The Effects of Wild Type p53 Tumor Suppressor Gene Expression on the Normal Human Cervical Epithelial Cells or Human Epidermal Keratinocytes Transformed with Human Papillomavirus Type 16 DNA

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The inactivation of p53 and p105RB by viral proteins or by mutations plays a key role in the oncogenesis of cervical carcinoma. The E6 and E7 proteins of HPV type 16 can bind to p53 and p105RB tumor suppressor gene products, respectively. In the present study, we tested a simple in vivo model that could explain the interactions between HPV E6 oncoprotein and p53 tumor suppressor protein. Our results showed that the life span of normal cervical epithelial cells was increased up to 4.5 times when transfected with expression vector containing E6/E7 ORF of HPV type 16. However, these cells did not divide after second crisis. Therefore, we employed an established human epidermal keratinocytes, RHEK-1. When transfected with an expression vector containing E6 ORF of HPV type 16, RHEK-1 cells showed anchorage independent growth character. When RHEK-E6 cells were transfected with wild type p53 expression vector, the growth rate of the RHEK-E6 cells was diminished. After 48 hours of transfection, many cells showed apoptotic signal but no more apoptotic signal was observed thereafter. These results suggested that the overexpression of the wild type p53 could overcome the dysfunction of the p53 on the cell cycle regulation imposed by E6 protein although not being of physiological condition.

Key Words: Human papillomavirus, p53 tumor suppressor gene, apoptosis

Among the 60 different types of human papillomaviruses (HPVs) isolated from a variety of squamous epithelial lesions, eighteen types have been shown to be associated with

anogenital tract lesions (DeVilliers, 1989). Some of them, such as HPV 6 and HPV 11, are associated with the benign proliferative tumors (e.g., condyloma acuminatum) which have a low risk for malignant progression. However, HPV16, HPV18, HPV31, HPV33, and HPV35 are associated with potentially precancerous genital tract lesions and anogenital cancers (zur Hausen and Schneider, 1987). The cloned DNA of high risk HPVs could transform established rodent fibroblasts (Yasumoto *et al.* 1986), primary rodent cells (Phelps *et al.* 1988; Storey *et al.* 1988), and primary human cells (Dürst *et al.* 1987; Pirisi *et al.* 1987). In cervical carcinomas and in cell lines derived from cervical carcinomas, the E6 and E7 open reading frames (ORFs) of the high risk HPVs are found to be intact and are actively tran-

scribed implicating the involvement of E6 and E7 genes in the malignant phenotype (Baker *et al.* 1987). It has been shown that E6 and E7 proteins can bind to p53 and p105RB tumor suppressor gene products, respectively (Dyson *et al.* 1989; Werness *et al.* 1990), although the role of these viral proteins in the oncogenesis at the molecular level is not clearly understood. Recently, it was suggested that the inactivation of p53 and p105RB by viral proteins or by mutations plays a key role in the oncogenesis of cervical carcinoma(Scheffner *et al.* 1991).

In this study, we tested a simple *in vivo* model that could explain the interactions between HPV E6 oncoprotein and p53 tumor suppressor protein. The HPV containing cancer cell lines were excluded in this study, because they might have multiple underlying genetic defects. Our results showed that when transfected with an expression vector containing E6/E7 ORF of HPV type 16, the life span of normal cervical epithelial cells was increased up to 4.5 times but these cells did not divide after second crisis. Therefore, we employed an established human epidermal keratinocytes, RHEK-1. When transfected with the expression vector containing E6 ORF of HPV type 16, they showed an anchorage independent growth character. When these cells were transfected with wild type p53 expression vector, all the cells were detached off during selection. And transient transfection assay up to 96 hours showed that the growth rate of RHEK-E6 cells was diminished. After 48 hours of transfection, many cells showed an apoptotic signal but no apoptotic signal was observed thereafter. These results suggested that overexpression of wild type p53 could overcome the dysfunction of the p53 on the cell cycle regulation imposed by E6 protein although not being of physiological condition.

MATERIALS AND METHODS

Reagents

Mouse anti-E6 protein monoclonal antibody (Ab) was purchased from Oncogene Science

(Uniondale, NY, USA). Mouse anti p53 (DO-1) monoclonal Ab was purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Restriction enzymes, Taq DNA polymerase and other molecular biology grade reagents were purchased from Boehringer Mannheim GmbH Biochemica (Mannheim, Germany). Other chemicals were obtained from Sigma Chem. Co. (St Louis, USA).

Cells

Cervical tissues, without gross evidence of pathology, were obtained from total hysterectomies taken from patients diagnosed as myoma of uterus. Tissues were washed twice with Hank's balanced salt solution (HBSS) and minced. The tissue was incubated in HBSS containing collagenase (1 mg/ml) and dispase (2.4 unit/ml) for 1 hour at 37℃. Under dissection microscope, epithelial sheets were separated from stroma, and then dissociated into single cells by incubation in $1 \times$ trypsin/EDTA (Gibco BRL, Gaithersburg, MD, USA) for 30 minutes at 37℃. The cells were washed with HBSS, resuspended with keratinocyte growth medium (KGM, Clonetics Co., San Diego, CA, USA) and plated onto $25 \, cm^2$ tissue culture flask.

RHEK-1, human epidermal keratinocytes were grown in Dulbecco's modified eagles media (DMEM) containing 10% fetal calf serum (FCS), $5 \, \mu g/mL$ hydrocortisone, 100 units /ml penicillin, and $100 \, \mu g/ml$ streptomycin (Rhim *et al.* 1984).

PCR primers

To amplify the E6 and E7 ORF DNA fragments of HPV type 16 using the PCR technique, primers were synthesized using DNA synthesizer (Model 381A, Applied Biosystem Inc., Foster city, CA, USA). The sequences of the oligonucleotides are as follows;

E6 ORF amplification primers
5'-CTCGGATCCATGCACCAAAAGAG-3'
 (nt 83-96) and
5'-CTCGGATCCAAGCTTATGATTACAGCT-3'(nt 552-563)
E7 ORF amplification primers
5'-CTCGGATCCATGCATGGAGATACA-3'

(nt 562-576) and
5'-CTCGGATCCAAGCTTGATCAGCCA-3'
(nt 866–874).

To rule out the possible infection of HPVs in normal human cervical epithelial cells, HPV E6 consensus primers (Resnick *et al.* 1990) were synthesized and used for the detection of HPV DNA by PCR. The sequences of primers are as follows:

HPV E6 consensus primers
WD72 5'-CGGTCGGGACCGAAAACGG-3'
WD76 5'-CGGTTSAACCGAAAMCGG-3'
WD66 5'-AGCATCCGGTATACTGTCTC-3'
WD67 5'-WGCAWATGGAWWGCYGTCTC-3'
WD154 5'-TCCGTGTGGTGTGTCGTCCC-3'
M=A/C; S=G/C; Y=C/T; W=A/T

PCR

Typical optimal PCR conditions were: template, 40 ng cloned HPV type 16 DNA; primers, 50 pmoles each; Taq DNA polymerase, 2.5 units; $10\times$ PCR buffer (100 mM Tris-HCl, pH 8.3, 500m M KCl, 20 mM $MgCl_2$, 0.1% (w/v) gelatin) $5\,\mu L$; each $200\,\mu M$ dNTP in a total volume of $50\,\mu L$. Samples were overlaid with mineral oil. Each cycle of PCR consisted of 30 seconds of template denaturation at 94℃, 40 seconds of annealing at 60℃, and 40 seconds of extension at 72℃. The cycle was repeated for 30 times and final extension was kept for 10 minutes at 72℃. For the detection of HPV DNA from normal cervical epithelial cells, 4mM $MgCl_2$ was used.

Recombinant plasmids

The PCR amplified E6 or E6E7 DNA was inserted into the BamHI cloning site of the retroviral expression vector, pZipNeo SV (X). It has a long terminal repeat of murine leukemia virus, and Neo^R gene. To express the wild type p53 protein, an expression plasmid pMAM (Clonetech Laboratories, Inc., Paolo Alto, CA, USA) which has dexamethasone inducible molony murine leukemia virus long terminal repeat (MMTV-LTR) was used.

The pZip vector and the PCR amplified E6 or E6E7 ORF DNAs were digested with BamHI and ligated with T4 DNA ligase. In-sert orientation was confirmed by appropriate restriction enzyme digestions. Each recombinant vector was named as pZipE6, and pZipE6E7, respectively (Fig. 1). XbaI digested p53 cDNA was subcloned into NheI digested pMAM expression vector and named as pMAM-p53.

Transfection

Approximately 40~60% confluent primary human normal cervical epithelial cells and RHEK-1 cells were transfected with each recombinant vector using lipofectin™ (Gibco BRL, Gaithersburg, MD, USA). For each 60 mm dish, a mixture of $5\,\mu g/1.5\,ml$ of pZip E6E7, or pZipE6 and $40\,\mu g/1.5\,ml$ of lipofectin reagent was added to the culture medium. The cells were incubated for 24 hours at 37℃. The medium was then replaced with fresh KGM, then cultures were incubated for additional 48 hours. To select transfectants, the cells were incubated in the appropriate media containing $100\,\mu g/ml$ G418 (neomycin analogue) for 48 hours, and cultured in fresh media before subculture.

Southern hybridization

High molecular weight chromosomal DNA was obtained from transfected and cloned cells (Sambrook *et al.* 1989a). DNA was digested with HindIII and electorphoresed in 0.8% agarose gel and transferred to nylon membrane. E6E7 ORF DNA was labeled with α-^{32}P-dCTP using random primed DNA labeling kit (Boehringer Mannheim GmbH Biochemica, Mannheim, Germany). Hybridization was performed in the 42℃ hybridization oven for overnight. The membrane was washed (Sambrook *et al.* 1989b) and exposed to X-ray film for overnight.

Immunohistochemical staining

Transfected cervical epithelial cells were incubated with mouse anti cytokeratin antibody (AE-1/AE-3) followed by biotinylated antimouse IgG Ab and then incubated with alkaline phosphatase labeled streptavidin. After incubation, alkaline phosphatase substrate solution(100 mM Tris-HCl, pH 9.5, 100

A.
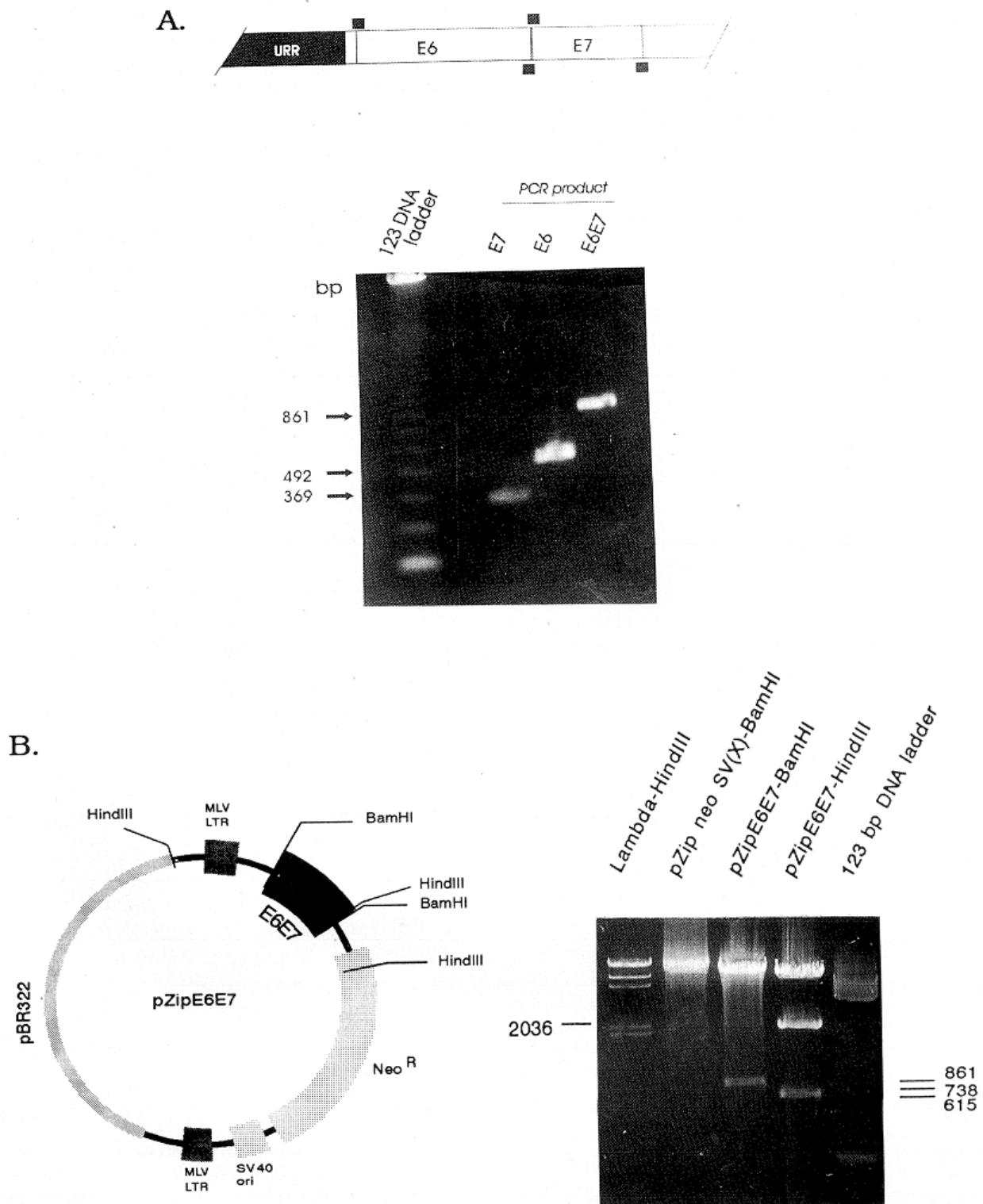
Fig. 1. PCR amplification of E6, E7, or E6E7 ORF of HPV type 16 and subcloning of amplified PCR products.

A. Schematic diagram of PCR primers for amplification of E6, E7, and E6E7 ORF of HPV type 16. Agarose gel (1.5%) electrophoresis of PCR product. Size of amplified PCR products was indicated: E7, 369bp; E6, 492bp; E6E7, 861bp.

B. Structure of recombinant vector used to transfect human cervical keratinocytes (left), and agarose gel (1.5%) electrophoresis of pZip neo SV(X) digested with BamHI and pZip E6E7 digested with BamHI or HindIII.

mM NaCl, 5 mM MgCl₂), 0.33 mg/ml nitro-bluetetrazolium(NBT), 0.16 mg/ml 5-bromo-4-chloro-3-indolyl phosphate were added as chromogens and then counterstained with hematoxylin. RHEK-1 cells transfected with pZipE6 were stained with mouse anti E6 protein monoclonal Ab followed by biotinylated anti-mouse IgG Ab and then incubated with peroxidase conjugated streptavidin. After incubation, 3, 4-diaminobenzidine (DAB) was added and counterstained with hematoxylin.

Soft agar colony assay

Soft agar colony assay(Pecoraro *et al.* 1989) was performed to check the ability of anchorage independent growth of RHEK-1 or RHEK-E6 cells.

Western blot analysis

The expression of p53 in RHEK-1, RHEK-E6, pMAM transfected RHEK-E6 cells, and pMAM-p53 transfected RHEK-E6 cells was assayed by Western blot technique as described by Towbin *et al.* (1979). Total proteins were extracted with RIPA buffer (150 mM NaCl, 1.0 % NP-40, 0.5% deoxycholic acid, 0.1% SDS, 50 mM Tris-HCl, pH8.0) and concentration of total protein samples was measured by Bio-Rad protein assay kit. Equal amount of proteins was mixed with the sample loading buffer (Laemmli, 1970) and the mixture was boiled at 100℃ for 5 minutes. The mixture was subjected to SDS-polyacrylamide (12%) gel electrophoresis. Proteins in the gel were electro-transffered to a nitrocellulose (NC) membrane and was blocked with 10% non fat dried milk in 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.1% tween-20 (TBST) and was incubated for 1 hour with mouse anti p53 monoclonal Ab (1:1000 dilution) and then washed 2 times with TBST for 15 minutes each. The NC membrane was incubated with anti mouse IgG-hoarseradish peroxidase conjugate (1:2000 dilution) in TBST. To generate a signal, the NC membrane was incubated with enhanced chemiluminescence (ECL) detection reagents for 1 minute, and the membrane was exposed to Hyperfilm-ECL (Amersham International plc, Buckinghamshire, UK).

Analysis of cell growth rate

RHEK-E6 transfectant cells(1×10⁵ cells/dish) were seeded into a 60 mm dish and transfected with 1 μg of pMAM or pMAM-p53. Immediately after transfection, cells were treated with 1×10⁻⁶M dexamethasone and then were harvested after 48, 72, and 96 hours of incubation. Number of cells was counted with hemocytometer and the viability of the cell was evaluated by the ability to exclude tryphan blue. Each experiment was done in triplicate.

Analysis of apoptosis

ApopTag™ (Oncor, Gaithersburg, MD, USA), an in situ apoptosis detection kit, was used to check the apoptosis in the RHEK-E6 cells
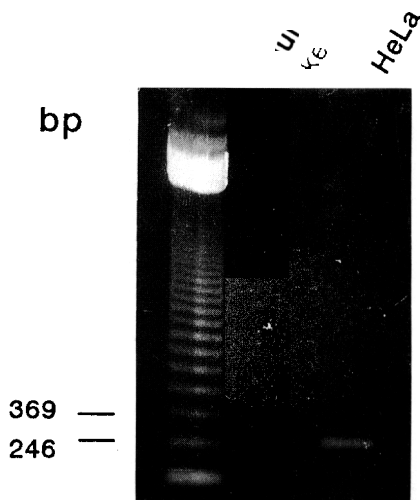
Fig. 2. PCR to rule out the possible infection with human papillomavirus in the normal cervical epithelial cells.
PCR was done by using the E6 consensus primers (described in the text) and lysates of normal cervical epithelial cells or lysates of HPV 18 positive HeLa cells.

A.

B.
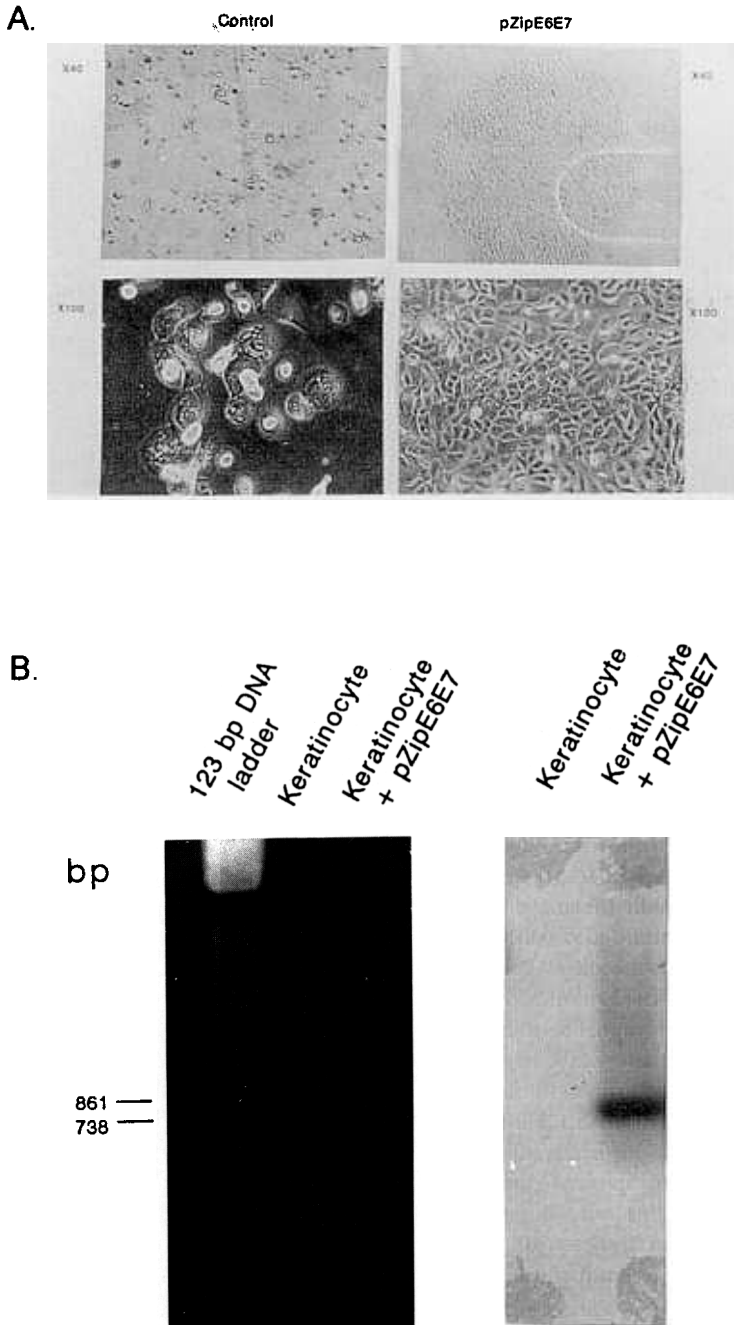
Fig. 3. *Colony formation of pZipE6E7 transfected human cervical kerationocytes and identification of HPV DNA in transfected cervical epithelial cell.*

A. Human cervical kerationocytes transfected with pZipE6E7 divided actively and formed colnies on 4th week of crisis period after 5th passage from primay culture (right). In contrast, control cells lost ability to proliferate and were senescensed after 4th passage (left).

B. PCR amplification of control and E6E7 transfected cell (left), and Southern blot analysis of PCR products (right). Nylon membrane was hybridized with ^{32}P labeled E6E7 ORF fragment of HPV type 16.

transfected with pMAM-p53 expression vector. All the procedures were performed according to the manufacturer's instructions. Prior to visual inspection, cells were counterstained with methyl green.

RESULTS

Culture of primary human normal cervical epithelial cell

Normal cervical epithelial cells were detached off after 4th passage with differentiated morphology and average time per passage was 7 days. Immunohistochemical staining with anti keratin antibody (Ab) revealed that the cultured cells were keratinocytes (data not shown). And PCR with HPV E6 consensus primers showed that there was no HPV in the cultured cells (Fig. 2).

After transfection with pZipE6E7 and G418 selection, cells could be passaged 4 times and then experienced crisis for 6 weeks. During this crisis period, the cell's ability to divide diminished markedly and almost all the cells had the differentiated morphology. After cri-sis, some cells could divide and form the colony with the same shape as normal keratinocyte and could passage 4 times more (Fig. 3A right). Then the cells reentered into second crisis and there was no growth thereafter. Normal control cells could survive until 2 weeks but, thereafter all the cells were detached off (Fig. 3A left).

The PCR result for demonstrating the presence of transfected E6E7 ORF DNA in the transfected cells showed 815 bp amplified DNA on agarose gel electrophoresis (Fig. 3B left) and the Southern hybridization result showed that the amplified DNA was the same DNA as used in transfection (Fig. 3B right). Cells transfected with expression plasmid containing HPV type 16 E6E7 ORF DNA showed prolongation of life span up to 4.5 times(18 weeks) compared with that of normal control cervical epithelial cells but the cells did not divide anymore. Therefore, the cells could not be used in the further experiments and we employed RHEK-1 human epidermal keratinocyte as equivalent cells. RHEK-1 cells transfected with pZipE6 were named as RHEK-E6 and used hereafter.
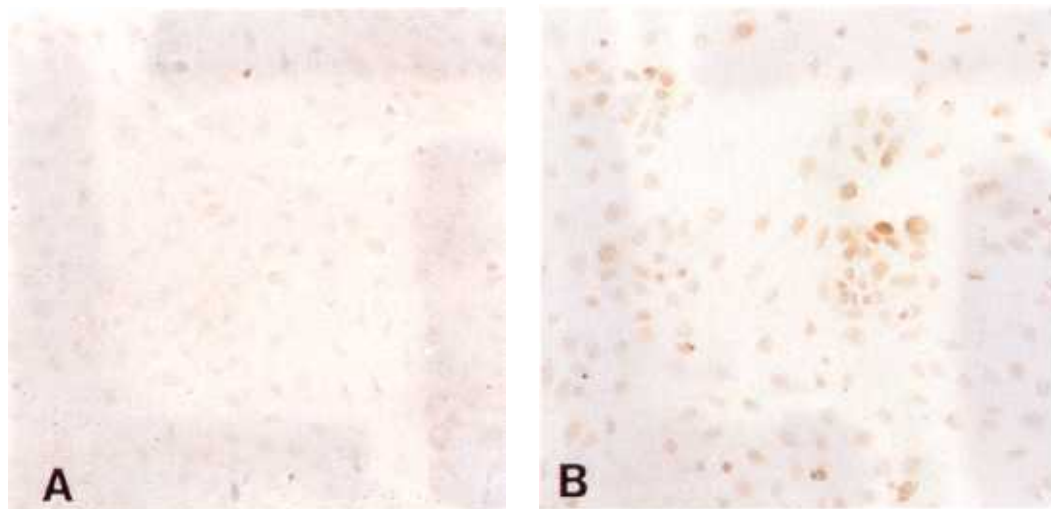
Fig. 4. Immunohistochemical staining with monoclonal anti E6 protein Ab.
A. RHEK-1 cells.
B. RHEK-E6 transfectant cells.
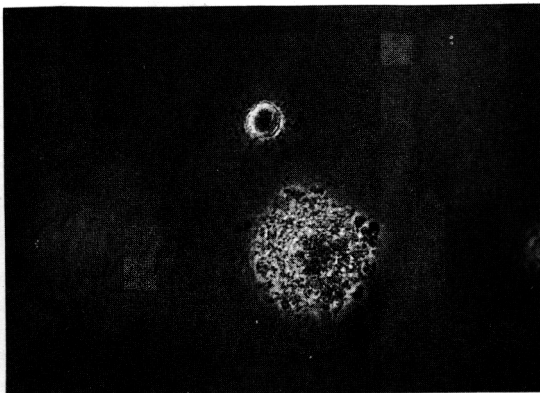
RHEK-E6 transfectant cells

Southern hybridization was performed to demonstrate presence of the transfected HPV DNA in the transfected RHEK-E6 cells. Because there was no signal in this analysis, PCR was done. Amplified 500 bp DNA was observed in RHEK-E6 transfected cells, but there was no amplified DNA in normal RHEK-1 cells (data not shown). Immunohistochemical staining with anti E6 protein monoclonal Ab showed that RHEK-1 cells were not stained (Fig. 4A) but RHEK-E6 cells showed a brown colored signal(Fig. 4B).

Anchorage independent growth of RHEK-E6

Soft agar colony assay showed that RHEK-1 cells formed colonies when transfected with E6 ORF DNA of HPV type 16 and had an anchorage independent growth character(Table 1, Fig. 5).

Effect of exogenous wild type p53 on RHEK-E6 cells

RHEK-E6 cells were transfected with pMAM or pMAM-p53 and cultured for 48 hours in the presence of 1×10^{-6}M dexamethasone. Total proteins were extracted from each

Table 1. Colony forming efficiency* of RHEK-E6 cells

Cells	Colony forming efficiency of initial seeding density of 5×10^5 cells/plate
RHEK-1	0
RHEK-E6	1.17

*: Colony forming efficiency $= \dfrac{\text{No. of colony}}{\text{Seeded cell No.}} \times 100$

Colonies were counted at 4 weeks after seeding.

Fig. 6. Western blot analysis for p53 tumor suppressor protein.
Total cellular proteins were extracted with RIPA buffer from each culture. Equal amount of proteins (20 μg) was subjected to SDS-PAGE(12%) and transferred to NC membrane, and then incubated with anti p53 monoclonal Ab followed by incubation with anti mouse IgG-hoarseradish peroxidase conjugate. Signal was developed with ECL. Lane 1: Total proteins extracted from RHEK-1; Lane 2: Total proteins extracted from RHEK-E6; Lane 3: Total proteins extracted from RHEK-E6 transfected with pMAM expression vector; Lane 4: Total proteins extracted from RHEK-E6 transfected with pMAM-p53 expression vector.

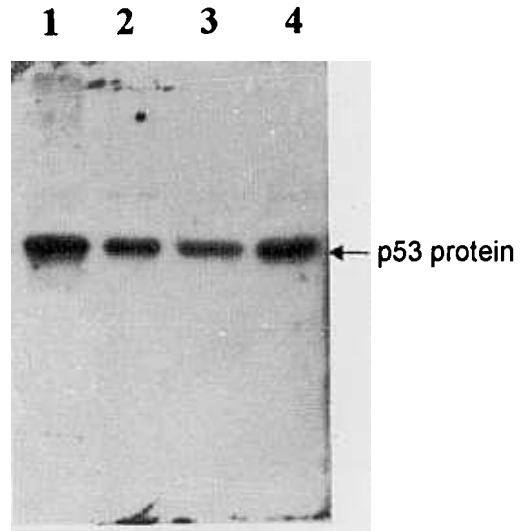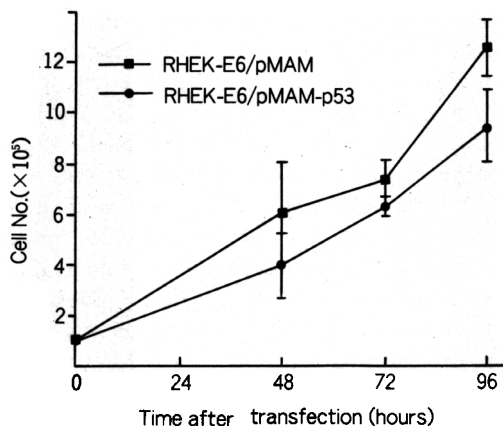

Fig. 5. Soft agar colony assay of RHEK-E6 cells.
RHEK-E6 transfectant cells showed many colonies characteristic of anchorage independent growth.

culture dish, and Western blotting was performed using anti p53 monoclonal Ab. It showed that p53 protein in RHEK-E6 cells (Fig. 6, lane 2) was less expressed than that of RHEK-1 cells(Fig. 6, lane 1), and amount of


Fig. 7. *Effect of exogenous wild type p53 expression on the growth rate of RHEK-E6 transfectant cells. Cells (1×10⁵) were seeded into 6 well culture dish and then transfected with pMAM or pMAM-p53. Number of cells was conuted at 48, 72, and 96 hours after transfection using a hemocytometer. Each point shows mean±SD.*

p53 protein was increased when RHEK-E6 cells were transfected with pMAM-p53(Fig. 6, lane 4). To monitor the effect of exogenous wild type p53 on cell growth, 1×10^5 cells were seeded into 60 mm culture dish and were cultured for 48, 72, and 96 hours in the presence of dexamethasone. Growth rate of pMAM-p53 transfectant cells was diminished up to 26.4% compared with that of the control cells at 96 hours(Fig. 7).

After 48, 72, and 96 hours of transfection with pMAM-p53, RHEK-E6 cells were stained with ApopTag™ kit. Signal that represented apoptosis was observed in many cells at 48 hours after transfection(Fig. 8), but cells at 72, and 96 hours after transfection showed no apoptotic signal (data not shown). Accordingly, the diminution of growth rate in pMAM-p53 transfectant cells might result from apoptosis caused by expression of the exogenous wild type p53.

DISCUSSION

Here, we report that the E6 and E7 protein of HPV type 16 could prolong the life span of normal cervical epithelial cells up to 4.5 times,
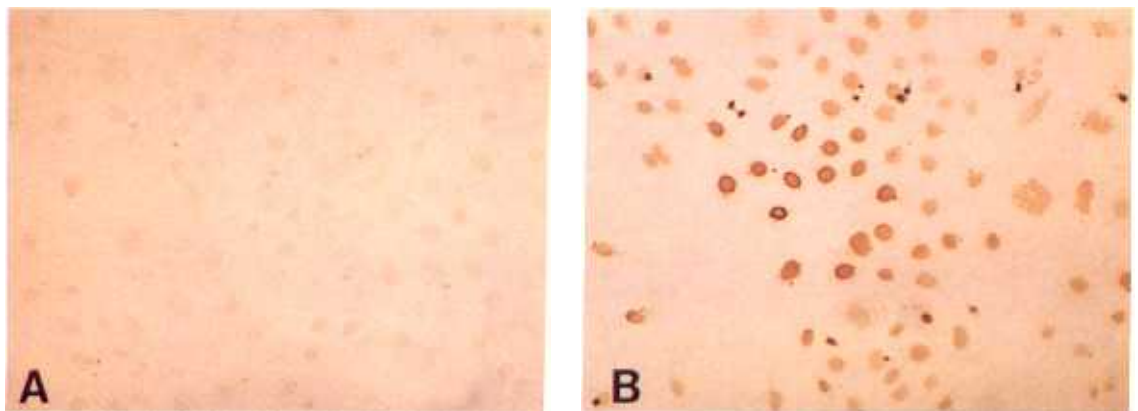
Fig. 8. *Apoptosis in the RHEK-E6 transfected with wild type p53.*
A. RHEK-E6 cells transfectd with pMAM.
B. RHEK-E6 cells transfected with pMAM-p53. After 48 hours of transfection, cells were stained with ApopTag™ kit and then counterstained with methyl green before viewing.

but the cells did not divide after the second crisis. The E6 protein could induce anchorage independent growth character of RHEK-1 cells and exogenous wild type p53 expression could diminish the growth rate of RHEK-E6 transfectant cells.

It has been proposed that there are two distinct stages, M1 and M2, in the process of immortalization of human fibroblast induced by simian virus 40 (SV 40) large T antigen (Shay *et al.* 1991). M1, large T antigen dependent stage, is associated with increased life span before crisis, whereas M2, large T antigen independent stage, is associated with the crisis. Large T antigen dependency of M1 suggest that M1 is more closely related with overcoming tumor suppression function of p53 and pRB proteins which bind large T antigen. The E6 and E7 of HPV can also bind p53 and pRB protein, respectively, and inactivate their suppressor activity. In our study, we could observe increased life span of keratinocytes transfected with expression vector containing E6E7 compared with that of normal keratinocyte, which imply that E6E7 transfected keratinocytes overcome M1 restriction such as in the case of fibroblast immortalized by large T antigen. But the fact that E6E7 transfected kera-tinocyte could not acquire the indefinite life span suggests that it may require other factors to overcome the M2 restriction.

RHEK-1 cells are human epidermal keratinocyte transformed by adenovirus type 12 (Ad12)-SV40 hybrid and express SV40 large T antigen and small t antigen. The cells do not express Ad12 transforming protein, and they do not have an ability either to form tumor when injected into nude mice or to form colonies on soft agar colony assay (Rhim *et al.* 1984).

It was reported that SV40 large T antigen could form a complex with the p53 protein (Lane and Crawford, 1979) and stabilize the p53 protein which had short half life (Oren *et al.* 1981; Reich *et al.* 1983). Interaction between SV 40 large T antigen and p53 might induce immortalized phenotype of RHEK-1.

Yustsudo *et al.* (1988) reported that tumorigenicity and anchorage independent growth are not always associated; the E6 protein is responsible for tumorigenicity and the E7 protein for the stimulation of cell growth, and both ORFs are necessary for full transformation. In our study, RHEK-E6 transfectant cells could form many colonies on soft agar colony assay but RHEK-1 cells could not. This fact suggested that the expression of HPV E6 could induce character of anchorage independent growth of RHEK-1 cells. The growth might result from intracellular oncogene complementation between SV 40 large T antigen and E6 ORF of HPV type 16. We could not find the HPV DNA in the RHEK-E6 on Southern blot analysis but could find HPV DNA fragment on PCR, and immunohistochemical staining with anti E6 protein monoclonal Ab showed a brown signal at the cytoplasm and nucleus of RHEK-E6 but no signal at the RHEK-1 cells. Bayever *et al.* (1988) reported that they used pZip Neo SV (x) retroviral vector to transfer the Neo^r gene into bone marrow cells, and following infection, bone marrow cells of multiple hematopoietic lineage displayed resistance for the duration of cultures. However, upon DNA analysis of cells surviving in G418, the Neo^r gene was not detected under conditions where single copy genes could readily be seen. In our work, we detected the expression of E6 protein in the RHEK-E6 transfectant but the levels of expression were variable because we pooled colonies that appeared after G418 selection and heterogeneous cell populations might have different activities of transcription and translation.

RHEK-E6 pooled transfectant cells were transfected transiently with pMAM-p53 to monitor the effect of exogenous wild type p53 on RHEK-E6 cells. Upon Western blot analysis, p53 protein in RHEK-E6 cells was less expressed than that of the RHEK-1 cells because the E6 protein encoded by high risk HPVs promote the degradation of p53 protein via ubiquitin dependent proteolytic pathway (Scheffner *et al.* 1991). pMAM-p53 transfectant cells grew continuously but their growth rate was reduced compared with that of RHEK-E6 cells. Woodworth *et al.* (1993) reported that HPV immortalized keratinocyte cell lines and three HPV positive cervical carcinoma derived

cell lines expressed significantly less (< 3 fold) p53 protein than normal keratinocytes, and the up regulation of wild type p53 (> 5 fold) by retrovirus infection did not significantly inhibit growth or restore normal epithelial differentiation in any line. Thus, down regulation of total cellular p53 levels by E6-mediated binding and degradation is not required to maintain the immortal phenotype in these cell lines.

Recent studies suggested that normal intracellular function of p53 protein is to arrest cell growth after DNA damage, thus allowing DNA repair to occur prior to cell division (Kastan *et al.* 1991; Lane 1992). And another line of recent reports suggested that p53 is not required for normal development, but in certain cellular environments (DNA damage, cellular stress), p53 expression is stimulated. In turn, p53 binds to Cip1 (Harper *et al.* 1993)/WAF1 (El-Deiry *et al.* 1993) regulatory elements and transcriptionally activates its expression. The Cip1/WAF1 protein binds to and inhibits cyclin dependent kinase which has been implicated in the control of G1 to S phase transition in mammals and thus prevents phosphorylation of critical cyclin dependent kinase substrates and blocking cell cycle progression. Induction of the wild type p53 in cells result in G1 arrest or apoptosis (El-Deiry *et al.* 1994).

Noble *et al.* (1992) reported that the induction of p53 expression resulted in a decreased growth rate and a decreased proportion of S phase cells in A459 lung carcinoma cells. And Shaw *et al.* (1992) reported that the induction of wild type p53 resulted in progressive detachment of transfected human colon tumor derived cell line EB and developed morphologic feature of apoptosis. DNA from both attached and detached cells was degraded into a ladder of nucleosomal sized fragments. Yonish-Rouach *et al.* (1991) reported that the introduction of wild type p53 resulted in rapid loss of cell viability in a way of characteristic apoptosis when murine myeloid leukemic line M1 was transfected with temperature sensitive mutant p53.

Our results from this study showed that although not being of physiological condition,

the expression of exogenous wild type p53 protein overcame the dysfuction of p53 on the cell cycle regulation imposed by E6 protein and eliminated the cells by apoptosis. These results suggested us that cancers originated from the dysfunction of p53 protein (by viral oncoprotein or point mutation) can be treated with the introduction of wild type p53 into affected cells. Therefore, further study will be needed to develop appropriate expression vector system which is delivered specifically to the affected cells and of which regulation is precisely controlled.

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