

Modulation of MDR/MRP by wild-type and mutant p53

Letter to the Editor

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Loss of wild-type p53 activity and acquisition of a multidrug resistance (MDR) phenotype, two apparently key factors in the resistance of human cancers to chemotherapy, may be interrelated. Wild-type p53 represses expression of both the *MDR-1* gene (encoding the prototypical MDR protein P-glycoprotein [PGP]) and *MRP-1* (encoding the MDR-associated protein) (1, 2). Using a temperature-sensitive p53 mutant, Sullivan et al. (3) recently claimed in the *JCI* that the introduction of a mutant p53 variant promoted MRP expression and activity in a prostate cancer cell line. Our experience with transfection of p53 into other tumor cell lines casts their data in a different light.

We transfected five previously characterized (4) human malignant glioma cell lines with the murine temperature-sensitive p53^{V135A} mutant, which is dominant-negative over endogenous wild-type p53 at 38.5°C but assumes wild-type properties at 32.5°C, medi-

ating the strong induction of p21 expression and causing G2/M arrest (5, 6). Control cell lines (hygro) were transfected with the same plasmid lacking a p53 cDNA insert. Changes in PGP or MRP protein levels were examined 48 hours after the cells were placed at the permissive temperature of 32.5°C (Table 1, upper part). At 32.5°C, expression of p53^{V135A} caused a decline in PGP expression and activity in two cell lines (LN-18 and LN-308) but left these parameters unaffected in two cell lines (U87MG and LN-229). p53^{V135A} caused an increase in PGP activity but no significant change in PGP expression in T98G cells. These changes mediated by p53^{V135A} at wild-type conformation did not correlate with the endogenous p53 status of the cell lines. Under these same conditions, MRP expression and activity were increased by expression of p53^{V135A} in three of the five cell lines (U87MG, T98G, and LN-229) and unaffected in two cell lines (LN-18 and

LN-308) at 32.5°C. As with PGP, no correlation emerged between the p53^{V135A}-mediated changes at 32.5°C and endogenous p53 status.

Since several studies have reported that mutant p53 may activate MDR gene expression (1, 7–9), probably by both dominant-negative and gain-of-function mechanisms, changes mediated by p53^{V135A} in mutant conformation (38.5°C) were also carefully considered (Table 1, lower part). At 38.5°C, p53^{V135A} enhanced PGP expression and activity in three of the five cell lines (U87MG, T98G, and LN-229), whereas the opposite pattern was observed in the other two cell lines (LN-18 and LN-308). Similarly, p53^{V135A} had heterogeneous effects on MRP expression and activity. At 38.5°C, p53^{V135A} reduced MRP expression and activity in LN-18 cells but increased MRP expression and activity in T98G and LN-229 cells.

We also examined the modulation of glioma cell sensitivity to vin-

Table 1

Modulation of PGP and MRP expression and activity by p53^{V135A} at permissive (32.5°C) and restrictive (38.5°C) temperatures

Temperature	Cell line	p53 status ^A	Transfection ^B	PGP expression	PGP activity	MRP expression	MRP activity
				SFI ^C	Transport ratio ^D	SFI ^C	Transport ratio ^D
32.5°C	LN-18	mutant	hygro	9.99	7.5	3.45	1.92
			p53 ^{V135A}	2.85 ^E	3.5 ^E	3.65	1.73
	U87MG	wild-type	hygro	2.78	1.5	2.54	1.8
			p53 ^{V135A}	2.61	1.46	3.37 ^F	2.18 ^F
	T98G	mutant	hygro	1.49	1.62	7.54	3.50
			p53 ^{V135A}	1.57	2.45 ^F	13.4 ^F	5.58 ^F
	LN-229	heterozygous with wild-type activity	hygro	5	2.08	1.8	1.51
			p53 ^{V135A}	4.3	1.97	2.3 ^F	1.75 ^F
	LN-308	deleted	hygro	5.3	4.7	4.8	1.98
			p53 ^{V135A}	4 ^F	2.47 ^F	4.5	2.35
38.5°C	LN-18	mutant	hygro	18.7	8.2	3.9	2.27
			p53 ^{V135A}	7.7 ^F	5 ^F	2.95 ^F	1.99 ^F
	U87MG	wild-type	hygro	1.73	1.4	3.56	1.95
			p53 ^{V135A}	2.79 ^E	2.03 ^E	3.32	1.91
	T98G	mutant	hygro	1.47	1.4	9.02	4.17
			p53 ^{V135A}	1.76 ^F	1.79 ^F	11.34 ^F	4.65 ^F
	LN-229	heterozygous with wild-type activity	hygro	5.6	2.3	1.7	1.38
			p53 ^{V135A}	6.45 ^F	3.2 ^E	2.8 ^E	1.58 ^F
	LN-308	deleted	hygro	7	5.42	5.4	2.04
			p53 ^{V135A}	5.7 ^F	4.92 ^F	5.4	2.22

^AFor details, see ref. 4. ^BThe cells were transfected by electroporation and selected in hygromycin-containing medium (6). ^CPGP and MRP protein levels were determined by flow cytometry using specific monoclonal antibodies MRK-16 and MRPr1 (Hözel Diagnostics, Cologne, Germany) and were expressed as specific fluorescence indexes (SFIs) derived from calculating mean fluorescence with specific antibody divided by fluorescence obtained with control antibody (13, 14). ^DPGP and MRP transport activity were determined by rhodamine and fluorescein expulsion assays and expressed as ratios of fluorescence (probe retention) in the presence vs. absence of a MDR-1 inhibitor, verapamil (VPM) or a MRP inhibitor, indomethacin (INDO) (13, 14) (^E*P* < 0.01, ^F*P* < 0.05, *n* = 3, *t*-test, p53^{V135A} compared with hygro).

Table 2

Modulation of VCR cytotoxicity in the absence or presence of verapamil or indomethacin by p53^{V135A} at permissive (32.5°C) and restrictive (38.5°C) temperatures

Temperature	Cell line	Transfection	EC ₅₀ VCR (nM) ^A	EC ₅₀ VCR (nM)		VPM effect		EC ₅₀ VCR (nM)		INDO effect	
					+ VPM	[EC ₅₀ - VPM/EC ₅₀ + VPM]	+ INDO	[EC ₅₀ - INDO/EC ₅₀ + INDO]			
32.5°C	LN-18	hygro	71.1	4.36	16.3 ^F	58.3	1.22				
		p53 ^{V135A}	433.7 ^C	96.4	4.5 ^E	347	1.25				
	U87MG	hygro	22.3	7	3.2 ^E	8.3	2.7				
		p53 ^{V135A}	85 ^D	30.6	2.8 ^E	56.7	1.5				
	T98G	hygro	7.9	0.01	755.9 ^F	0.017	466.2 ^F				
		p53 ^{V135A}	>1200	>1200	NA ^B	>1200	NA				
LN-229	hygro	15.4	1.66	9.3 ^F	5.92	2.6					
	p53 ^{V135A}	75.6 ^C	23.6	3.2 ^E	75.3	1					
LN-308	hygro	12.8	0.0354	360.6 ^F	10.5	1.22					
	p53 ^{V135A}	4.4 ^C	0.0343	128.4 ^F	3.26	1.35					
38.5°C	LN-18	hygro	143.8	8.56	16.8 ^F	128.4	1.12				
		p53 ^{V135A}	92 ^D	2.37	38.8 ^F	88.5	1.04				
	U87MG	hygro	53.4	8	6.7 ^E	22.3	2.4				
		p53 ^{V135A}	43.8 ^D	3.1	14.1 ^F	21.9	2				
	T98G	hygro	44.4	0.212	209.3 ^F	0.0473	938.6 ^F				
		p53 ^{V135A}	42.2	2.64	16 ^F	0.0625	674.7 ^F				
LN-229	hygro	18.5	0.9	20.6 ^F	15.4	1.2					
	p53 ^{V135A}	17	0.404	42 ^F	1.3	1.5					
LN-308	hygro	250	4.36	57.4 ^F	227.3	1.1					
	p53 ^{V135A}	47.6 ^C	0.21	226.4 ^F	43.3	1.1					

^AThe cells were seeded at a density of 10⁴ cells per well in 96-well plates, adhered for 24 hours, maintained at 38.5°C or adapted to 32.5°C for 4 hours, and exposed to VCR in the absence or presence of verapamil (VPM; 20 μM) or indomethacin (INDO; 100 μM) for 72 hours. Survival was assessed by crystal violet staining. ^BWild-type p53^{V135A}-expressing T98G cells become highly resistant to VCR, and VCR resistance is not overcome by VPM or INDO (^C*P* < 0.01, ^D*P* < 0.05, *n* = 3, *t*-test, p53^{V135A} compared with hygro; ^E*P* < 0.05, ^F*P* < 0.01, *n* = 3, *t*-test, effect of VPM or INDO).

cristine (VCR), a classic substrate of both PGP and MRP, by p53^{V135A} at 32.5°C or 38.5°C (Table 2). At 32.5°C, p53^{V135A} induced dramatic resistance to VCR in four of the five cell lines, although LN-308 cells were sensitized to this drug. Further, we asked how p53^{V135A} affected the ability of a MDR-1 inhibitor, verapamil (VPM), or a MRP inhibitor, indomethacin (INDO), to enhance VCR sensitivity. At 32.5°C, p53^{V135A} uniformly decreased the sensitizing properties of VPM compared with isogenic control transfectants (hygro) under identical experimental conditions. In contrast, p53^{V135A} had little effect on the sensitizing effect of INDO. Note that INDO has biologically relevant effects only in T98G cells, as predicted from the strong expression of MRP in these cells but not in the other cell lines (Table 1). At 38.5°C, p53^{V135A} uniformly increased VCR sensitivity in the glioma cell lines, albeit moderately in all but LN-308 cells. Moreover, p53^{V135A} enhanced the ability of VPM to increase VCR sensitivity in four of the five cell lines (the exception being T98G), whereas the effects of INDO on VCR sensitivity were largely unaffected at 38.5°C. All

studies reported here were performed with pooled transfectants, precluding cloning artifacts as an explanation for unexpected data.

Our data highlight several important issues that need to be considered when using temperature-sensitive p53 mutants to study cancer cell sensitivity to chemotherapy or irradiation. First, it is imperative to always examine isogenic, empty vector control cell lines under identical experimental conditions. Shifting the control cells from 38.5°C to 32.5°C alone significantly (a) reduced PGP expression and activity in LN-18 and LN-308 cells but increased PGP expression in U87MG cells, (b) reduced MRP expression in four of the five (LN-18, U87MG, T98G, and LN-308) and MRP activity in two of the five (LN-18, T98G) cell lines, and (c) increased VCR sensitivity in all cell lines. There is no information in the work of Sullivan et al. (3) indicating whether such controls were performed and whether temperature had any effect on MRP expression or activity in the cells examined in their study. Importantly, heat may transcriptionally activate MDR expression (10), but hyperthermia has also been suggested as one strategy to overcome MDR-type drug resistance

(11), further supporting the need for careful controls when examining the modulation of drug resistance using temperature-sensitive p53 mutants.

Second, in contrast to the proposed negative regulation of MDR transcription by wild-type p53 (7), we found that p53^{V135A} at wild-type conformation (32.5°C) fails to reduce PGP expression and activity in three of the five cell lines. Moreover, in the two cell lines in which p53^{V135A} reduced PGP expression and activity at 32.5°C, LN-18 and LN-308, the same effects were observed at 38.5°C, where p53^{V135A} assumes mutant conformation and would be expected to enhance rather than reduce MDR expression and activity. Thus, these observations dissociate p53^{V135A}-dependent modulation of MDR from the conformation of the sequence-specific DNA-binding domain of p53^{V135A} in cell lines lacking p53 function. On the other hand, the effects of p53^{V135A} in p53 wild-type cell lines, U87MG and LN-229, conform to current concepts of MDR modulation by p53 (1). This is because p53^{V135A} had no effect on MDR expression at wild-type conformation (32.5°C) but enhanced MDR expression and activity at 38.5°C, acting as a dominant-negative mutant (5). Finally, although

activation of rat MDR1b transcription by wild-type p53 has been described (12), the only cell line responding to p53^{V135A} with moderately enhanced PGP activity at 32.5°C, T98G, behaved accordingly at 38.5°C, where p53^{V135A} assumes mutant conformation.

Third, in contrast to the proposed negative regulation of MRP gene transcription by wild-type p53 (2), p53^{V135A} at wild-type conformation (32.5°C) not only failed to reduce MRP expression and activity in any of the cell lines examined but enhanced MRP expression and activity in three of the five cell lines. Again, p53^{V135A} also promoted MRP expression and activity in two of these three cell lines at 38.5°C. Since no such effect was seen in U87MG cells at either temperature, and since p53^{V135A} promoted MRP expression and activity in LN-229 cells at both temperatures (Table 1), the modulation of MRP by p53 is different from the modulation of MDR by p53 in glioma cells. Moreover, these data contrast sharply with the effects of temperature-sensitive p53 on MRP in a prostate carcinoma cell line (3). Thus, there is striking heterogeneity of the MDR/MRP responses to p53 alterations among cell lines of the same histogenetic origin, and studies of p53 and MDR/MRP interactions must not be extrapolated from one cell type to another.

Fourth, previous studies have shown that two critical hydrophobic amino acids in the NH₂-terminal domain of p53, as well as the oligomerization and non-sequence-specific DNA-binding domains, are required for the tran-

scriptional activation of the MDR gene by tumor-derived p53 mutants (8, 9). These two amino acids and the mentioned domain of p53 are preserved on p53^{V135A}. The uniform effects of p53^{V135A} on T98G cells at both temperatures, for instance, could be attributed to such effects. On the other hand, most of the effects of p53^{V135A} observed here could not have been predicted by the current understanding of MDR/MRP regulation by p53, suggesting that other genetic alterations, which differ among the glioma cell lines, determine how the MDR/MRP systems are modulated by p53^{V135A} at either conformation.

Fifth, the use of temperature-sensitive p53 mutants for the study of chemoresistance requires careful consideration of controls (6). Thus, we show here that although p53^{V135A} in its mutant conformation at 38.5°C uniformly increased VCR sensitivity in the glioma cell lines, it had heterogenous, indeed opposing, effects on MDR/MRP expression and activity. Clearly, p53-dependent changes in cellular sensitivity to the classic MDR and MRP substrates are not due exclusively to the modulation of MDR or MRP expression.

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Reply to Modulation of MDR/MRP by wild-type and mutant p53

Sullivan et al. reply, (1) Our paper demonstrated that the expression of MRP1 at the level of transcription, translation, and function was repressed by wild-type p53 in human prostate cancer, human melanoma, and human colon carcinoma cell lines. In these experiments, we used either transfection with a human p53 temperature-sensitive mutant, or isogenic cell lines in which p53 was inactivated by either the E6 protein of human papilloma virus 16/E6 or a dominant-negative p53 mutant in the case of the colorectal cancer transfec-

tants. We demonstrated that shifting the control cells from 38°C to 32°C had no effect on MRP expression or function (Figures 3–7). Previously, Wang and Beck reported the ability of wild-type p53 to repress MRP1 promoter activity (2). We did not investigate the expression of MDR1, since we had previously shown that P-glycoprotein was not expressed in over 90 human prostate cancer specimens obtained at the time of surgery (3).

The findings described in the letter of Bähr and colleagues in glioma cells are different from our paper and from those reported in the literature. It may be as the authors suggest that different genetic alterations exist in individual glioma cell lines; these would require further elucidation.

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