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H-*ras* Transfection in Mink Lung Epithelial Cells May Induce "Atypical" Multidrug Resistance

Gordon C. Wishart, Jane A. Plumb, Demetrios A. Spandidos and David J. Kerr

IT IS now well recognised that a correlation exists between oncogene expression and prognosis in certain tumour types, e.g. *erb*-B2 and breast cancer [1]. Since oncogene transfection of immortalised cells can result in relative resistance to a range of antineoplastic drugs [2] this association could be explained by the development of cellular drug resistance in the transfected cells. In a previous study we examined patterns of drug cross resistance in mink lung epithelial cells transfected with c-*myc*, H-*ras* proto-oncogene and activated H-*ras* in an attempt to predict the potential underlying mechanisms of resistance. Cytotoxic drug sensitivity was measured in the transfected lines using a tetrazolium based microtation assay [3]. In this study the mink lung line transfected with activated H-*ras* developed a clinically relevant two-fold resistance to doxorubicin and vincristine but not etoposide (Table 1). This particular pattern of resistance is consistent with that found in multidrug resistant (MDR) cell lines suggesting that the H-*ras* transfected cells may be expressing the *mdr*-1 gene and its protein product P-glycoprotein (P-gp). We have therefore examined P-glycoprotein expression in the parent line and the three transfected lines by immunohistochemistry using a monoclonal antibody to P-gp, C219 [4].

The cell lines were obtained from the parent cell line Mv1Lu by insertion of mutated (T24) and non-mutated H-*ras*1 and human c-*myc* genes in high expression vectors by a modification of the calcium phosphate precipitation technique [5]. These cell lines, named MLMC (*myc*), H06N1 (normal H-*ras*) and H06T1 (mutated H-*ras*), were expanded from a single clone and their mink origin confirmed by chromosomal analysis.

The immunohistochemical technique is described more fully elsewhere [6]. In brief, acetone-fixed cytospin preparations of all cell lines were incubated with the primary antibody C219 which was applied at a final concentration of 10 μg/ml. The secondary antibody was a rabbit anti-mouse immunoglobulin conjugated to alkaline phosphatase and used at a working concentration of 1:20 for 45 minutes. The colour reaction was developed using a substrate solution based on fast red producing a red reaction in positive cells. As a positive control we used the small cell carcinoma of lung line H69/LX10, shown previously to have high expression of P-gp [7] and in negative controls the primary antibody was substituted by an irrelevant antibody (Clonab LN-C).

Table 1. Cytotoxic response of parent and transfected cell lines following 24 h exposure to a range of antineoplastic drugs

	ID_{50} (μmol/l)			
Cell line	Cisplatin	Doxorubicin	Vincristine	Etoposide
Mv1Lu	0.41 (0.05)	2.7 (0.3)	2.4 (0.3)	1.73 (0.05)
MLMC	0.46 (0.04)	3.4 (0.3)	3.7 (0.9)	0.81 (0.05)
HO6N1	0.34 (0.03)	2.6 (0.5)	4.9 (0.6)	0.31 (0.02)
HO6T1	0.50 (0.04)	4.4 (0.3)	4.9 (0.2)	0.56 (0.02)

Mean (S.E.).
ID_{50} is the drug concentration which kills 50% of cells.

Our results show no evidence of P-gp expression in the activated H-*ras* transfected line or the two other derived lines in the presence of appropriate positive and negative controls. A number of cell lines which exhibit the MDR phenotype in the absence of P-gp expression have now been isolated [8] and the H-*ras* line may well fit in to this category.

In conclusion, we have isolated a H-*ras* transfected mink lung line which has a drug cross resistance pattern consistent with the MDR phenotype but which does not express P-gp. This pattern, often called "atypical" MDR, suggests that alternative biochemical pathways to P-gp must exist and these atypical MDR cell lines may prove useful in identifying them.

Correspondence to G.C. Wishart.
G. C. Wishart, J. A. Plumb and D. J. Kerr are at the CRC Dept of Medical Oncology, Alexander Stone Building, Garscube Estate, Switchback Road, Bearsden, Glasgow G611BD, U.K.; and D. A. Spandidos is at the Hellenic Institute Pasteur, Athens, Greece.
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