PERGAMON

European Journal of Cancer 36 (2000) 270–272

European Journal of Cancer

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Letters

Comments on: *Multidrug resistance-associated protein (MRP) expression is correlated with expression of aberrant p53 protein in colorectal cancer*, Fukushima Y, Oshika Y, Tokunaga T, *et al.*, *Eur J Cancer* 1999, **35**, 935–938. Mutant p53 and high expression of MRP are associated in acute myeloblastic leukaemia

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Received 11 October 1999; accepted 25 October 1999

We were interested to read of the association between the expression of aberrant p53 and multidrug resistance-associated protein (MRP) in tissues from patients with colorectal cancer, published recently in the *European Journal of Cancer* [1]. Our laboratory has made a similar observation in clones from patients with acute myeloid leukaemia (AML). We have previously reported point mutations in the *TP53* gene in some cases of AML [2]. We subsequently compared the expression of MRP as well as that of other drug-resistant proteins, *P*-glycoprotein (*P*gp) and the lung resistance protein (LRP) and the Bcl-family proteins: Bcl-2, Bax and Bcl-x in cryopreserved samples from these patients and in cases with wild-type *TP53* DNA. Pgp, LRP, MRP, Bcl-2, Bax and Bcl-x were measured by flow cytometry using previously published methodology [3–5]. Each mutant case ($n=5$) was age- and sex-matched with at least two with wild-type p53 cases ($n=12$). MRP, Pgp and LRP expression were measured using the Kolmogoroff/Smirnoff (K/S) statistic to ascribe the quantitative D value, on a scale of 0–1, to the difference between test and control distributions [4]. For Bcl-2, Bcl-x and Bax relative fluorescence was recorded [3,5]. Statistical analysis of differences was performed using the Mann–Whitney U test. We found that MRP overexpression was strongly ($P<0.001$, $n=16$) associated with mutations of the *TP53* gene. Pgp displayed a weak, but significant ($P<0.01$, $n=16$) association (see Table 1).

Our findings that mutant p53 and high expression of MRP are associated in AML samples add to those found by Fukushima and colleagues in colorectal cancer, as well as to previous discoveries in non-small cell lung cancer [6], suggesting that p53 control of MRP is a widespread mechanism. Wang and colleagues [7] have

Table 1
The relationship between multidrug resistance-associated protein (MRP) expression and mutations of the *TP53* gene in acute myloid leukaemia

Drug resistance protein expression		Mutant p53 cells ($n=5$) Median (range)	Wild-type p53 cells ($n=12$) Median (range)	*P* value
K/S statistic (D value)	MRP	0.90 (0.69–0.99)	0.67 (0.31–0.93)	<0.001
	Pgp	0.16 (0.08–0.72)	0.09 (0.02–0.48)[a]	<0.01
	LRP	0.62 (0.03–0.80)	0.6 (0.20–0.84)[a]	>0.1
Test/control	Bcl-2	10.40 (3.30–52.60)	13.36 (1.78–64.0)	>0.1
	Bcl-x	3.47 (2.60–130.10)	5.9 (2.97–20.70)	>0.1
	Bax	33.53 (6.05–208.00)	38.78 (7.40–211.40)	>0.1

K/S statistic, Kolmogoroff/Smirnoff; Pgp, P-glycoprotein; LRP, lung resistance protein.
[a] $n=11$.

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PII: S0959-8049(99)00286-5

demonstrated that wild-type p53 markedly suppresses MRP promoter activity to downregulate gene expression in both human and mouse cell lines. Only one report in the literature indicates a lack of association between MRP and abnormal p53 in gastric cancer, but this study reported 100% positivity for MRP [8], so would have been insufficiently sensitive to detect interclonal variation. We also found that all AML samples, i.e. those with wild-type as well as mutant p53 express at least some MRP, indicating that p53 inhibition of MRP is incomplete.

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Response from T. Tokunaga *et al*.

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We thank J. Turzanski and colleagues for their interest in our recent paper [1,2]. There are distinct differences from our study in the estimation of associations between various drug-resistant proteins including multidrug resistance-associated protein (MRP), P-glycoprotein (Pgp), lung resistance protein (LRP) and Bcl-family members and alterations in *TP53*. They carefully studied such associations in clones from patients with acute myeloid leukaemia (AML). Whereas, we examined the expression of *MRP* mRNA in 104 non-small cell lung carcinomas by reverse transcription–polymerase chain reaction (RT–PCR) and determined that all these cancers express MRP mRNA. Thus, we agree with Turzanski and colleagues that wild-type p53 is insufficient to inhibit native MRP expression. Nooter and colleagues [3] have postulated that a ubiquitous low level expression of *MRP* in various tissues plays an essential role in cellular physiology. To detect interclonal variation in *MRP* gene expression, we used Northern blotting [4] and immunohistochemical analyses [2]. Flow cytometric analysis is inadequate for solid tumour specimens that inevitably contain stromal tissues but contrastingly is a suitable and quantitative method for the analysis of single cell populations such as blood clones or clonogenic cancer cell lines. Turzanski and colleagues' results on the overexpression of MRP proteins associated with mutations of the *TP53* gene were convincing and encouraged us.

They additionally state that the expression of Pgp in acute leukaemia cells showed significant association with mutant *TP53*. We reviewed the association between Pgp overexpression and mutation of the *TP53* gene in 107 non-small cell lung cancers, 54 colorectal cancers, 30 osteosarcomas and 64 brain tumours. Regretfully, we did not confirm any significant association between them in any type of tumour. We previously reported that Pgp overexpression is related to acquired multidrug resistance in non-small cell lung cancer and osteosarcoma cell lines *in vivo*, whilst no marked overexpression was found in the expression of MRP protein [5,6]. These results suggest that alteration of MRP expression is probably found in cases with acquired *TP53* mutations.

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