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Inhibition of Cervical Cancer Cell Growth by Gene Silencing of HPV16 E6 Induced by Short-interfering RNA

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The Human Papilloma Virus (HPV) infection has been strongly associated with pathogenesis of uterine cervix carcinoma. HPV type 16, a causative agent of uterine cervix carcinoma, encodes the E6 and E7 oncogenes, expression of which is pivotal for malignant transformation and maintenance of malignant phenotypes. To develop a gene therapy for HPV-related carcinoma, We investigated the effect of E6 short-interfering RNA (E6 siRNA) on the expression of this oncogene and on the growth of HPV 16-related uterine cervix carcinoma cells. SiHa cells, a uterine cervix carcinoma cell line, which contain a single copy of HPV 16 integrated in the chromosome and express the E6 and E7 oncogenes. Before 24 hr of transfection, cells were seeded and transfected with control plasmid or E6 siRNA-expressing plasmid. The mRNA was analysed by reverse transcriptase polymerase chain reaction (RT- PCR). The cell growth rate was investigated by MTT method. The E6 mRNA level in SiHa cells was decreased in HPV 16 E6 siRNA-expression vector transfected cells and a decrease in the growth of these cells was also observed. From these results. it is evident that E6 siRNA played a role in suppression of growth of SiHa cells and has a fair chance as a candidate for gene specific therapy for HPV related uterine cervix carcinoma.

Key Words : HPV 16, E6, siRNA, SiHa cell, Uterine cervix carcinoma

INTRODUCTION

The average length of the life of a person is increasing and to follow up the pace the occurrence rate of cancer and chronic metabolism disease is also increasing. Uterine cervix carcinoma, commonly known as woman cancer is a commonly known type of carcinoma and its prevalence rate is very high in Korea (Ministry of Health and Welfare, 2003). It is revealing enough to know the fact that it has close relation to cervix cancer and is originated due to human papilloma virus infection (Munoz, 2003). Gissman *et al*. (1977) first characterized four different isolates of Human papilloma virus (Gissman *et al*, 1977). Since then about seventy subtypes of this virus have been found. About forty of them are double stranded DNA virus which were isolated from the infected organs of generation (Gissman *et al*, 1977; Matsukura and Sugase, 2001). The HPV type 16 and 18 which infect the organs with mucosa tropic group are the high risk group and the HPV type 6, 11, 42, 43 and 44 which are discovered from the invasive carcinoma cell have a possibility of sharing the low risk group inside the epithelium and is mainly discovered together from the low-end lesion region cell (Lorincz *et al*, 1992). Carcinogenesis by the high risk group, HPV type 16 and 18 by the E6 and E7 oncogene expression is mortal as it suppresses the function of the p53, which is a tumor

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suppressor protein (Havre *et al*, 1995; Wells *et al*, 2000). The E6 oncogene is important for existence of carcinogenesis of the cervix cell due to HPV type 16 because E6, E7 oncogene expression increases in the cervix cancer cells (Havre *et al*, 1995). It follows hereupon that treatment of the cervix cancer caused by HPV, involves the E6 or E7 gene expression silencing, and multi branched methods for this test were attempted. Fire *et al.* (1998) while searching for anti-sense RNA from the C. elegans, discovered the interference RNA (RNAi) which destroys the double stranded mRNA (dsRNA) having a complementary base sequence. He used a specific homologous base sequence of RNA and it destroyed the mRNA. It was confirmed that the knock-down condition is actually due to hindrance of the expression of the gene in the animal cell (Bass, 2000). Consequently the knock-down induction technique which uses a interference RNA in actual condition is a tool which is becoming powerful specially in cancer therapy and in treatment of virus characteristic diseases which is evident from various research in this field (Tuschl, 2001). McCaffrey *et al.* (2002) used the short interference RNA (siRNA) against hepatitis C virus in mouse and the protein synthesis of the hepatitis virus was suppressed. Similarly, according to Gitlin *et al.* (2002) poliovirus siRNA infected to the poliovirus decreases the progeny virus remarkably from the cell. Possibility of gene cure of cancer was augmented very recently, and is Jacque *et al* (2002). reported that the HIV-1 virus siRNA suppresses the reproduction of the HIV-1 virus. The place of RNAi, having the becoming the aim of gene therapy of the dominant mutant oncogene, amplified oncogene and the virus cancer genes etc. (Shuey *et al*, 2003; Ge *et al*, 2003). Cioca *et al.* (2003) have reported that the c-raf gene and bcl-2 gene RNAi induce the apoptosis in myeloied leukemia cell line. According to Aoki *et al.* (2003) RNAi is stronger than anti-sense RNA suppressing gene expression in cancer cell line. The RNAi thus has a very promising prospect in field of gene therapy of cancer.

In order to observe the actual condition which is required to suppress the E6 oncogene expression of HPV type 16, known to be involved with cervix cancer occurrence, the HPV 16+ Uterine carcinoma cell line (SiHa Cell) is transfected with HPV type 16 E6 RNAi and the following study is done to understand the effect on E6 gene expression on growth of the cell.

MATERIALS AND METHODS

1. Cell culture

Uterus cancer cell from a person infected with the human papilloma virus type 16 (SiHa cell, HPV 16+) was obtained from the Korean cell line bank. The SiHa cell was cultured in a 6-well cell culture courage by innoculation in the Dulbecco's modified eagle medium (DMEM, Gibco BRL, GrandIsland, NY, USA) along with 5% fetal bovine serum, streptomycin (100 U/mL) and penicillin (100 U/mL). It was incubated in CO_2 incubator at 37℃, 5% CO_2. After 24 hours of incubation, the cells were transfected with HPV 16 E6 short interference RNA (siRNA) expression plasmid construct. Lipofectamine (Life Technology Co., USA) was used for transfection and it was done according to the test conditions of the manufacturing company. After the 24 hours of the transfection, collected cell, separated RNA and conducted reverse transcription polymerase chain reaction (RT-PCR).

Also it removed medium and washed with Dulbecco's phosphate buffer solution (PBS) from transfected cell, add 100 μL SDS (sodium dodecyl sulfate) - loading buffer (50 mM Tris-HCl : pH 6.8, 2% SDS, 0.1% bromphenol blue, 10% glycerol), conducted immunoblot which is electrophoresis and incubated for 5 min at 95℃.

2. Creation of E6 siRNA plasmid vector

The base sequence of human papilloma virus E6 siRNA,

HPV 16 E6 siRNA forward strand, 5'-GAAUGUGUGUACUGCAAGCdTdT-3' and reverse strand, 5'-GCUUGCAGUACACACAUUCdTdT-3' was obtained from Bioneer company (Seoul, Korea). The HPV 16 E6 siRNA DNA forward strand and reverse strand were mixed at 1:1 ratio and RT-PCR was performed at 95℃ for 10 minutes and 2 hours at room temperature. The reaction mixture for E6 siRNA plasmid vector construct (HPV E6-siRNA expression plasmid) consisted of 10x dilution ligation buffer (1 μL), ddH_2O (4.5 μL), pSUPER (3 μL), insert DNA (E6-siRNA expression DNA) 1 μL and T4 DNA ligase (0.5 μL) and incubated for 12 hours at 16℃. The plasmid vector construct was amplified by transforming it into competent cells, cultivating the cells and extracting the HPV 16 E6-siRNA expression plasmid vector using plasmid DNA isolation kit (Trizol, Life Technology Co., USA) (Fig. 1).

3. Transfection test

The SiHa cell were seeded in 24-well cell culture courage at 1×10^5 cells/well. After 24 hours of cultivation, the cells were transfected with control expression plasmid and HPV 16 E6-siRNA expression plasmid by using Lipofectamine (Life Technology Co., USA). The HPV 16 E6-siRNA constructed expression plasmid pSUPER-HPV 16 E6 (0.25 μg/μL) 10 μL, pEGFP-C2 (0.46 μg/μL) 0.5 μL, and DMEM (serum free) 89.5 μL was mixed with Lipofectamine blend (lipofectamine 6 μL and DMEM 94 μL) and incubated for 30 minutes at room temperature. Free DMEM 800 μL is added to each of the 24-wells then it is incubated for 5 hours at 37℃. Then the medium is changed to DMEM containing 10% fetal bovine serum. Control group (control expression plasmid) are used pSUPER-HPV 16 E6 (0.25 μg/μL) substitution pBluescript II SK (0.4 μg/μL), the dosage and method executed are identical to the previous method.

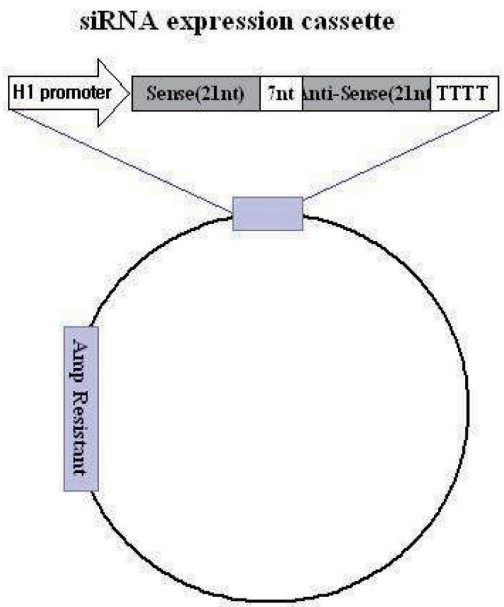

Fig. 1. Construction of siRNA expression vector.

4. RNA isolation

The RNA was extracted from the cultivated cells after removing the culture fluid, the cells were collected and homogenized after addition of TRI Regant (Quiagen GmbH, Co., Hilden, Germany). To it was added 0.2 mL chloroform and after mixing, incubated at room temperature for 15 minutes, Then it was centrifuged at 12,000x g for 15 minutes at 4℃, and the supernatant was collected. To it was added 0.5 mL isopropanol and incubated for 5~10 minutes at room temperature. Then it was centrifuged at 12,000x g for 15 minutes at 4℃ and the upper layer was discarded., The pellet was washed with 1 mL 75% ethanol for 5 minutes and the supernatant was discarded., 50 μL of 0.1% DEPC solution was added and heated at, 55~60℃ to dissolve the pellet.

5. cDNA Synthesis and PCR

The transcription reaction was made using random hexamer primers and transcription enzyme RAV-2 (TaKaRa Code No. 2610, TaKaRa Korea biomedical Co., Korea) First, RNA (10 μL), primer (2 μL) were incubated at 95℃

for 5 minutes and then in ice for 5 minutes. then the mixture was centrifuged at 10,000 rpm for 5 seconds and then mixed with distilled water controlled with the DEPC (20 μL), PCR buffer (10 μL), 0.1 M dTT (5 μL),10 mM dNTP (2 μL), RNAsin (1 μL) and reverse transcriptase (1 μL) and incubated at 37℃ for 1 hour. The PCR mixture consisted of DEPC controlled distilled water (30.5 μL), 10x PCR buffer (55 μL), 25 mM $MgCl_2$ (6 μL), 10 mM dNTPs (1 μL), forward primer (1 μL), reverse primer (1 μL), cDNA template (5 μL), *Taq* polymerase (5 U/μL, 0.5 μL). The conditions of the reaction were as follows, denaturation at 95℃ for 45 seconds, annealing at 50℃ for 45 seconds, and enzyme polymerization 72℃ for 1 minute and this cycle was repeated 30 times and the reaction mixture was stored at 4℃ after completion. In order to confirm the amplification PCR product, 10 μL of it was mixed with 2 μL of gel loading buffer (0.25% bromophenol blue tracking dye in 25% Ficoll) and run on a 1.5% agarose gel at 100V for 30 minutes in TAE buffer (0.04 M tris-acetate, 0.001 M EDTA) in a mini-gel electrophoresis unit (MUPID-2). The agarose gel was stained with ethidiume bromide solution and the required DNA band was identified after exposure to the UV-transilluminator. The band density of the control group and the test group was compared and measured with image analyzer (ID ver.2.1, Amersham Pharmacia Biotech Co., Uppsala, Sweden) measured (Table 1).

Table 1. The nucleotide sequences of primer

Primer		Sequence	Product size
GA3PDH	For	GCA CAG GGA CAT AAT AAT GG	584
	Rev	CGT CCA AGA GGA TAC TGA TC	
E6	For	ACC GAA AAC GGT CGA ACC GA	308
	Rev	AAT AAT GTC TTT ATT CAC TA	
P53	For	TGT TCA CTT GTG CCC TGA CT	310
	Rev	AGC AAT CAG TGA GGA ATC AG	

6. Western blot

The cells were washed with Dulbecco's PBS once and to it was added 100 μL SDS-loading buffer (50 mM Tris-HCl, pH 6.8, 2% SDS, 0.1% bromphenol blue and 10% glycerol) and the cells were lysed at 95℃ for 5 minutes. Electrophoresis was carried out in a 10% polyacrylamide gel in Tris buffer (pH 8.8, 0.025 M Tris, 0.192 M glycine and 0.1% SDS) at 80V for 2 hours. After electrophoresis Western blotting was performed. The proteins were transferred to a nitrocellulose membrane using transfer buffer (25 mM Tris, 192 mM glycine and 20% methanol) at 20V from for transfer 12 hours. The membrane was blocked with 5% non fat milk in TBS (0.1% Tween 20 in pH 7.4 Tris-based saline buffer) for 1 hour 5% and washed with TBS for 2 times. The membrane was incubated with Anti-rabbit polyclonal anti-p53 Ab (1:1200 dilution, Santa Cruz Biotechnology, Inc., USA) with 2% non fat milk/TBS for 12 hours at 4℃. After 4 times washing with TBS, horseradish peroxidase-labeled goat anti-rabbit IgG (1:2000 dilution), in 2% non fat milk/TBS was added for 1 hour and again washed for 4 times with TBS. The Membrane was developed using chemiluminescence solution (Amersham Pharmacia Biotech Co., Uppsala, Sweden), and exposed to X-ray film for 30 seconds to 1 minute. The band intensity was measured by image analyzer.

7. Measurement of Cell growth

The SiHa were cultured in 24-well cell culture courage, 1×10^5 cell/well. After 24 hours of cultivation, the cells were transfected with control expression plasmid and HPV 16 E6-siRNA constructed expression plasmid using Lipofectamine. After 24 hours the cells were trypsinized and dispensed into a 96 well cell culture courage at 5×10^4 cell/well, After 24 hours of plating, the cell viability was measured using the MTT (3-(4,5-dimethylthiazol-2-4yl)-2,5-diphenyltetrazolium bromide) method (Carmichael *et al*, 1987). The MTT the last consistency became and 1 mM

it added during 4 hours of incubation, the culture medium was removed and the cells were washed 3 times with Hank's balanced salt solution (HBSS), Then 200 μL of dimethyl sulfoxide (DMSO) was added and measured at 540 nm both for normal control & control expression plasmid. Compared to normal group and control expression plasmid group the si RNA transfected cells showed an extinction of about 50% (Table 2).

RESULTS

1. The effect of E6 siRNA on E6 gene expression in the SiHa cell

The SiHa cell has HPV 16 virus DNA incorporated inside the chromosomal DNA. Hence, to know the effect of HPV 16 E6 siRNA (short interference RNA) on E6 gene expression the SiHa cell was transfected with HPV 16 E6 siRNA and E6 mRNA was measured by RT-PCR (Fig. 2). The total RNA was isolated after 24 hours of transfection and cDNA was synthesized by RT-PCR, and analysed by electrophoresis. By measuring the density of the DNA band of E6 gene and comparing it with standard contrast gene glyceraldehyde-3-phosphate dehydrogenase (GA3PDH) gene, the ratio was calculated. E6/GA3PDH ratio of the normal control group which not transfected is 0.8, transfected with plasmid vector character, control group which it 0.7, and in the cells transfected with E6 siRNA expressions plasmid vector, (the test group) is 0.1. It is evident from these result that E6 siRNA plasmid construct controls the E6 gene expression in the test group and the E6 gene expression is remarkably diminished (Fig. 2).

Table 2. Effect of HPV16 E6 siRNA on the growth of SiHa cells

Groups	Cell growth (A : 540nm)
Normal control	0.871±0.010
Control plasmid	0.749±0.013
siRNA plasmid	0.365±0.009

Values are mean±SD, n= 5.

2. The effect of E6 siRNA on the p53 gene expression of SiHa cell

The HPV 16 virus E6 oncoprotein acts by ubiquitin-proteasome pathway and disjoints the p53. Consequently to study the effect HPV 16 E6 siRNA on p53 gene expression, the SiHa cell was cultured and after transfection with HPV 16 E6 siRNA, p53 mRNA volume was isolated and after synthesis of cDNA by RT-PCR it was analysed by electrophoresis. The density of the DNA bands (Fig. 3) were measured and the p53 gene was compared with the GA3PDH gene which is the standard control gene and the ratio was calculated. p53/GA3PDH ratio in the non transfected normal control group is 0.9, in cells transfected with the vector plasmid control group is 0.8 and the test group which is transfected with siRNA expression plasmid vector is 0.9. From the above results we can say that there is no effect of E6 si RNA on p53 gene expression in SiHa cell (Fig. 3).

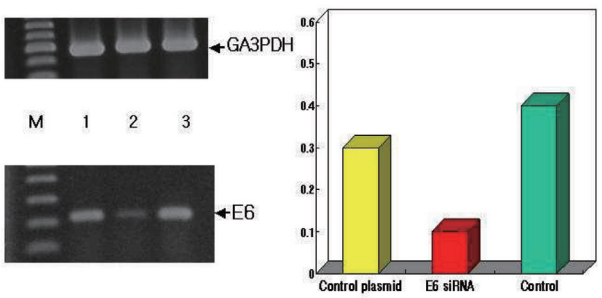

Fig. 2. Suppression of HPV16 E6 transient expression by siRNA. SiHa cells were transfected with a HPV E6 siRNA expression plasmid for 24 hr and then total RNA was prepared and expression level of E6 mRNA was analyzed by RT-PCR.
M: molecular weight control,
lane 1 : Control plasmid transfection,
lane 2 : HPV E6 siRNA expression plasmid transfection,
lane 3: Non-transfected control

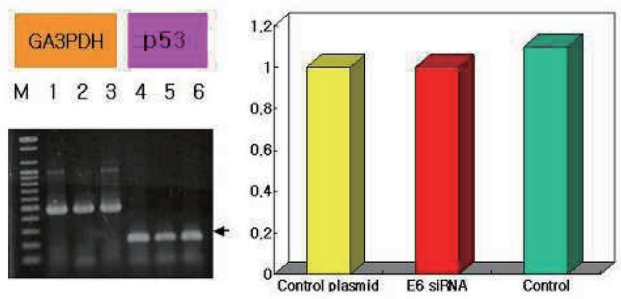

Fig. 3. Effects of E6 siRNA on p53 expression in SiHa cells. SiHa cells were transfected with a HPV E6 siRNA expression plasmid for 24 hr and level of p53 mRNA was analyzed by RT-PCR.
M: molecular weight control, lane 1,4: Control plasmid transfection, lane 2,5: HPV E6 siRNA expression plasmid transfection, lane 3,6: Non-transfected control.

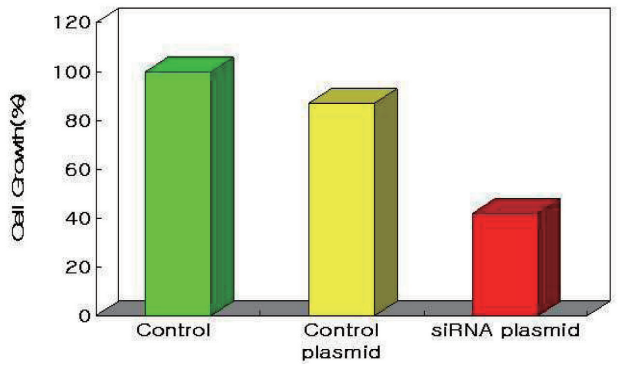

Fig. 4. Suppression of SiHa cell growth by siRNA. Effect of HPV 16 E6 siRNA on the monolayer growth of SiHa cells. SiHa cells were transfected with 2.5 μg control plasmid or E6 siRNA-expression plasmid for 24 hr, then trypsinized, and dispensed into a 96-well plate at $5x10^4$ cells per well, and cell viability was determined 24 hr after plating by MTT assay.

3. The effect of E6 siRNA on growth of SiHa cell

To study the effect of HPV 16 E6 siRNA on SiHa cell growth, the SiHa cell was cultured and transfected with HPV 16 E6 siRNA and number of cells were estimated by the MTT method after 48 hours of cultivation. The conversion of MTT to insoluble formazan was measured using spectrophotometer. In the non transfected normal control group formazan is 100%. The Formazan ratio of the control plasmid vector transfected group its 87% of normal control group, and that of the E6 siRNA expression plasmid vector transfected group is 42% of normal control group. Hence it is seen that the formazan creation quantity is diminished in the test group which is transfected with E6 siRNA expression plasmid vector which suggests that the number of viable cells was diminished due to cell death (Fig. 4).

DISCUSSION

While deaths from uterine cervix carcinoma ranking are decreased during past fifty years in advanced countries, it is still high in Korea. There are many known risk factors which cause uterine cervix carcinoma. Among these, an individual the experience of first sexual intercourse at age below 20, or individuals who have multiple sexual relationships has sexual relationships with many people are more prone to this disease. Human papilloma virus is considered as an important causative factor of uterine cervix carcinoma and the virus can be transmitted by sexual intercourse.

HPV of high risk group (type 16, 18, 31, 33) is related to cancer, while HPV of low risk group (type 6, 11, 42, 44) is related to condyloma. The HPV of high risk group integrates into host genome DNA, while the HPV of low risk group integrates into episomal virus DNA in condyloma and has precancer focus. In addition, the HPV of high risk group (E6 and E7 gene of HPV type 16) has the ability of being transformed in experimental culture and combined with specific virus oncogene. E6 oncoprotein of HPV type 16 and 18 bind to p53, tumor suppression gene, and promotes proteinolytic degradation (Munoz, 2003; Lorincz *et al*, 1992; Matsukura and Sugase, 2001).

HPV is known to effectively cause a carcinoma among tumor inducible virus in human. Carcinogenesis mechanism as known shows that HPV DNA integrates into hu-

man DNA, and then E6 and E7 protein of HPV can bind to p53 and pRB, which are tumor suppressive proteins, and inactivate them in host cell, so can cause the cancer (Havre *et al*, 1995; Wells *et al*, 2000).

E6 and E7 protein inactivates regulation of cell cycle which is the function of tumor suppression gene, prevents repair of damaged DNA and promotion, induction and differentiation of apoptosis. E6 protein is known to be very important not only for maintenance of tumor but also for genesis of tumor (Wells *et al*, 2000; Kiyono *et al*, 1997).

In order to observe the effect of inhibition of E6 gene expression in tumor cell, we used E6 siRNA (E6 short interference RNA) to SiHa (HPV 16+) cell and obtained the result of inhibition of E6 gene expression. SiHa cell is originated from uterine cervix carcinoma and HPV 16 DNA is integrated into genomic DNA of cell so it can express continuously the E6 and E7 gene of HPV (Schwarz *et al*, 1985).

From this study, level of E6 mRNA level as detected by RT−PCR was reduced in E6 siRNA transfected cell. This result suggests that E6 siRNA leads to E6 mRNA reduction.

E6 protein is known to having close relationship with p53 protein in host cell. In this study, we did experiment to observe p53 gene expression in E6 siRNA transfected cell using RT−PCR and western blot, but the band intensity was not different from control, the untransfected cell. p53 a tumor suppression gene is located in short arm of chromosome 17. It consists of 375 amino acids and has a very short half−life (Quintanilla−Martinez *et al*, 2001). Normal p53 has an ability to suppress cell growth, especially can induce a reversible cell cycle inhibition at G1 and S phase of cell cycle (Taylor and Stark 2001). This inhibition of cell cycle can cause irreversible apoptosis (Tang *et al*, 2002; Bykov and Wiman 2003). Mutated p53 can occur in variety of human carcinoma, and it has weak or no ability of cell cycle inhibition (Hussain and Harris, 1999).

Loss of p53 function in uterine cervix carcinoma can be caused by interaction with HPV E6 protein of high risk group. Oncogene E6 of HPV 16 type bind to p53 so promote p53 degradation (Braun *et al*, 2004), which leads to loss of p53 functions in HPV positive carcinomas On the other hand, p53 mutant frequently occur in HPV negative carcinoma (Hashiguchi *et al*, 2004; Braun *et al*, 2004).

SiHa cell, which is originated from uterine cervix carcinoma, has one copy of HPV 16 DNA in chromosome. E6 and p53 gene expression in this cell line is normal and mutant p53 is not occurred (Schwarz *et al*, 1985). So, SiHa cell is appropriate to observe the interrelation of E6 gene expression and p53 protein change by E6 siRNA.

RNA is physiologically important and plays many roles such as mRNA in gene transcription, rRNA and tRNA in translation and SnRNA and ScRNA in splicing and protein targeting. Double strand RNA can degrade mRNA, inhibit translation and transcription. This phenomenon of degradation of mRNA which is complementary homologous base sequence is RNA interference (RNAi) (Gitlin *et al*, 2002; Bass, 2000).

RNA can act in regulation of gene expression, anti sense RNA and RNA interference are well known mechanisms of post transcriptional gene silencing (PSTG) (Cogoni and Macino, 2000). Basic mechanism of RNA interference is to selectively degrade specific mRNA which is complementary to dsRNA so silencing the gene expression (Bass, 2000; Plasterk and Ketting, 2000).

RNA interference was first discovered at 1998 by Fire etc. from study of anti−sense RNA in *C. elegans*. After this, same phenomenon was discovered in Drosophila and plant, and has been recently reported in mammalian cells and human cells (Gitlin *et al*, 2002; Hammond *et al*, 2002).

Knock−out animal is the best model to study functional genomics but it costs lots of time and money. It is reported in mammalian and human cells that knock−down of specific gene expression by degrading mRNA using RNAi method can be an alternative method (Gitlin, 2002). RNA knock−down technique using RNAi will not only be the

most powerful tool in biological studies but also can be applied to treatment of cancer and virus-related disease (Jacque *et al*, 2002; Shuey *et al*, 2002).

RNAi can be achieved by fragment of dsRNA called siRNA (short interfering RNA) which has 21-23 nucleotides (Cogoni and Macino, 2000). Prevention of virus through RNAi seems to be very useful tool in plant, parasites and animal cell.

McCaffrey *et al.* (2002). confirmed that protein synthesis of hepatitis virus is inhibited by siRNA in study of hepatitis C virus at mice. Gitlin *et al.* reported that the production of progeny virus was reduced by poliovirus siRNA in poliovirus contaminated cell. Jacque *et al.* reported that HIV-1 virus siRNA inhibited replication of HIV-1 virus. Ge *et al.* found that, in embryo cells and chicken embryo, production of influenza virus was dramatically decreased by effect of siRNA. These reports, support the fact that RNAi can be used effectively in treatment of virus.

Mean while, silencing of gene expression using RNAi could be used in gene therapy, such as treatment of mutant gene and inhibition of gene expression that is abnormally expressed (Shuey *et al*, 2002). Regarding gene therapy of cancer using RNAi, dominant variation cancer gene, multiple cancer gene and viral cancer gene could be the target of gene therapy using RNAi (Shuey *et al*, 2002).

According to Cioca *et al.* (2003) siRNA of c-raf and bcl-2 gene induces cell apoptosis in myeloid leukemia cell line. Aoki *et al.* (2003) showed inhibition of gene expression by siRNA is more potent than anti-sense RNA in human cancer cell line, so RNAi using siRNA is the interesting field in gene therapy of cancer.

In this study, suppression of E6 mRNA was observed using HPV 16 E6 siRNA in cervix cancer cell line, SiHa cell. This result suggests that the amount of E6 protein was reduced by HPV 16 E6 siRNA so inhibition of p53 was diminished and apoptosis was induced. In addition, inhibition of cell growth by HPV 16 E6 siRNA means that HPV 16 E6 siRNA can inhibit growth of cancer cell so it can be used for target gene in gene therapy of cancer. We should confirm whether same result can be observed from in vivo assay or not.

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