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p53 Status and Gene Transfer Experiments Using CMV Enhancer/Promoter

S. Allamane, D. Ratel, P. Jourdes, F. Berger, A. L. Benabid, and D. Wion
INSERM U318, CHU Michallon, 38043 Grenoble cedex 09, France

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Comparison of transfection efficiencies between different commercial reagents or methods is a matter of major concern in the field of gene therapy. Transfection efficiencies are usually evaluated by the quantification of a reporter gene expression (i.e., luciferase or lacZ) whose expression is usually driven by the CMV promoter. However, this experimental approach does not consider the possible effects of the transfection on the activity of the promoter used to drive reporter genes expression. Using p53 null fibroblasts we show that transfection efficiency estimated by the use of pCMV-luc or pCMV-βgal plasmids may be dramatically affected by the cell p53 status. These data highlight the fact that differences in p53 levels may be one of the parameters involved in the variation of transfection efficiencies observed with different cell lines. Furthermore, they point to the fact that comparison of transfection efficiencies should distinguish differences in the efficiency of transfection from differences in the level of transcription of the transgene. Finally they suggest that the known p53 down-regulation of the CMV promoter should be considered in order to avoid the nonintentional construction of transfer vectors in which the expression of a transgene down-modulates its own promoter.

Key Words: **p53; gene therapy; gene transfer efficiency.**

Introduction of foreign DNA in eukaryotic cells is a basic method in molecular and cellular biology and is often used as the first step to devise new strategies for gene therapy. A consequence of this, is the increasing number of new protocols or commercial reagents claiming to achieve the highest transfection efficiency available. Invariably, comparison of transfection efficiencies between different transfection reagents uses a reporter vector containing either the luciferase or β-galactosidase reporter genes downstream a promoter. However, it appears that most of the promoters used in these studies are repressed by p53. Thus, SV40 immediate early promoter/enhancer, RSV-LTR, human CMV immediate-early promoter/enhancer, HSV thymidine kinase and β-actin promoters have all been described to be down-modulated by wild type p53 (1–6). A first important point regarding these experiments reporting the down-regulation of viral promoters by p53 is that they have been conducted about ten years ago with the aim to study the involvement of p53 in the regulation of the activity of various promoters and not to evaluate the effects of endogenous levels of p53 on gene transfer and gene therapy approaches. Hence, the potential consequences of endogenous levels of wt. p53 on gene transfer efficiency may have been underestimated in the gene therapy context. Furthermore, these experiments on the down-modulation of promoters by p53 were most often performed using cells transfected with p53 expression vectors which therefore overexpress wt p53.

MATERIAL AND METHODS

Mouse heterozygous for the null allele (7) were a kind gift of Dr. E. May. Genotyping was performed as previously described. PCR was performed on DNA extracted from mouse tail with primers designed to distinguish the wild type and null p53 alleles: a sense oligonucleotide from the fourth intron of the murine p53 gene (5′-ATTCTCTGCATCTCTCCAGGGGAC-3′) and an antisense nucleotide from the fifth exon (5′-CATCACCATCGGAGCCAGCGCTCATG-3′) amplified a 760-bp fragment of the wild-type allele. The same sense oligonucleotide and an antisense oligonucleotide from the POL II sequence of the knock-out construct (5′-TTTACGGAGCCCTGGTCGATG-3′) amplified a 390-bp fragment from the null allele (10).

Mouse primary fibroblasts were isolated from new-born mice using a standard procedure consisting in an enzymatic dissociation of minced tissue. Cells were then cultured in DMEM supplemented with 10% fetal bovine serum. *In vitro* transfection were performed with Fugene (Boehringer Mannheim) according to the manufacturer's instructions.

RESULTS AND DISCUSSION

To investigate whether endogenous levels of wt.p53 may affect gene transfer efficiency, fibroblasts from either $p53^{+/+}$, $p53^{+/-}$ or $p53^{-/-}$ mouse (7) were transfected *in vitro* with a pCMV-βgal (Figs. 1A and 1B) or a pCMV-luc plasmid (Fig. 1C). Data presented in Fig. 1 clearly demonstrate that endogenous

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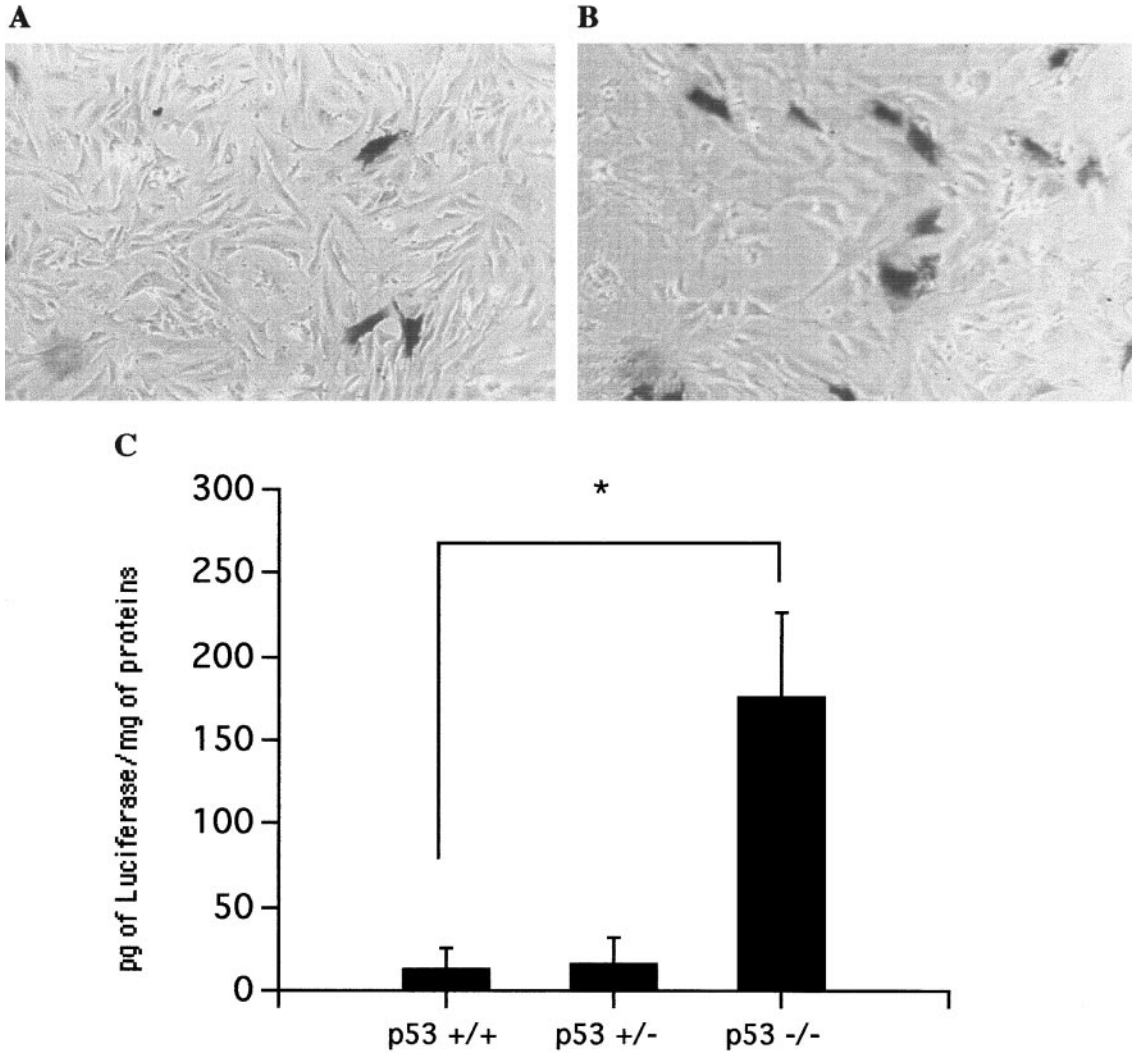

FIG. 1. Comparison of the expression of βgal in mouse $p53^{+/+}$ (A) and $p53^{-/-}$ (B) mouse fibroblasts. Mouse fibroblasts from mouse $p53^{+/+}$ (A) and $p53^{-/-}$ (B) were cultured and transfected as described. Twenty-four hours after transfection with a pCMV-βgal reporter plasmid, cell cultures were processed with X-gal to reveal βgal-expressing cells. (C) Influence of p53 endogenous levels on the expression of a reporter gene controlled by the CMV promoter. Mouse $p53^{+/+}$, $p53^{+/-}$ and $p53^{-/-}$ fibroblasts were transfected with a pCMV-luc reporter vector. Twenty-four hours after transfection cell lysates were assessed for luciferase activity. Each bar represents the average from six independent experiments performed in triplicate with two different batches of plasmid (*$P < 0.001$).

levels of p53 are sufficient to dramatically decrease gene transfer efficiency, as reported by β-galactosidase staining or luciferase activity. It is important to mention that these results which were obtained with the widely used CMV promoter, may have more widespread consequences if we consider that almost all of the promoters used in gene transfer experiments such as SV40, RSV-LTR, HSV thymidine kinase, HIV type 1 LTR, and β-actin promoters/enhancers have been described to be repressed by p53 (1–6). A first consequence of this situation is that differences of the p53 status of transfected cells could be one of the parameters explaining the variation in transfection efficiency observed between different cell lines or tissues when these p53 down-modulated promoters are used to drive the expression of transgenes. Another point is that data presented here raise the possibility that differences in transfection efficiencies reported with different commercial reagents or methods may be in part related to their ability to induce or repress p53 functions, and this especially since a rise in p53 protein levels has been associated with transfection (8). This potentially artifactual situation reflects the fact that reporter gene constructs used to compare different transfection efficiencies were initially designed to report the transcriptional activity of promoters following transfection and not to report a "transfection efficiency". Thus, it is essential, when a reporter gene is used to compare two methods of

transfection (i.e., to assess a transfection efficiency), to include an internal control that will distinguish differences in the efficiency of transfection from differences in the level of transcription. This could be achieved by the use of two different reporter genes controlled by the same promoter, one of which being stably transfected to assess the effects of transfection on the promoter activity, while the other will be transiently transfected and used to evaluate both transfection efficiency and promoter activity. Thus, transfection efficiency will result from the ratio between these two reporter activities. In the absence of the use of such engineered cell lines or transgenic animals, the reliability of data comparing different transfection reagents or methods remains questionable. Finally, results presented here, highlight the importance of the couple promoter-transgene in gene therapy. Thus, even if it is obvious that several transcription factors may cooperate in the regulation of a given promoter, it can be expected that the use of p53-inhibited promoters such as CMV to control the expression of a transgene triggering an increase in p53 levels should lead to a paradoxal feed-back inhibition of transgene expression. This unwanted situation might be encountered in p53-oriented cancer therapies using p53 expression vectors or when the transgene encodes a cytokine able to activate p53 (9).

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