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Growth Inhibitory Effect of Human Cervical Cancer Cells with the Direct Transfer of Liposome Complexed Recombinant pRcCMVp53

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We investigated cell growth inhibitory effect of the wild type p53 gene into the cervical cancer cells via the recombinant adenoviral plasmid pRcCMVp53 with lipofectin. Inhibition of the growth of cervical cells as determined by a cell count assay. The cells were inoculated at density of 10^4 cells/well in each 12 well plate, 24hr before infection. At each indicated point, cells in three wells on each well plate were trypsinized and counted. The mean cell counts of triplicated well after transfection at day 1~6 was checked. Inhibition of the growth of cervical cells were checked by ELISA assay, MTTassay. The cells inoculated at densities of 5×10^3/well in each 96 plate 24hr before infection. At each indicated point cells of three wells were transfected for 6days, harvested and counted by liquid scintillation counter the mean cpm per triplicate wells were plated for ELISA and MTT assay. One-way ANOVA F-test and multiple comparison Tukey method was used for statistical analysis. Inhibition of the growth of cervical cells in cell count showed CaSki(89%), SiHa(59.2%), HeLa(86%), HeLaS3(78%), C33A(91%) and HT3(74%). ELISA assay showed CaSki(70%), SiHa(53%), HeLa(73%), HeLaS3(50%), C33A(67%) and HT3(73%). MTT assay showed HeLa(33%), HeLaS3(28%), SiHa(75%),CaSki(38%),C33A(33%) and HT3(53%). These results indicate that transfection of cervical cancer cells with the wild type p53 gene via pRcCMVp53 with lipofectin is a potential novel approach to the gene therapy of cervical cancer.

Key words : p53, pRcCMVp53, cervical cancer, gene therapy

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Diverse sequence variants of human papillomavirus type 16 E6 in primary cervical cancers from Korea

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This study was performed to examine the DNA sequence variations in E6 ORF of HPV 16 in Korean women. Forty-two cases of invasive cervical cancer (ICC) tissues containing HPV 16 DNA confirmed by polymerase chain reaction (PCR) from Korean women were subjected to scrutinize the E6 gene mutations. PCR-amplified products were sequenced by the fluorescent dideoxy termination method and opposite strand sequencing was performed as required. A total of 23 of 151 codons contain 27 nucleotide substitutions. Among nucleotide change found at 27 sites in total, 10 sites were previously reported and 12 of 17 newly found sites resulted in amino acid change. The carboxy terminus contains the longest segment of highly conserved amino acids (27 bases). Thirty of 42 (71%) ICC contained HPV 16 E6 variants and the most frequently found variants (6 cases) showed change only at nucleotide position 178 (GAT to GAG, D25E). Over half of ICC (16/30) showed nucleotide change at position 178 which is frequently included within the HLA-A2.1 interaction nanomer. Ten ICC showed change at 548 (ACC to GCC, T149A) which may affect the p53 binding and degradation. Six ICC showed change at 350 (TTG to GTG, L83V) and 5 showed change at 335 (CAT to TAT, H78Y). In some extent, these frequently found sites have been suggested to be included within the T-cell peptide epitopes by previous researchers. Our data suggest that in case of the attempt to develop therapeutic HPV vaccine which might include the E6 epitope targeting Korean women, special regard should be paid to the sequence diversities of HPV 16 E6 gene.