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Progression toward tumor cell phenotype is enhanced by overexpression of a mutant p53 tumor-suppressor gene isolated from nasopharyngeal carcinoma

(neoplastic phenotype/negative dominance/DNA transfection/mouse JB6 cells/human Saos-2 cells)

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ABSTRACT **We recently reported the detection of a heterozygous G → C point mutation at codon 280 of p53 in nasopharyngeal carcinoma, which causes an Arg → Thr substitution. To test whether this mutant p53 has gained function as an oncogene, we overexpressed the mutant p53 in nontumorigenic cells of two model systems: (*i*) human Saos-2 cells lacking endogenous p53 and (*ii*) mouse JB6 variants that bear endogenous wild-type p53. Although they have no growth advantage over the neomycin controls in monolayer culture, human Saos-2 transfectants overexpressing mutant p53 do show enhanced progression to tumor cell phenotype, as assayed by anchorage-independent growth and *in vivo* tumorigenicity. The enhancement is seen only in transfectants expressing higher levels of p53 protein. In the mouse JB6 system, the mutant p53 functions dominantly in the presence of endogenous wild-type p53 to enhance progression of preneoplastic promotion-sensitive cells toward anchorage-independent phenotype. Mouse JB6 transfectants of mutant p53 are, however, not tumorigenic in nude mice. We conclude from these studies that the G → C point mutation of p53 at codon 280 is a gain-of-function mutation that appears to operate dominantly and that the mutant p53-thr^{280} has only moderate oncogenic activity. This mutation may cooperate with other yet-to-be isolated genes in the genesis of nasopharyngeal carcinoma.**

Mutation of the p53 tumor-suppressor gene has been found to be the most frequent molecular alteration in human cancers (1, 2). Most p53 mutations are missense mutations clustered in five evolutionarily conserved regions (1, 2). The outcomes of p53 mutations can be divided into three categories: (*i*) gain-of-function mutations: mutant p53 gains oncogenic activity; (*ii*) loss-of-function mutations: mutant p53 loses tumor-suppressor function; and (*iii*) silent mutations: like wild-type p53, mutant p53 has tumor-suppressor function (3–7).

We have recently detected an infrequent heterozygous G → C mutation at codon 280 of p53 in nasopharyngeal carcinoma (NPC) (8). This mutation causes an Arg → Thr substitution. To test whether this mutation is a gain-of-function mutation, we transfected the mutant p53, p53-thr^{280}, into cells of two model systems: (*i*) human Saos-2 cells lacking endogenous p53 and (*ii*) mouse JB6 variants bearing wild-type p53. The Saos-2 cell line is a human osteogenic sarcoma cell line with both p53 alleles deleted (9) that is weakly or nontumorigenic (ref. 10; American Type Culture Collection catalog). This no-p53-background cell line facilitates the examination of phenotypic changes caused by introduced exogenous p53. The mouse JB6 variants were originally established from untreated BALB/c primary mouse epidermal cell culture (11, 12). P$^-$ (promotion resistant), P$^+$ (promotion sensitive), and Tx (transformed) variants represent a series of cell lines ranging from earlier to later stages of preneoplastic progression. We have previously reported that P$^-$ variants gain the P$^+$ phenotype upon transfection with activated promotion-sensitivity sequences (*pro* genes) isolated from P$^+$ cells (13, 14) or at a low frequency by phorbol 12-myristate 13-acetate (PMA) treatment and that P$^+$ variants gain the transformed phenotype irreversibly upon PMA treatment (11, 12) or after transfection with a transformation-associated DNA sequence isolated from NPC (15). The JB6 variants, therefore, provide a useful system for testing the role of a gene during induction of multistage carcinogenesis (oncogenes) or in the reversion of transformed phenotype to P$^+$ or P$^-$ phenotype (tumor suppressors). Due to their endogenous wild-type p53 status, JB6 variants also provide an opportunity for assessing the negative dominance of an introduced mutant p53 gene.

In this study, we have examined gain or loss of tumor cell phenotype measured by anchorage-independent growth and *in vivo* tumorigenicity in nude mice after transfection of p53-thr^{280} and/or wild-type p53 into human Saos-2 cells or JB6 variants. We report here that overexpression of p53-thr^{280} enhances progression toward tumor-cell phenotype, as tested in both systems, and that overexpressed wild-type p53 does not cause reversion of phenotype in P$^+$ or transformed JB6 variants.

MATERIALS AND METHODS

Construction of Vectors Expressing Wild-Type or Mutant p53 Proteins and Sequencing Verification. Wild-type or mutant p53-thr^{280} cDNAs containing the entire open reading frame were obtained from NPC cell line CNE$_2$A15 by reverse transcriptase-PCR technique (8). p53 cDNAs generated by PCR were *Eco*RI-digested and subcloned into mammalian expression vector pMM, under transcriptional control of the metallothionein promoter and inducible by Zn^{2+} (M. Birrer, National Cancer Institute, personal communication). The entire length of p53 cDNA subcloned was sequenced to ensure the sense orientation and either wild-type or codon-280 G → C mutant status. In mutant p53 cDNA there are four reverse transcriptase- or PCR-generated silent mutations at codons 16 and 331 (G → A, Gln → Gln), codon 277 (T → C, Cys → Cys), and codon 389 (T → C, Pro → Pro).

Cell Culture, DNA Transfection, and Neomycin Selection. The Saos-2 cell line, known to lack endogeneous p53 (9), was from the American Type Culture Collection and was cultured

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Abbreviations: NPC, nasopharyngeal carcinoma; PMA, phorbol 12-myristate 13-acetate; FCS, fetal calf serum.

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in McCoy's 5A medium (Whittaker Bioproducts)/15% fetal calf serum (FCS). Mouse JB6 variants P^-, P^+, and Tx cells were cultured in 5% FCS/minimal essential Eagle's medium (Whittaker). The p53 status in all three variants was examined by reverse transcriptase-PCR-direct sequencing of the entire coding region of p53 and was verified to be wild type (Y.S., K.N., G. Hegamyer, Z. Dong, and N.C., unpublished work). Plasmid DNA (2–4 μg) expressing wild-type or mutant p53 protein was transfected by using Lipofectin reagent (BRL) into Saos-2 cells or JB6 variants at 50–70% confluence in 60-mm dishes. The transfected cells were subjected to drug selection by including Geneticin (G418; GIBCO) in the culture medium (500 μg/ml for Saos-2 and P^+ cells, 250 μg/ml for P^- cells, and 1000 μg/ml for Tx cells, RT101). Each individual clone (eight clones per transfection group) was ring-isolated and expanded in the presence of G418 and analyzed for expression by RNA blotting. The two clones having the highest introduced p53 mRNA expression per group were chosen for characterization after verification of protein expression by immunoprecipitation.

RNA Analysis and Immunoprecipitation. Northern analysis of total RNA was done as described (16). Subconfluent cells were either directly labeled or pretreated with $ZnSO_4$ (75 μM for Saos-2 cells and 25 μM for JB6 variants) for 2 hr before 1-hr methionine starvation in a methionine-free RPMI 1640 medium/5% dialyzed FCS. Cells were then Trans[^{35}S]-labeled at L-methionine (0.2 mCi/ml; 1 Ci = 37 GBq; ICN) for 2 hr. Labeled cells were lysed on ice for 30 min in phosphate-buffered saline/2% Nonidet P-40/0.2% SDS/0.5% sodium deoxycholate/1 mM sodium orthovanadate/5 mM NaF/5 mM sodium pyrophosphate/1 mM phenylmethylsulfonyl fluoride/leupeptin at 1 μg/ml/aprotinin at 0.2 unit/ml. Samples having equal trichloroacetate-precipitable radioactivity (3×10^7 cpm) were immunoprecipitated with anti-p53 pAb240 (Oncogene Science, Manhasset, NY) for 2 hr at room temperature. Immune precipitates were analyzed by electrophoresis on a SDS/10% polyacrylamide gel, followed by autoradiography.

Soft-Agar Assay and *in Vivo* Tumorigenicity. Fifty thousand single Saos-2 cells, after 2 hr of 75 μM $ZnSO_4$ pretreatment, or 10,000 JB6 cells were suspended in 0.33% agar/10% FCS in a 60-mm dish with (JB6 cells only) or without PMA (16 ng/ml, 2.56×10^{-8} M). Colonies of eight or more cells were counted after 26 days for Saos-2 cells or 14 days for JB6 cells (11). For *in vivo* tumorigenicity, 1×10^7 Saos-2 cells, pretreated with 75 μM $ZnSO_4$ for 2 hr, or 2×10^6 JB6 variants, were injected s.c. into the upper back of athymic nude mice (Harlan–Sprague–Dawley), and tumor formation was scored every week.

Statistical Analysis. Statistical significance was tested by ANOVA. Newman–Keuls multiple comparison procedure at a 0.05 significance level was used to assess the differences from the neomycin control in soft-agar colony numbers generated by transfectants.

RESULTS

Overexpression of p53-thr^{280} in Human Saos-2 Cells Enhances Progression to Tumor Cell Phenotype. There are two advantages to using Saos-2 cells as recipients in this study. (*i*) They lack endogenous p53 and (*ii*) although they are derived from an osteogenic sarcoma, Saos-2 cells show behaviors characteristic of preneoplastic cells, including indefinite lifespan and inability to form tumors in nude mice (American Type Culture Collection catalog). Thus, these cells have considerable capacity to progress to a more transformed phenotype. The Saos-2 cells were transfected with p53-thr^{280} and/or wild-type p53 and subjected to neomycin selection. Initially the wild-type p53 transfectants showed a dramatic decrease of growth rate in monolayer, whereas double transfectants (with both wild-type and p53-thr^{280}) showed growth rates similar to that with p53-thr^{280} alone, a rate comparable to neomycin-only controls (doubling time ≈ 40 hr). After stable transfectants were selected and expanded from the single and double transfectants, only p53-thr^{280} was expressed, as shown by a single immunoprecipitated band (Fig. 1; compare with Fig. 3). Wild-type p53 protein was not expressed in single or double transfectants (data not shown). Two mutant transfectants H-7-6 and H-7-1 expressed high levels of mutant p53 protein (*Upper*). One of the mutant transfectants, H-7-F, expressed detectable mutant p53 only after Zn^{2+} induction (*Lower*). Zn^{2+} pretreatment also induced expression of the other two clones (*Lower*). The levels of protein expression paralleled mRNA expression (data not shown). No p53 expression occurred in the neomycin-control clone H-5-1. A human epidermoid carcinoma cell line, A-431, harboring a codon-273 mutation in p53 (17) was included as a positive control. These three mutant p53 transfectants along with the neomycin-control clone were used for characterization of possible progression to tumor cell phenotype caused by the introduced p53-thr^{280}. Fig. 2 shows that soft-agar-colony yield approached zero in the neomycin-control H-5-1. The mutant p53 transfectants, however, significantly increased soft-agar colony formation ≈3- and 9-fold in the high expressors. The increase of soft-agar colony number is seen only at higher levels of mutant p53 protein expression: clones H-7-6 and H-7-1 produced more colonies than clone H-7-F, suggesting a threshold effect.

To examine the malignancy of p53-thr^{280} transfectants, we assayed for *in vivo* tumorigenicity in nude mice (for results, see Table 1). The neomycin control and clone H-7-F, showing a very low level of p53 expression, did not cause tumor formation 13 weeks after inoculation. The two clones having high-level expression of mutant p53 formed tumors. One of these clonal lines, H-7-1, formed tumors in nude mice after 2 weeks; the other line, H-7-6, formed a tumor after 11 weeks. The tumors reached a maximum size of 3.5 mm. One of the H-7-1 tumors had shrunk, and the other had disappeared by week 13. In addition, clone H-9-a, a double transfectant (cotransfected with wild-type p53 under vector pMM and p53-thr^{280} under vector pcos-7-neo, a long terminal repeat-

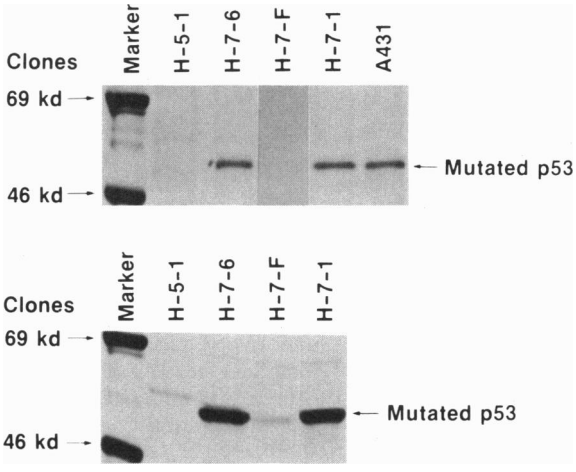

FIG. 1. Expression of introduced mutant p53-thr^{280} protein in human Saos-2 cells. Human Saos-2 cells were transfected with pMM-p53-thr^{280} (lines H-7-6, H-7-F, and H-7-1) or pMM-neo (line H-5-1) as control. Stable transfectants were G418 selected, expanded, and immunoprecipitated with pAb240. (*Upper*) No $ZnSO_4$ treatment. A human carcinoma cell line, A-431, known to contain mutant p53 (17) was included as a positive control. (*Lower*) Transfectants were subjected to 75 μM $ZnSO_4$ pretreatment for 2 hr before [^{35}S]methionine labeling. The film was exposed for 2 days, except for transfectant H-7-F (*Upper*), which was exposed for 3 days. kd, kDa.

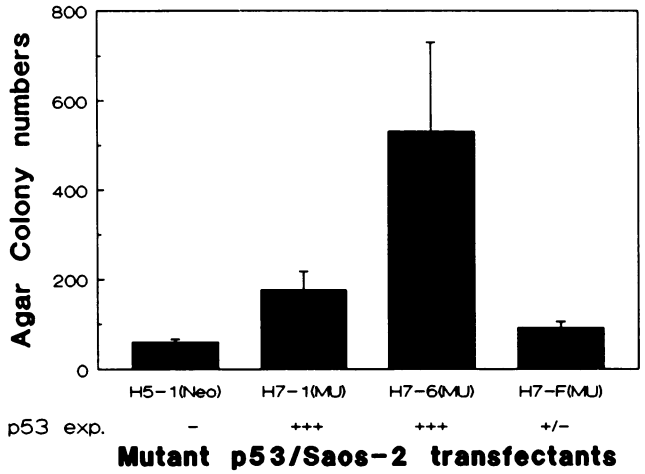

FIG. 2. Overexpression of p53-thr^{280} in Saos-2 cells enhances their ability to form soft-agar colonies. Fifty thousand single Saos-2 cells were suspended in 0.33% agar/10% FCS in a 60-mm dish after 2 hr Zn^{2+} treatment to induce p53 expression (exp.). Colonies of eight or more cells were counted after 26 days. Values are means ± SEMs from three independent experiments. The level of p53-thr^{280} protein expression is indicated.

driven vector) expressed only mutant p53 protein at moderate levels. The H-9-a cells showed ≈3-fold increase in agar colony numbers and formed a tumor in nude mice (1/6 at week 10; data not shown). Histopathology analysis of the nude mice tumors showed that all were undifferentiated malignancies (data not shown). Thus, although the p53-thr^{280} transfectants exhibit a long latent period for tumor formation and tumors tend to regress, overexpression of this mutant p53 did enhance the expression of tumor cell phenotypes, as assayed by anchorage-independent growth and *in vivo* tumorigenicity.

Overexpression of p53-thr^{280} in JB6 P$^+$ Cells Enhances Progression Toward Tumor-Cell Phenotype in Soft Agar but Not in the Nude Mouse. Because the mutation we observed in NPC was a heterozygous one, a goal of this study was to test whether p53-thr^{280} can function dominantly to override the wild-type form. Given the NPC heterozygosity, p53-thr^{280} should be dominant if it is to play any role in NPC genesis. That Saos-2 double transfectants initially grow as fast as the p53-thr^{280} transfectants, whereas wild-type transfectants initially grow very slowly, suggests possible dominance of the mutant form in governing cell-growth rate. To further study this question of p53-thr^{280} dominance, we transfected it into JB6 preneoplastic P$^+$ cells, which express endogenous wild-type p53. We asked whether p53-thr^{280} would, in the presence of the wild-type form, cause progression toward transformed phenotype. Similarly, we also transfected p53-thr^{280} into P$^-$ cells to see whether it could change them to P$^+$ cells. Fig. 3 shows that mutant p53 protein was expressed in P$^+$ transfectants 8-d and 8-4 (lanes 10 and 11) at levels similar to that of the endogenous wild-type p53 (indicated as mouse p53). Expression of mutant p53 protein in P$^-$ cell transfectants 9-G and 9-a was, however, low (lanes 8 and 9). No expression of mutant p53 was seen in the neomycin controls (lane 2, 12-a and lane 1, 13-2). Human carcinoma lines A-431 and A-427 were used as positive controls for mutant (17) and wild-type p53 (18), respectively. Unlike human Saos-2 transfectants, JB6 variants did not show increased p53 protein expression after Zn^{2+} treatment (data not shown). Using our experimental conditions, antibody pAb240 (designated as "mutant-specific" by Oncogene Science) immunoprecipitates both human and mouse p53 of either wild-type or mutant form (Fig. 3 and data not shown).

After confirmation of introduced p53 expression, we examined transfectants for possible progression beyond P$^+$ or P$^-$ phenotype by soft-agar assay in the presence or absence of PMA. Fig. 4 shows that without PMA the P$^+$ mutant p53 transfectant 8-d formed significantly more soft-agar colonies than the neomycin-control 12-a ($P < 0.05$). The enhancement seen with transfectant 8-4 was not statistically significant. With PMA, however, both transfectants 8-d and 8-4 formed significantly more colonies than the neomycin-control transfectant 12-a ($P < 0.01$), suggesting partial progression. When compared with parental cell line Cl41.5a, neomycin controls have repeatedly yielded more agar colonies (1.3- to 2.0-fold greater than line Cl41.5a, comparable to the value shown for transfectant 12-a). The P$^-$ mutant p53 transfectants 9-G and 9-a maintained the P$^-$ phenotype shown by lack of agar colony formation both with and without PMA (data not shown). The results indicate that, although the effect is moderate, p53-thr^{280} does function apparently dominantly in the presence of endogenous wild-type p53 to enhance progression of preneoplastic P$^+$ cells toward tumor cell phenotype *in vitro* and that p53-thr^{280} at the level expressed in P$^-$ transfectants is not oncogenic enough to cause progression from P$^-$ to P$^+$ cells, as indicated by nonresponsiveness to PMA.

The phenotype of P$^+$ mutant p53 transfectants was further examined by an *in vivo* tumorigenicity assay in nude mice. Table 2 shows that tumor formation was not seen up to 8 weeks after inoculation in any of the P$^+$ p53 transfectants. For a typical transformed JB6 line, tumor formation would be seen within 2–3 weeks after inoculation (ref. 16 and Table 2, Tx transfectants). The results indicate that although mutant p53, in the presence of endogenous wild-type p53, causes partial progression *in vitro*, the p53-thr^{280} P$^+$ transfectants are not fully transformed.

Overexpression of Wild-Type p53 in JB6 Cells Has No Effect on Either Tumor Cell or P$^+$ Phenotype. Wild-type p53 has repeatedly been shown to suppress tumor cell growth and tumorigenicity (19–23, 31). To determine whether wild-type p53 in excess of endogenous levels could suppress tumor-cell phenotype of transformed mouse JB6 variants or reverse the P$^+$ phenotype to P$^-$ phenotype, we overexpressed wild-type p53 in transformed-cell line RT101 to produce transfectants 10-1 and 10-b (Fig. 3, lanes 6 and 7) and a neomycin control (transfectant 14-2 in lane 3); and in P$^+$ cells to produce transfectants 11-2 and 11-1 (Fig. 3, lanes 4 and 5) and a neomycin control (transfectant 12-a in lane 2). In all p53

Table 1. Overexpression of the mutant p53 in Saos-2 cells causes tumor generation in nude mice

Clone	Transfectant	p53 protein*	Tumor yield, no. positive/total					
			2 wk	6 wk	10 wk	11 wk	12 wk	13 wk
H-5-1	neo-control	−	0/6	0/6	0/6	0/6	0/6	0/6
H-7-1	p53-thr^{280}	+++	2/6	3/6	3/6	3/6	3/6	2/6
H-7-6	p53-thr^{280}	+++	0/6	0/4	0/4	1/4	1/4	1/4
H-7-F	p53-thr^{280}	±	0/8	0/8	0/7	0/7	0/7	0/7

Ten million cells, pretreated with 75 μM $ZnSO_4$ for 2 hr, were injected s.c. into the upper back of nude mice, and tumor formation was scored every week.
*See Fig. 1.

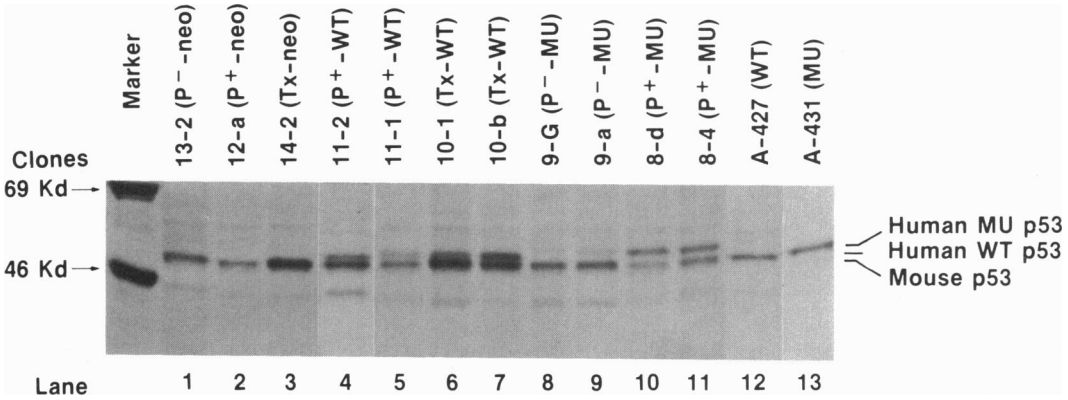

FIG. 3. p53 protein overexpression in mouse JB6 variants. JB6 variants Cl30.7b (P^-) or Cl41.5a (P^+) cells were transfected with pMM-p53-thr^{280}, and Cl41.5a (P^+) or RT101 (Tx) cells were transfected with pMM-WTp53. pMM-neo was also transfected into each of JB6 variants as a control. Stable transfectants were G418 selected, expanded, and immunoprecipitated with pAb240. Human carcinoma cell lines A-427 (wild-type control, lane 12) and A-431 (mutant control, lane 13) were included. Migrations of human p53, wild type (WT) or mutant (MU), and endogenous mouse p53 are indicated. Also indicated are molecular mass markers.

transfectants the level of introduced p53 expression was comparable to that of endogenous wild-type p53 (Fig. 3, human wild-type p53 and mouse p53). Compared with parental RT101 Tx cells or the neomycin-control 14-2, wild-type p53 transfectants 10-1 and 10-b have the same growth rate in monolayer culture (data not shown) and equal ability to form soft-agar colonies (≈7000 colonies per 10^4 cells) and to generate nude mouse tumors with similar latent period and yields (Table 2 and ref. 16). P^+ wild-type p53 transfectants 11-1 and 11-2, when compared with the neomycin-control 12-a, show similar PMA-induced soft-agar colony yields and lack of nude mouse tumor formation (Fig. 4 and Table 2). These findings indicate that overexpression of human wild-type p53 does not reverse the phenotype in JB6 tumorigenic or P^+ variants expressing endogenous wild-type p53.

DISCUSSION

In several recent studies of mutant p53, an additional gene, such as an activated *ras* oncogene, was used to test whether the mutant p53 could cooperate to fully transform primary cells. The results demonstrated gain of cotransformation function (4, 24–26). In a loss-of-function study, loss of growth-inhibitory properties of wild-type p53 has been found in several mutant p53 proteins seen in Li–Fraumeni patients, as tested in Saos-2 cells (6). In simian virus 40-immortalized bronchial epithelial BEAS-2B cells, overexpression of p53-ala^{143} resulted in acquisition of neoplastic phenotype (5). We have shown here that a different mutant p53, p53-thr^{280} isolated from NPC, can by itself enhance progression to or toward tumor cell phenotype, as shown in two cell lines, human Saos-2 and mouse JB6 P^+ variants.

In human Saos-2 cells lacking endogenous p53, introduction of p53-thr^{280} caused an enhanced progression to both anchorage-independent phenotype and *in vivo* tumorigenicity. The enhancement is seen only in the transfectants expressing higher levels of p53-thr^{280} protein. Of the two transfectants with high mutant p53 expression, H-7-6 transfectants formed more soft-agar colonies, whereas H-7-1 cells are more tumorigenic, a discrepancy within the range of clonal variability found among tumor cells. The nontumorigenic status of the neomycin control in this study agrees with the observation described in the American Type Culture Collection catalog but disagrees with a published report (10) in which 100% tumor yield was seen in neomycin-control Saos-2 cells at 12 weeks after inoculation. The disagreement may be explained by differences in the site of tumor injection (contralateral flanks, ref. 10, vs. upper back, this report), in the genetic background of nude mice used (not available from

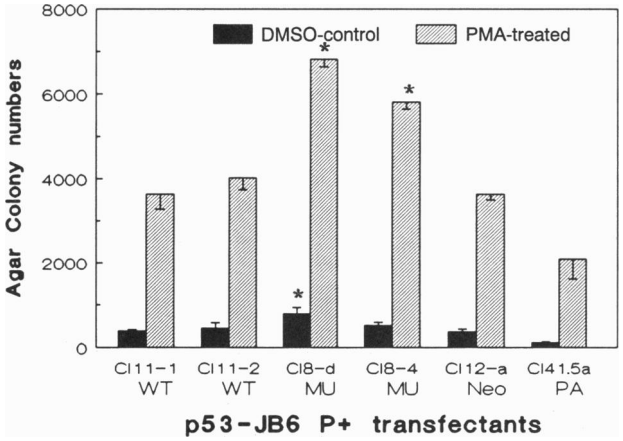

FIG. 4. Overexpression of p53-thr^{280} enhances both basal and PMA-induced anchorage-independent growth of JB6 cells. Ten thousand single cells of p53-thr^{280} (MU) transfectants Cl8-d and Cl8-4, wild-type p53 (WT) transfectants Cl11-1 and Cl11-2, neomycin-control (Neo) Cl12-a, or parental (PA) line Cl41.5a were suspended in 0.33% agar/10% FCS in a 60-mm dish without [but with 0.03% dimethyl sulfoxide (DMSO)] or with PMA (16 ng/ml, 2.56×10^{-8} M), respectively. Colonies of eight or more cells were counted after 14 days (11). Values are means ± SEMs from three independent experiments. ANOVA at 95% significance was used to test statistical significance. *, Significant difference from the neomycin-control line Cl12-a.

Table 2. *In vivo* tumorigenicity of p53-JB6 transfectants

Clone	p53 transfectant*	p53 protein†	Tumor yield, no. positive/total 3 wk	4 wk	6 wk	8 wk
11-2	P^+-WT	++	0/6	0/6	0/6	0/6
11-1	P^+-WT	+	0/6	0/4	0/4	0/4
8-d	P^+-MU	++	0/6	0/6	0/6	0/6
8-4	P^+-MU	++	0/6	0/6	0/6	0/6
12-a	P^+-neo	−	0/5	0/5	0/5	0/5
10-1	Tx-WT	++	8/8	8/8		
10-b	Tx-WT	++	7/8	8/8		
14-2	Tx-neo	−	8/8	8/8		

Two million single cells were injected s.c. into the upper back of nude mice, and tumor formation was scored every week.
*WT, transfected with wild-type human p53; MU, transfected with p53-thr^{280}, each in vector pMM.
†See Fig. 3.

ref. 10), or in genetic drift during passaging of the cells. Nevertheless, whether the Saos-2 cells are considered weakly tumorigenic or nontumorigenic, the above studies show a progression-enhancement effect of overexpressed mutant p53.

Dominance of p53-thr^{280} over wild-type p53 in governing Saos-2 cell growth may be occurring in double transfectants early during G418 selection after transfection. Failure to establish a stable wild-type only-p53 transfectant suggests the intolerance of Saos-2 cell growth to wild-type p53 protein, an observation also reported by others (27). Dominance of mutant p53-thr^{280} also appears to be occurring in JB6 P$^+$ transfectants harboring wild-type p53 because these cells show enhanced anchorage-independent growth. Two observations suggest a dosage effect of dominance. (*i*) P$^+$ transfectants having a level of mutant p53 expression similar to that of endogenous p53 have partially progressed toward tumor phenotype *in vitro*, as shown by enhanced soft-agar colony formation with or without PMA. (*ii*) P$^-$ transfectants having a lower mutant p53 expression than that of endogenous wild-type p53 retained P$^-$ phenotype. Dosage effects have been seen in a human colorectal cancer cell line cotransfected with wild-type and mutant p53 and assayed for transcriptional regulation by using a chloramphenicol acetyltransferase reporter (28). A similar experiment in Saos-2 cells cotransfected with p53-thr^{280} and wild-type p53 showed partial-to-complete mutant dominance at 1:1 or greater ratios (Z. Dong, Y.S., and N.C., unpublished observation). The tolerance of JB6 variants for introduced wild-type p53 expression (in contrast to human Saos-2 cells) and the expression of tumorigenicity in the presence of wild-type p53, at or in excess of endogenous levels, suggest that other gene(s) acting dominantly in JB6 variants may override the endogenous or exogenous wild-type p53 to maintain tumor cell phenotype.

Although p53-thr^{280} enhanced the expression of tumor cell phenotype in both human and mouse systems, the oncogenic activity of this mutant p53 is not high compared with activated Ha-*ras*, which causes full transformation of both JB6 P$^+$ cells and P$^-$ cells (P. Woolley and N.C., unpublished observation). We now have preliminary data showing that when cotransfected with latent membrane protein, an Epstein–Barr virus gene product expressed in NPC (29), p53-thr^{280} transfectants of P$^+$ or P$^-$ cells gained the ability to grow in soft agar with high efficiency (Y.S. and N.C., unpublished observation). This gene cooperation may resemble the *in vivo* situation in which both Epstein–Barr virus infection and other factors, such as p53 mutation, may be involved in NPC carcinogenesis (8, 30).

We conclude from this study that the G → C point mutation at codon 280 of p53 found in NPC is a gain-of-function mutation. When overexpressed, the mutant p53-thr^{280} enhanced progression toward tumor cell phenotype and appeared to function dominantly over the wild-type p53. Hence, the p53-thr^{280} found in a heterozygous state in NPC (8) might enhance progression. The mutant p53 itself, however, is not fully oncogenic. If this mutant does contribute to the genesis of NPC, it apparently requires other cooperating genes.

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