research paper

haematologica 2002; 87:136-142
http://www.haematologica.it/2002_02/136.htm

Co-transfection of p16^{INK4a} and p53 genes into the K562 cell line inhibits cell proliferation

HONG-BING RUI, JIN-ZI SU
Fujian Hypertension Research Institute,
First Affiliated Hospital, Fujian Medical University,
Fuzhou, P.R. China

Correspondence: Hong-Bing Rui, MD, Department of Hematology, First Affiliated Hospital, Fujian Medical University, Fuzhou 350005, P. R. China. Phone: international +86.0591.3323969.
E-mail: fjrhb@sina.com

Background and Objectives. The tumor suppressor genes p53 and p16^{INK4a}, both of which act in tumor surveillance, are homozygously deleted in the human leukemia cell line K562. This study was performed to assess whether co-transfection of the p16^{INK4a} and p53 genes could inhibit K562 cell proliferation.

Design and Methods. p16^{INK4a} and p53 genes were co-transfected into K562 cells with liposome, and the expression of the transfected genes was detected by Western-immunoblotting and immunocytochemistry. The effect of the p16^{INK4a} and p53 transfected cell culture was quantified by trypan blue staining, and the number of recovered viable cells was assessed every day after transfection. Cells were analyzed for expression of annexin V in order to detect apoptosis. Differentiation of transfected K562 cells was measured by the benzidine oxidation test, and the cell cycle was analyzed by flow cytometry.

Results. After co-transfection, there were 23% and 28% p53 and p16^{INK4a} positive cells respectively. Co-transfection with p16^{INK4a} and p53 genes significantly inhibited cell proliferation when compared to transfection with either p16^{INK4a} or p53 gene. The percentage of cells expressing the apoptosis-related cell surface antigen annexin V was significantly higher in p53 and p16^{INK4a} transfected cells than in p53 or p16^{INK4a} transfected cells (6.24±0.37% vs 4.88±0.17%, $p<0.05$ and vs 2.78±0.26%, $p<0.05$, respectively). p16^{INK4a} and p53 co-transfection significantly increased the number of cells in G1 phase and decreased that in S phase.

Interpretation and Conclusions. Expression of wild-type p16^{INK4a} and p53 genes in K562 cells results in reduced proliferation and apoptosis. Introduction of exogenous p16^{INK4a} and p53 genes into K562 cells might contribute to the clinical treatment of leukemia.
©2002, Ferrata Storti Foundation

Key words: p16^{INK4a}, p53, transfection, tumor suppressor, leukemia.

Tumor suppressor genes p53 and p16^{INK4a}, both of which act in tumor surveillance,[1-3] are the most common targets for genetic alterations in human cancer.[2,4] Wild-type p53 is a critical element in suppressing cell proliferation in response to DNA-damaging agents by inducing G1 arrest or activating programmed cell death.[5,6] In the presence of DNA damage, wild-type p53 accumulates in the nucleus and arrests the cell cycle via the cyclin-dependent kinase (CDK) inhibitor p21$^{WAF1/CIP1}$. p21 arrests the cell cycle in the G1 phase, and expression of p21 induces leukemic cell differentiation. Particularly, p53 appears to affect the rate and efficiency of the excision repair system that plays a critical role in DNA repair.[7-9] Loss of p53 function leads to genomic instability and tumor cell resistance to anti-cancer therapy, including chemotherapeutic drugs and radiation.[10-14] p16^{INK4a} is a cyclin-dependent kinase inhibitor that acts upstream of the retinoblastoma (Rb) protein to promote cell-cycle arrest. It binds specifically to and blocks the function of the cyclin dependent kinases CDK4 and CDK6.[15] When associated with D-type cyclins, CDK4 and CDK6 promote passage through the G1 phase of the cell cycle by phosphorylation and functional inactivation of the Rb gene product, pRb.[16,17] Therefore, p16^{INK4a} and other members of the INK4 (inhibitors of cyclin dependent kinase 4) family (p15^{INK4b}, p18^{INK4c} and p19^{INK4d}) are all capable of imposing a G1 cell cycle arrest that is dependent on the presence of functional pRb.[2,3,18] Deletion of p16^{INK4a} gene may drive the cell cycle from G1 to S phase and cell pro-

liferation will be enhanced.[2,15]

Previous reports demonstrated homozygous deletions of p16^{INK4a} and p53 genes in the human erythroleukemic cell line K562.[19,20] Some studies showed that transfer of p16^{INK4a} or p53 gene alone could inhibit K562 cell proliferation.[21-23] However, no study on the effect of co-transfection of p53 and p16^{INK4a} genes into K562 cells has been reported so far. In this study, we have assessed the effects of co-transfection of both p53 and p16^{INK4a} genes into K562 cells.

Design and Methods

Cells and culture conditions

The human erythroleukemic cell line K562 (a gift from the Fujian Institute of Hematology) was cultured at 37°C in RPMI-1640 medium (Gibco BRL), supplemented with 10% fetal calf serum (FCS) in 5% CO_2 humidified atmosphere.

Plasmid constructions

A human p16^{INK4a} cDNA (kindly provided by Dr. Gordon Peters, Imperial Cancer Research Fund, London, UK), flanked by *BamH1* and *EcoR1* restriction sites, was transferred into the mammalian expression vector pcDNA3 (Introgene, San Diego, USA) under the cytomegalovirus (CMV) promoter to obtain plasmid pcDNA3p16. Plasmid pC53-SN3 (a gift from Dr. Ming Liang, Fujian Institute of Hematology, P. R. of China) contains human p53 cDNA under the CMV promoter. Plasmid pcDNA3LacZ was constructed by putting the 3.4kb *Bam H1* fragment of β-galactosidase (β-gal) gene from plasmid pAdCMVnLacZ (a gift from Dr. Liya Yi, Hubei Medical University, P.R. of China) into the unique *Bam H1* site of pcDNA3. Plasmid pCNeo-SN3 (also a gift from Dr. Ming Liang, Fujian Institute of Hematology) carries a neomycin phosphotransferase gene. pcDNA3LacZ and pCNeo-SN3 served as control plasmids.

Transfections

Transfection was carried out with liposome (Transfast™ transfection reagents, Promega) according to the manufacturer's recommendations. Briefly, the DNA-liposome complex, freshly obtained by adding 3 μL liposome to 1 μg plasmid DNA (0.5 μg pcDNA3p16 and 0.5 μg pC53-SN3) solution diluted in 400 μL RPMI-1640 without FCS, was added to the 2×10^5 cells in 24-well plates. Incubations were maintained at 37°C for 1 hour before adding another 1 mL of complete medium containing 10% FCS. Cells were cultured continuously. For mock experiments, 0.5 μg pcDNA3LacZ and 0.5 μg pCNeo-SN3 were co-transfected. Meanwhile, K562 cells were also transfected with either pcDNA3p16 or pC53-SN3 alone.

Detection of p16^{INK4a} and p53 gene expression

Expression of transfected p16^{INK4a} and p53 was detected by Western immunoblotting. Briefly, cells were adjusted to a final density of 1×10^7 cells/mL, protein was prepared by adding 2 × electrophoresis sample buffer (Tris 0.25mol/L pH 6.8, 2% sodium dodecyl sulfate [SDS], 4% σ-mercaptoethanol, 10% glycerol and 0.5% bromophenol blue) from equal columns of cell suspension. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) gel was prepared. After boiling samples for 5 minutes, 25 μg of proteins and markers of molecular weight were run on a 12.5% SDS-PAGE and electrophoretically transferred to a sheet of nitrocellulose paper. p16^{INK4a} was detected using the rabbit polyclonal antibody to p16^{INK4a} protein (Maxim Biotech Inc.), and p53 using the mouse monoclonal antibody to p53 protein (Maxim Biotech Inc.). Protein detection was visualized by the streptoavidin/biotin/peroxidase technique.

Expression of p16^{INK4a} and of p53 in cells was detected by immunocytochemistry using the UltraSensitive™ S-P Kit (Maxim Biotech Inc.). Cells were harvested 48 hours after transfection and placed on silane-coated slides; the slides were then fixed with 4°C acetone. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase for 10 min. Non-specific conjugation was blocked with non-immune rabbit serum in Tris buffered saline for 10 min, following which the mouse monoclonal antibody to p53 or rabbit polyclonal antibody to p16^{INK4a} (Maxim Biotech. Inc.) was layered on the slides. After incubation at 4°C overnight, the slides were washed with PBS and processed with streptoavidin/biotin/peroxidase reagents to detect and amplify the signal. The peroxidase was developed using diaminobenzidine.

Growth inhibition assay

The effect of the p16^{INK4a} and p53 transfected cell culture was quantified by trypan blue staining. The number of recovered viable cells was assessed every day after transfection. Results were expressed as the mean cell number of three wells.

Apoptosis detection

Cells were analyzed for expression of annexin V in order to detect apoptosis. Cells were washed once in cold PBS and then resuspended to 1×10^6

© Ferrata Storti Foundation

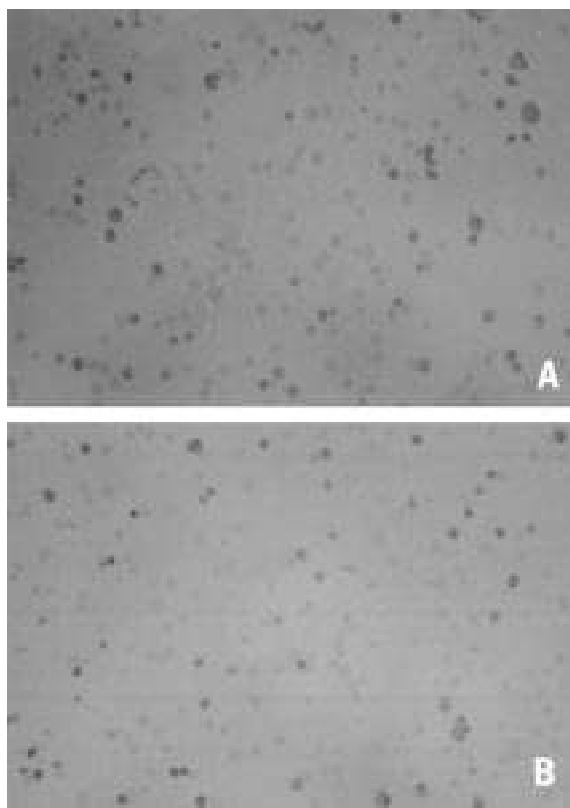

Figure 1. Immunocytochemistry of K562 cells co-transfected by p16^{INK4a} and p53 genes (a) detected by mouse monoclonal antibody to p53; (b) detected by rabbit polyclonal antibody to p16^{INK4a}. The cells containing brown granules were considered to be the positive ones.

cells/mL. Next, 100 μL of the cell suspension were incubated for 15 min at room temperature in the dark with 5 μL of annexin V-FITC (PharMingen, San Diego, CA, USA) and 10 μL propidium iodide (PI), and then taken to flow cytometric analysis in a FACScan flow cytometer (Becton Dickinson, USA).

Assessment of differentiation

The benzidine oxidation test was performed as described previously.[24] Briefly, 0.5×10^6 transfected cells were incubated with 5μm hemin for 4 days. Cells were then washed twice in 1×PBS, and finally resuspended in 0.9% NaCl. After 30 min incubation with benzidine, reagent solution (to 1 mL of 0.2% tetramethylbenzidine in 0.5 M acetum, 20 μL of 30% H_2O_2 are added just prior to use) was added to start the reaction. After a 30 min incubation in the dark at room temperature, 200 cells were counted and the number of cells containing oxidized tetramethylbenzidine (visualized as cells containing blue crystals) was taken as indicative of peroxidase activity and thus reflecting hemoglobin production.

Cell cycle analysis

Cells were washed with PBS, then 0.2 mL nuclear isolation medium containing propidium iodide was added (50 μg/mL propidium iodide, 0.6% NP40, 100 μg/mL RNase, in PBS). The cells were incubated at room temperature in the dark for 60 min before the addition of 0.4 mL PBS and then taken to flow cytometric analysis in a FACScan flow cytometer (Becton Dickinson, USA). Cell-Fit software (Becton Dickinson) was used for cell cycle analysis.

Statistical analysis

Chi-squared tests were used for analysis of categorical variables. All the results were expressed as mean ± standard deviation and all p values were two-tailed and were considered statistically significant when they reached the probability level <0.05.

Results

Transfected exogenous p16^{INK4a} and p53 expression in K562 cells

p16^{INK4a} and p53 expression was detected 48 hours after transplantation. Immunocytochemistry results showed that p16^{INK4a} and p53 proteins were located in the nucleus, and only partially in the cytoplasm. p53 and p16^{INK4a} expressing cells were, respectively, 23% and 28%. After co-transfection 23% and 28% of cells expressed respectively (Figure 1). In the transfections with p53 vector alone, 25% of cells expressed the protein, while in the cells transfected with p16^{INK4a} vector, 31.5% expressed the protein. In mock experiments, pcDNA3LacZ and pCNeo-SN3 co-transfected cells expressed neither p53 nor p16^{INK4a} protein. By immunoblotting, the cells co-transfected with the two tumor suppressor genes showed detectable levels of p16^{INK4a} and p53 protein, while mock- transfected cells showed none (Figure 2).

Inhibition of cell growth

The effects of co-transfection on cell growth were assessed by enumerating the viable cells every day after transfection. As shown in Figure 3, cell growth of p53 and p16^{INK4a} vector co-transfected cells was significantly inhibited compared with that of cells co-transfected with control vectors. For cells transfected with p53 alone, moderate inhibition was observed. However, in p16^{INK4a} transfected cells, only a mild inhibition was seen.

©Ferrata Storti Foundation

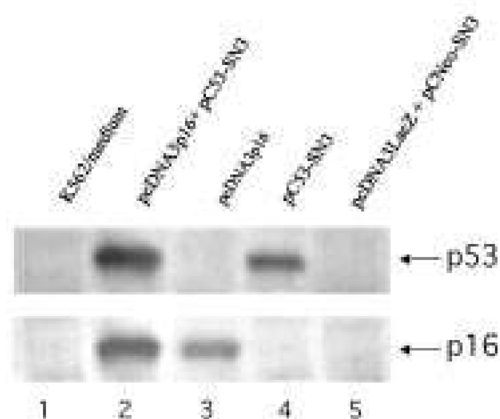

Figure 2. Western immunoblotting detection of p16INK4a and p53 expression in transfected K562 cells. Cells co-transfected with plasmids pcDNA3p16 and pC53-SN3 showed detectable levels of p16INK4a and p53 protein, and pcDNA3p16 or pC53-SN3 transfected cells also showed detectable p16INK4a or p53 protein, while K562/medium and mock vectors transfected cells showed none.

No significant inhibition was seen in cells transfected with control vectors.

Wild-type p16INK4a and p53 induce cell apoptosis

To determine whether the cell growth inhibition was attributable to apoptosis, the cells were analyzed for expression of annexin V by FACS analysis concomitantly with PI. As shown in Table 1, cells transfected with p53 and p16INK4a vectors showed higher rates of apoptosis.

Wild-type p16INK4a and p53 promote the capacity for hemoglobin production

After 4 days with 5μm hemin, the transfected cells were subjected to a benzidine oxidation test. This test is performed to determine the peroxidase activity of the cells, which reflects their content of hemoglobin. As shown in Figure 4, p16INK4a and p53 increased the differentiation sensitivity of the K562 clones 7-fold compared with control vectors, 1.6-fold compared with p16INK4a and 1.3 fold compared with p53 alone.

Cell cycle arrest mediated by p53 and p16INK4a

As shown in Table 2, p53 and p16INK4a co-transfection significantly increased the number of cells in G1 phase and decreased that in S phase. Either p53 or p16INK4a transfection also appreciably increased the number of cells in G1 phase. In contrast, no G1 arrest was observed in the control group. These results suggest that the p53 and p16INK4a proteins suppress the growth of the tumor cells by mediating G1 arrest in cell lines that do not express p53 and p16INK4a.

Discussion

p53 and Rb proteins are tumor suppressors which negatively regulate different steps in cell-cycle progression.[16] In turn, their activities are modulated by p19ARF and p16INK4a proteins encoded by the INK4a/ARF locus.[23] Together, the loci encoding these four proteins are probably the most commonly inactivated in cancer.[2,4] Because the p16INK4a protein is a CDK4 inhibitor, deletion of this gene may increase the activity of CDK4, resulting in hyperphosphorylation of the Rb protein, thus driving the cell cycle from G1 to S phase and enhancing cell proliferation. Restoring the expression of p16INK4a in p16INK4a negative cell lines arrests the cells at G1 phase, thus inhibiting cell proliferation.[2,15] One of the functions of p53 is cell cycle arrest at the G1/S boundary to allow repair of damaged DNA before DNA replication or induction of apoptosis, when DNA damage is too severe.[5] Expression of wild-type p53 in p53-deficient tumor cell lines renders them more susceptible to induction of apoptosis by radiation or DNA-damaging chemotherapeutic drugs.[25]

p53 mutations, accompanied by p16INK4a muta-

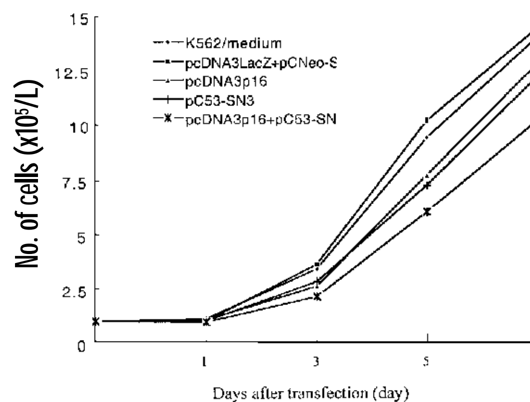

Figure 3. Cell growth inhibition of K562 cells co-transfected with p16INK4a and p53 genes. Cell growth of p16INK4a and p53 co-transfected cells was significantly inhibited (—*—). The most obvious effect appeared at day 3~5.

©Ferrata Storti Foundation

Table 1. Expression of the apoptosis-related cell surface antigen annexin V in p16[INK4a] and p53 transfected K562 cells (mean±SD). Values shown are percentage of cells expressing Annexin V.

	Annexin V
K562/medium	1.75±0.17
pcDNA3LacZ+pCNeo-SN3	1.81±0.13
pcDNA3p16	2.78±0.26[#]
pC53-SN3	4.87±0.17[#]
pcDNA3p16+pc53-SN3	6.24±0.37*

**p<0.05 vs pC53-SN3/ pcDNA3p16, #p<0.05 vs pcDNA3+pCNeo-SN3, SD: standard deviation.*

Table 2. Effect of p16[INK4a] and p53 co-transfection on K562 cell cycle (mean±SD).

	cell cycle %		
	G0/G1	*S*	*G2/M*
K562/medium	37.64±.52	60.93±2.55	1.43±0.98
pcDNA3LacZ+pCNeo-SN3	37.71±0.78	60.6±0.86	1.60±0.59
pcDNA3p16	42.10±1.49[#]	55.39±1.26[#]	2.52±0.49
pC53-SN3	46.44±1.99[#]	51.60±1.73#	1.95±0.29
pcDNA3p16+pc53-SN3	52.56±1.38*	46.30±1.68*	1.14±0.38

**p<0.05 vs pC53-SN3/ pcDNA3p16, #p<0.05 vs pcDNA3+pCNeo-SN3. SD: standard deviation.*

tions, have been found in many cancer cell lines. Though restoration of wild-type p16[INK4a] or p53 function alone could inhibit the proliferation and colony formation of some human cancer cell lines,[22,26-28] tumors with both p16[INK4a] and p53 mutations might also display aggressive characteristics owing to defect in the p53 and Rb pathway. Our study showed that both p53 and p16[INK4a] expression in K562 cells, a human leukemia cell line with homozygous deletion of p53 and p16[INK4a] genes, induced obvious growth arrest, as indicated by the decrease in the percentage of cells in the S phase of the cell cycle. The expression of annexin V implied that the cell growth inhibition was attributable to apoptosis. Either p53 or p16[INK4a] expression alone also inhibited K562 cell growth, whereas p16[INK4a] protein showed mild

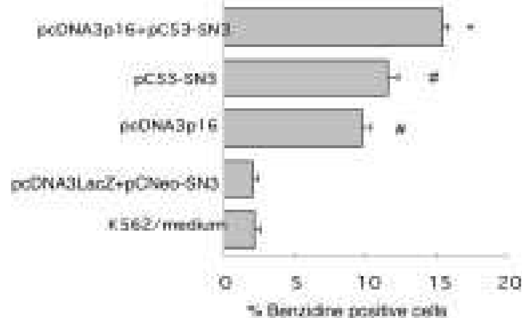

Figure 4. Effects of p16[INK4a] and p53 on differentiation of K562 cells, assayed by the capacity to oxidize benzidine. Expression of wild-type p16[INK4a] and p53 induced K562 cell differentiation. ****p*<0.05 vs pC53-SN3/pcDNA3p16, #*****p*****<0.05 vs pcDNA3LacZ+pCNeo-SN3.**

inhibition. A role of p53 or p16[INK4a] protein in G1 arrest is consistent with previous studies.[15,29-31] Our data have important implications for the multi-gene therapy of tumors.

In these experiments, we used liposome-coated DNA for p16[INK4a] and p53 gene transfer. Cationic liposome-DNA complexes have been used *in vitro* and *in vivo* as gene delivery vehicles as an alternative to viral vectors.[32,33] Some of the advantages of liposomes are that they can carry large pieces of DNA, they are not immunogenic, they are safe relative to viral vectors, and large scale production of liposomes is relatively straightforward.[33] The efficiency of transfection, assessed by measuring the percentage of cells expressing p16[INK4a] or p53 protein, was found to be too low to enable the therapeutic plasmid to inhibit all cell growth. No more than 30% of all transfected K562 cells expressed both p16[INK4a] and p53. Even considering either p16[INK4a] or p53 transfected cells, there were still no more than 35% positive cells. Because of the low transfer efficiency, most K562 cells did not take in these two genes, so that the effect of cell growth suppression was not satisfactory. Further studies are required to investigate whether the stable expression of both functional p53 and p16[INK4a] in mismatch deficient cells might improve the role of multi-gene therapy in cancer. These studies indicate that the transfection efficiency of liposomes needs to be significantly increased before they can be used successfully *in vivo*. Fortunately, novel liposomes improved transfection efficiencies and activities are being developed.[34,35] In previous reports of p16[INK4a] gene transfer using retroviruses, the viral titer obtained was low.[21] It was reported that murine NIH3T3 cells, from which most amphotropic packaging cell lines are

©Ferrata Storti Foundation

derived, have a homozygous deletion of $p16^{INK4a}$ gene, and that ectopic expression of $p16^{INK4a}$ in NIH3T3 cells resulted in G1 phase arrest.[36] This could explain why high viral titer producing PA317 clones were unable to be obtained, as PA317 clones with low-expression $p16^{INK4a}$ mRNA were probably selected, leading to a weak viral production. This finding will have to be taken into account if retroviruses are to be used as vectors for gene therapy using the $p16^{INK4a}$ gene. There are also several disadvantages associated with retroviral delivery of the p53 gene, such as the potential for insertional mutagenesis, cell division for efficient infection, poor stability and low titer.[1] All of these limit the use of replication-deficient retroviruses to *ex vivo* gene therapy.

Adenoviruses have been widely used as vectors in various cell types, and can introduce foreign genes into non-replicating cells at high titer, but do not integrate into the genome.[1] Several investigators have reported the antitumor effects of adenoviruses encoding wild-type p53 or $p16^{INK4a}$ on various tumor cell lines.[37,38] However, some studies also reported the poor ability of adenovirus vectors to transduce acute myeloid leukemia cells (AML).[39,40] A modified adenovirus containing a heparin/heparan sulfate binding domain incorporated into the fiber protein of the adenovirus was investigated. It was reported that retargeting the adenovirus fiber protein to heparan sulfates can overcome the low efficiency of adenovirus in AML blast cells and may provide a useful tool for gene therapy approaches in AML.[41]Therefore, the adenovirus vector seems suitable for future p53 and $p16^{INK4a}$ gene therapy protocols in acute leukemia cells.

In conclusion, these data indicate that co-transfection of wild-type p53 and $p16^{INK4a}$ can inhibit K562 cell growth. Furthermore, the results suggest a possible role of multi-gene therapy in acute leukemias.

Contributions and Acknowledgments

HBR, JZS: conception and design; HBR: drafting and revisions. All the authors approved the final version of manuscript. The authors thank Dr. Guangshen Zhuo, First Affiliated Hospital, Fujian Medical University, China, for his helpful discussion.

Funding

This study was supported by a Youth Fund from the First Affiliated Hospital, Fujian Medical University, P.R. of China.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

References

1. Nielsen L, Maneval D. p53 Tumour suppressor gene therapy for cancer. Cancer Gene Ther 1998; 5:52-63.
2. Ruas M, Peters G. The $p16^{INK4a}$/CDKN2A tumor suppressor and its relatives. Biochim Biophys Acta 1998; 1378: F115-77.
3. Sharpless NE, De Pinho RA. The INK4A/ARF locus and its two gene products. Curr Opin Genet Dev 1999; 9:22-30.
4. Haber DA. Splicing into senescence: the curious case of p16 and p19ARF. Cell 1997; 91:555-8.
5. Hansen R, Oren M. p53; from inductive signal to cellular effect. Curr Opin Genet Dev 1997; 7:46-51.
6. Ko LJ, Prives C. p53: puzzle and paradigm. Genes Dev 1996; 10:1054-72.
7. Jiang H, Lin J, Su ZZ, Collart FR, Huberman E, Fisher PB. Induction of differentiation in human promyelocytic HL-60 leukemia cells activates p21, WAF1/CIP1, expression in the absence of p53. Oncogene 1994; 9:3397-406.
8. Zhang Y, Xiong Y, Yarbrough WG. ARF promotes MDM2 degradation and stabilizes p53: ARF-INK4a locus deletion impairs both the Rb and p53 tumor suppression pathways. Cell 1998; 92:725-34.
9. Wang XW, Yeh H, Schaeffer L, Roy R, Moncollin V, Egly JM, et al. p53 modulation of TFIIH-associated nucleotide excision repair activity. Nat Genet 1995; 10:188-95.
10. Livingstone LR, White A, Sprouse J, Livanos E, Jacks T, Tlsty TD. Altered cell cycle arrest and gene amplification potential accompany loss of wild-type p53. Cell 1992; 70:923-35.
11. Yin Y, Tainsky MA, Bischoff FZ, Strong LC, Wahl GM. Wild-type p53 restores cell cycle control and inhibits gene amplification in cells with mutant p53 alleles. Cell 1992; 70:937-48.
12. Lowe SW, Bodis S, McClatchey A, Remington L, Ruley HE, Fisher DE, et al. p53 status and the efficacy of cancer therapy in vivo. Science 1994; 266:807-10.
13. Hollstein M, Sidransky D, Vogelstein B, Harris CC. p53 mutations in human cancers. Science 1991; 253:49-53.
14. Hollstein M, Rice K, Greenblatt MS, Soussi T, Fuchs R, Sorlie T, et al. Database of p53 gene somatic mutations in human tumors and cell lines. Nucleic Acids Res 1994; 22:3551-5.
15. Serrano M, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. Nature 1993; 366:704-7.
16. Weinberg RA. The retinoblastoma protein and cell cycle control. Cell 1995; 81:323-30.
17. Sherr CJ. Tumor surveillance via the ARF-p53 pathway. Genes Dev 1998; 12: 2984-91.
18. Sherr CJ, Roberts JM. Inhibitors of mammalian G1 cyclin-dependent kinases. Genes Dev 1995; 9:1149-63.
19. Ogawa S, Hirano N, Sato N, Takahashi T, Hangaishi A, Tanaka K, et al. Homozygous loss of the cyclin-dependent kinase 4-inhibitor (p16) gene in human leukemias.

© Ferrata Storti Foundation

Blood 1994; 84:2431-5.
20. Sugimoto K, Toyoshima H, Sakai R, Miyagawa K, Hagiwara K, Ishikawa F, et al. Frequent mutations in the p53 gene in human myeloid leukemia cell lines. Blood 1992; 79:2378-83.
21. Quesnel B, Preudhomme C, Lepelley P, Hetuin D, Vanrumbeke M, Bauters F, et al. Transfer of p16inka/CDKN2 gene in leukaemic cell lines inhibits cell proliferation. Br J Haematol 1996; 95:291-8.
22. Feinstein E, Gale RP, Reed J, Canaani E. Expression of the normal p53 gene induces differentiation of K562 cells. Oncogene 1992; 7:1853-7.
23. Chylicki K, Ehinger M, Svedberg H, Bergh G, Olsson I, Gullberg U. p53-mediated differentiation of the erythroleukemia cell line k562. Cell Growth Differ 2000; 11:315-24.
24. Bergh G, Ehinger M, Olofsson T, Baldetorp B, Johnsson E, Brycke H, et al. Altered expression of the retinoblastoma tumor-suppressor gene in leukemic cell lines inhibits induction of differentiation but not G1-accumulation. Blood 1997; 89:2938-50.
25. Banerjee D, Lenz HJ, Schnieders B, Manno DJ, Ju JF, Spears CP, et al. Transfection of wild-type but not mutant p53 induces early monocytic differentiation in HL60 cells and increases their sensitivity to stress. Cell Growth Differ 1995; 6:1405-13.
26. Okamoto A, Demetrick DJ, Spillare EA, Hagiwara K, Hussain SP, Bennett WP, et al. Mutations and altered expression of p16INK4 in human cancer. Proc Natl Acad Sci USA 1994; 91:11045-9.
27. Arap W, Nishikawa R, Furnari FB, Cavenee WK, Huang HJ. Replacement of the p16/CDKN2 gene suppresses human glioma cell growth. Cancer Res 1995; 55:1351-4.
28. Cheng J, Yee JK, Yeargin J, Friedmann T, Haas M. Suppression of acute lymphoblastic leukemia by the human wild-type p53 gene. Cancer Res 1992; 52:222-6.
29. Baker SJ, Markowitz S, Fearon ER, Willson JK, Vogelstein B. Suppression of human colorectal carcinoma cell growth by wild-type p53. Science 1990; 249:912-5.
30. Wang Y, Okan I, Szekely L, Klein G, Wiman KG. bcl-2 inhibits wild-type p53-triggered apoptosis but not G1 cell cycle arrest and transactivation of WAF1 and bax. Cell Growth Differ 1995; 6:1071-5.
31. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. Cancer Res 1991; 51:6304-11.
32. Felgner PL, Tsai YJ, Sukhu L, Wheeler CJ, Manthorpe M, Marshall J, et al. Improved cationic lipid formulations for in vivo gene therapy. Ann NY Acad Sci 1995; 772: 126-39.
33. Templeton NS, Lasic DD. New directions in liposome gene delivery. Mol Biotechnol 1999; 11:175-80.
34. Watabe A, Yamaguchi T, Kawanishi T, Uchida E, Eguchi A, Mizuguchi H, et al. Target-cell specificity of fusogenic liposomes: membrane fusion-mediated macromolecule delivery into human blood mononuclear cells. Biochim Biophys Acta 1999; 1416:339-48.
35. de Lima MC, Simoes S, Pires P, Gaspar R, Slepushkin V, Duzgunes N. Gene delivery mediated by cationic liposomes: from biophysical aspects to enhancement of transfection. Mol Membr Biol 1999; 16:103-9.
36. Quelle DE, Ashmun RA, Hannon GJ, Rehberger PA, Trono D, Richter KH, et al. Cloning and characterization of murine p16 and p15 gene. Oncogene 1995; 11:635-45.
37. Wills KN, Maneval DC, Menzel P, Harris MP, Sutjipto S, Vaillancourt MT, et al. Development and characterization of recombinant adenoviruses encoding human p53 for gene therapy of cancer. Hum Gene Ther 1994; 5: 1079-88.
38. Zhang WW, Fang X, Mazur W, French BA, Georges RN, Roth JA. High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus. Cancer Gene Ther 1994; 1:5-13.
39. Wattel E, Vanrumbeke M, Abina MA, Cambier N, Preudhomme C, Haddada H, et al. Differential efficacy of adenoviral mediated gene transfer into cells from hematological cell lines and fresh hematological malignancies. Leukemia 1996; 10:171-4.
40. Cantwell MJ, Sharma S, Friedmann T, Kipps TJ. Adenovirus vector infection of chronic lymphocytic leukemia B cells. Blood 1996; 88:4676-83.
41. Gonzalez R, Vereecque R, Wickham TJ, Vanrumbeke M, Kovesdi I, Bauters F, et al. Increased gene transfer in acute myeloid leukemic cells by an adenovirus vector containing a modified fiber protein. Gene Therapy 1999; 6:314-20.

©Ferrata Storti Foundation

Peer Review Outcomes

What is already known on this topic

A large body of evidence indicates that transformed cells show alterations which involve the mechanisms regulating cell cycle machinary. Such a phenomenon might cause an ineffective DNA repair, the accumulation of genetic damages and finally the progression towards a more aggressive genotype. Each passage from a phase to another is under positive control of a complex of cyclin and cyclin-dependent kineases as well as negative control of two families of CDK inhibitors, p21 and p16.

What this study adds

This study demonstrates that co-transfection with p16^{INK4a} and p53 genes significantly inhibited cell proliferation when compared to transfection with either p16^{INK4a} or p53 gene.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Prof. Achille Iolascon, who acted as an Associate Editor. The final decision to accept this paper for publication was taken jointly by Prof. Iolascon and the Editors. Manuscript received August 29, 2001; accepted December 9, 2001.

Potential implications for clinical practice

The gene therapy is targeted to introduce in the cell a protein that directly could control proliferation, differentiation or apoptotic pathway. This paper represents the first step to produce this therapeutic opportunity.

Achille Iolascon, Associate Editor