Original Article

Viral Etiology Relationship between Human Papillomavirus and Human Breast Cancer and Target of Gene Therapy

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Abstract

Objective To explore the viral etiology of human breast cancer to determine whether there are novel molecular targets for gene therapy of breast cancer and provide evidence for the research of gene therapy and vaccine development for breast cancer.

Methods PCR was used to screen HPV16 and HPV18 oncogenes *E6* and *E7* in the SKBR3 cell line and in 76 paraffin embedded breast cancer tissue samples. RNA interference was used to knock down the expression of HPV18 *E6* and *E7* in SKBR3 cells, then the changes in the expression of cell-cycle related proteins, cell viability, colony formation, metastasis, and cell cycle progression were determined.

Results HPV18 oncogenes *E6* and *E7* were amplified and sequenced from the SKBR3 cells. Of the patient samples, 6.58% and 23.68% were tested to be positive for HPV18 *E6* and HPV18 *E7*. In the cell culture models, the knockdown of HPV18 *E6* and *E7* inhibited the proliferation, metastasis, and cell cycle progression of SKBR3 cell. The knockdown also clearly affected the expression levels of cell cycle related proteins.

Conclusion HPV was a contributor to virus caused human breast cancer, suggesting that the oncogenes in HPV were potential targets for gene therapy of breast cancer.

Key words: Human papillomavirus; Human breast cancer; RNA interference; Gene therapy

Biomed Environ Sci, 2016; 29(5): 331-339 *doi: 10.3967/bes2016.043* *ISSN: 0895-3988*

www.besjournal.com (full text) *CN: 11-2816/Q*

INTRODUCTION

In 2014, breast cancer was estimated to be the second most common cause of death (15%) and the leading cause of new cancer diagnoses (29%) among women[1]. There are many theories about the causes of breast cancer[2-3], including human papillomavirus (HPV)[4-5]. The relationship between HPV and many kinds of cancers, such as cervical carcinoma and head-neck carcinoma, is well established[6-8]. The hypothesis that HPVs might contribute to human breast cancer is based on the immortalization of primary mammary epithelial cells by high-risk HPV. However, there are conflicting reports regarding the prevalence of HPV DNA in breast cancer in different countries[9-14]. Similarly, there are conflicting reports regarding the presence of an HPV18 oncogene in the SKBR3 breast cancer

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cell line[5,15].

Gene therapy is a novel research field and over 63% of gene therapy clinical trials are conducted for cancer treatment. RNA interference (RNAi) is a mechanism of post-transcriptional gene silencing in plants and animals[16-17]. The use of short hairpin RNA (shRNA) to inhibit the expression of defined target genes has cleared therapeutic potential. In the context of breast cancer, identifying a putative target gene is the foundation of efficient gene therapy.

In this study, we aimed to determine whether the *E6* and *E7* oncogenes from HPV16 and HPV18 contribute to the viral etiology of breast cancer in the SKBR3 cell line and primary breast cancer tissue samples. We then explored whether they are novel viral targets for gene therapy for breast cancer, providing evidence for the future research in gene therapy and vaccine development.

MATERIALS AND METHODS

Human Specimens and Cell Culture

Seventy-six paraffin-embedded breast cancer samples were obtained from Peking University People's Hospital. Of the patients included, 31.58% (24/76) were aged 25-45 years, 50.00% (38/76) were aged 46-65 years, 17.11% (13/76) were aged 66-85 years, and 1.32% (1/76) were aged ≥86 years. In terms of the sex distribution, 1.32% (1/76) were males and 98.68% (75/76) were females. Invasive ductal carcinoma was detected in 98.68% (75/76) of the samples, while invasive lobular carcinoma was detected in 1 (1/76) sample. Of the 75 invasive ductal carcinoma samples, 48.00% (36/75) were in stage I, 34.67% (26/75) were in stage II, and 17.33% (13/75) were in stage III. Written consent statements were obtained from all the patients before the study. This study was carried out in accordance with the Code of Ethics of the World Medical Association. The privacy rights of the human subjects were protected. Human breast cancer cells (SKBR3) and human embryonic kidney (HEK) 293 cell lines were obtained from the Cell Resource Center, Peking Union Medical College. The cell line was confirmed free from mycoplasma contamination by PCR and culture assays. The species origin of the cells was confirmed by PCR. The identity of the cell line was authenticated with STR profiling (FBI, CODIS). HEK293 cells were maintained in Dulbecco's Modified Eagle Media (HyClone, Utah, USA) supplemented with 10% FBS (Gibco, Massachusetts, USA) at 37 °C and 5% CO_2. The SKBR3 cells were maintained in 1640 (HyClone, Utah, USA) supplemented with 10% FBS (Gibco, Massachusetts, USA) at 37 °C and 5% CO_2.

Detection of HPV Oncogenes in SKBR3 Cells and Breast Cancer Tissue Samples

Detection of HPV Oncogenes in SKBR3 Cells

Specific primers (Table 1) were designed based on the mRNA sequence of HPV18 *E6*, HPV18 *E7*. The total RNA was extracted from the SKBR3 cells by using Trizol (Invitrogen, Massachusetts, USA). Briefly, each sample was dissolved in 1 mL of Trizol and 0.2 mL of chloroform was added. Then the tube was shaken vigorously for 15 s and centrifuged at 12,000 × g for 15 min. The RNA was extracted by washing the pellet with 1 mL of ice-cold isopropanol followed by cold 75% diethylpyrocarbonate (DEPC) treated ethanol. The ethanol was removed and the tube was briefly air dried. The RNA pellet was resuspended in nuclease-free water. The complementary DNA (cDNA) was synthesized from the total RNA by using the Goscript reverse transcription system (Promega, Wisconsin, USA). The total DNA was extracted by using the QIAamp DNA Blood Mini Kit (Qiagen, Dusseldorf, Germany) from 1×10^6 cells.

Table 1. Primer Sequences

Target	Primer Sequences (5'-3')
HPV16*E6*	F: GCAAGCAACAGTTACTGCGA R: CAACAAGACATACATCGACC
HPV16*E7*	F: TGCATGGAGATACACCTACATTG R: CCCATTAACAGGTCTTCCAAAGT
HPV18*E6*-322(66-387)	F: CACTTCACTGCAAGACATAGA R: GTTGTGAAATCGTCGTTTTTCA
HPV18*E6*-155(306-460)	F: AATAAGGTGCCTGCGGTGCC R: TGCGTCGTTGGAGTCGTTCC
HPV18*E7*-125(86-210)	F: AGCAATTAAGCGACTCAGA R: GCTTCACACTTACAACACAT
HPV18*E7*-100(80-179)	F: CGGTTGACCTTCTATGTCACGA R: TGTGTGACGTTGTGGTTCGG
RB-qPCR	F: ATTGGCGTGCGCTCTTGAGG R: GGGTGCTCAGACAGAAGGCG
Tp53-Mutant	F: GGTGACACGCTTCCCTGGATTGGC R: AGGCTGTCAGTGGGGAACAAGAAGTGG
MDM2	F: TGGAGGGTAGACCTGTGGGC R: GGATTCGATGGCGTCCCTGT
CCNA1	F: TGGGGTCCAGGCAGGTTTTG F: CTGATTCTTGTGATCCCCTGGC
CCND1	F: CAGTAGCAGCGAGCAGCAGAGT R: GCGGCCAGGTTCCACTTGAG
BCL2	F: CAGGATAACGGAGGCTGGGATG R: AGGGCCAAACTGAGCAGAGTC
β-*Actin*-qPCR	F: TGACCCAGATCATGTTTGAGA R: TACGGCCAGAGGCGTACAGG