of those with undifferentiated large cell carcinoma.

An essential aspect of the study of proteins associated with multidrug resistance is their implication in response to chemotherapy. Whereas the relationship is clearly established for certain hematologic tumors, the data for solid tumors, such as lung cancer, are disperse and inconclusive.[24-26]

The most interesting tumor to study with regard to the relevance of these proteins is undifferentiated small cell carcinoma, for its greater sensitivity to chemotherapy.[3] The early data that linked Pgp to poor response to chemotherapy in this tumor[29] have been accepted. Yet, although positive findings are more common,[26] some authors have not found Pgp and MRP1 expression to be associated with chemotherapeutic response.[28,30]

Indirect evidence on the impact of these proteins on chemotherapeutic response in lung cancer suggests that their expression has a bearing on prognosis in chemotherapy-treated patients, although it has not been possible to firmly establish whether resistance or some other unknown factor is to blame for the poor outcome. This is the case for the findings published by Segawa et al[30] on Pgp in undifferentiated small cell carcinoma and by Ota et al[31] on MRP1 in squamous cell carcinoma. In addition, a small randomized trial of the addition of verapamil to chemotherapy in 72 patients with non-small cell lung cancer found that response and survival were better for patients who received the Pgp modulator.[32]

A possible link between these proteins and the lack of response to chemotherapy has also been suggested in non-small cell carcinoma. In 61 patients with this tumor type who were treated with regimens that included vindesine and etoposide, Ota et al[31] found that the prognosis was worse for those who expressed elevated or moderate levels of MRP1. The pattern was more pronounced in patients with squamous cell carcinoma and less pronounced in those with adenocarcinoma. Similar results were reported by Oshika et al.[29] Lu et al,[33] in a study of 69 patients, found that patients expressing LRP had a significantly poorer response to therapy. Volm et al[34] used an in vitro test of response to doxorubicin in a study of 94 patients who had not previously been treated. According to the authors, results from that test bore a good relation to response in vivo. They found there was a significant association between Pgp expression and resistance, regardless of whether expression was weak or strong. This last study would suggest that a low expression of Pgp is sufficient to give rise to a resistant phenotype. The expression of LRP was also significant in 87 cases. Harada et al[33] studied cases of squamous cell carcinoma, also finding an association between LRP expression and therapeutic response.

Our results, like those in the literature, point to a possible association between proteins associated with multidrug resistance and chemotherapeutic resistance in lung cancer. Although our study is limited by the inclusion of only a small number of cases with an evaluable response, we can conclude that *a)* the expression of proteins associated with multidrug resistance is highly prevalent in lung cancer; *b)* of the 3 proteins associated with multidrug resistance studied (Pgp, MRP1, and LRP), the last is most often expressed; *c)* simultaneous expression of Pgp, MRP1, and LRP is seen most often in squamous cell carcinoma; *d)* Pgp is mainly coexpressed with LRP and is responsible for significant differences in response to chemotherapy; and *e)* the expression of the 3 proteins associated with

multidrug resistance studied is not associated with prognostic factors such as stage and does not seem to affect survival or risk of recurrence.

Well-designed prospective studies are needed. If possible they should be combined with function and comparisons of specific inhibitors in order to provide more conclusive results. However, the design and proper execution of such studies present certain difficulties. The questions posed are as follows: What is the best method for detecting proteins associated with multidrug resistance? What is the minimum concentration or amount of a protein necessary for causing resistance in a clinical situation? Do we have reliable ways to analyze the function of proteins associated with multidrug resistance? Are there specific inhibitors that are candidates for clinical application? Because lung cancer is a highly prevalent disease associated with high mortality and significant expression of proteins associated with multidrug resistance, it offers a good setting for this type of study.

It is increasingly evident that resistance to chemotherapy is the result of many factors and that it is highly unlikely that acting on a single mechanism of resistance will offer the key to overcoming the phenomenon as a whole. It is necessary to identify as many mechanisms as possible in the same patient simultaneously. With this information, resistance profiles can be defined and individualized treatment strategies can be based on them. That goal may seem far off now, but it may also be closer than we think.

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