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RESEARCH ARTICLE

FUNCTIONAL CHARACTERIZATION & PREPARATION OF E6 & E7 ONCOGENES OF HIGH RISK ONCOGENIC HUMANPAPILLOMA VIRUS-16

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ABSTRACT

In reliable to the fact that changes in amino acid sequence of HPV-16 E6 & E7 proteins might modify the transforming activity of the protein by disturbing the interactions with the EGFR proteins. It was proposed that those variations in the E6 & E7 proteins may affect the transforming potential of HPV-16 owing to change affinity for cellular transcription factors or for viral DNA. In this study, HPV-16 DNA was isolated from cervical cancer tissue using the specific primer designed by the Primer 3 plus software for the HPV antigen E2 gene. The amplified gene was ligated with T vector (pEGTMZ) and transformed into DH5α cells. The plasmid DNA obtained was then established by restriction digestion and sequence analysis which was found to be 99% similar to that obtained in GenBank. Dendrogram was constructed using ClustalW software (online) to get the similarity of the sequence with the existing sequence in the NCBI.

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INTRODUCTION

Globally, cervical cancer, one of the most common malignancies among women, is measured to be mainly caused by the oncogenic human Papilloma viruses (HPV 17, 18, etc) (Santos *et al.,* 2001; McGlennen, 2000). Although more than 70 various types of human Papilloma virus genome have been recognised, only few HPV types have been recognized as high risk types owing to their relationship with an genital cancers, particularly cervical cancer. It has been found that DNA of these high risk HPVs, chiefly HPV-16 and HPV-18, are present in about 93% of invasive cervical cancer patients (Thomas *et al.,* 2001). Despite the fact that the region of the Papilloma virus genome required for transformation was thought to be the long control region (LCR) and the E6 and E7 genes products also play vital role in either activating or repressing the transcription of the promoter (de Villiers *et al*., 1994; Bosch *et al.,*1995; Eriksson *et al.,* 1999). It has also been found that E6 and E7 promoters, which inactivate two important cellular tumour suppressors, the p53 and the pRB proteins, respectively, in HPV 16 is relatively repressed by E2. Although the role of HPV E2 proteins in transcriptional regulation has been studied extensively (Veress *et al*., 1999). Moreover, the characterization of these high risk HPV types based on their gene sequence remains as a truthful method to identify different variants of HPV and thus it might validate the modification in normal physiological function (Roberts *et al.,* 1997). As a result, the objective of the present study is to isolate HPV 16 DNA from the cervical cancer tissue and then transform it into DH5α cells. The study also aims to verify the sequence of the E6 and E7 gene expressed by E.coli with reference to the GENBANK.

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MATERIALS AND METHODS

Tissue sample collection

In this study, 10 histopathologically confirmed cervical cancer biopsies were collected from patients attending Out-door Patients' ward Department of Gynecology and Obstetrics, District Hospital, Noida. The samples were screened for the presence of HPV and further HPV-16 positive cervical cancer cases were recruited for the study of variant analysis. Fresh tumor biopsies were collected in chilled 1X phosphate buffered saline (PBS) and stored at - 70°C deep freezer for further processing.

Isolation of genomic DNA from cancer tissue

In order to isolate the genomic DNA, collected sample tissue was taken thawing has been performed so that the cervical scrape sample come to the room temperature, and homogenized in glass petri dish with help of scalper (minced in PBS). Transfer it to the microfuge tube. mix it by vortex. Pellet down the cells Centrifuge at 8000 rpm at 4°C for 10 minutes. Discard the supernatant. Add 400 µl 1x TE buffer and keep in shaker for 20 minutes at room temperature. Add 200 µl tissue lysis buffer & 8 µl proteinase K and mix by vortex. Seal the tube with para film & incubate overnight in water bath at 55°C. Remove the para film and vortex to dissolve the cells. Add 400 µl phenol and mix by inverting tubes several times. Centrifuge at 8000 rpm at 4°C for 15 minutes. Transfer upper aqueous layer into a fresh microfuge tube & discard the pellet. Add equal volume of phenol: chloroform: isoamylalcohol (PCI) (25:24:1) solution to the supernatant and mix by inverting several times. Centrifuge at 8000 rpm at 4°C for 15 minutes. Transfer upper aqueous layer into a fresh microfuge tube & discard the pellet. Add 600 µl chloroform: isoamylalcohol (24:1) solution to the supernatant and mix by inverting several times. Centrifuge at 8000 rpm at 4°C for 15 minutes. Transfer upper layer into a fresh microfuge tube & discard the pellet. Add $1/10^{th}$ volume Na-acetate and equal volume of isopropanol and mix by inverting several times. Incubate at -20°C for 1-2 hours. Thaw the sample to room temperature and centrifuge at 10000 rpm at 4°C for 15 minutes. Discard the supernatant and add 500 µl 70 % ethanol (chilled) to wash the pellet. Centrifuge at 8000 rpm at 4°C for 5 minutes. Discard the supernatant and air dry the pellet for 30 minutes to evaporate the ethanol. Dissolve the pellet in 20 µl TE buffer. Store at -20°C temperature till use.

Primer Designing and amplification of E6 and E7 genes

The specific primers were designed using Primer3 Plus software (http://www.bioinformatics.nl/ cgibin/ primer3plus/ primer3plus.cgi/) and the designed oligonucleotides were synthesized in Sigma Corporation USA. The details of primer for E6 used in our study are 2 gene amplifications are:

HPV-16 E6 (nt 99–178)

Forward primer: 5' CTGCAATGTTTCAGGACCCA 3'
Reverse primer: 5' TCATGTATAGTTGTTTGCAGCTCTGT 3'
Probe: FAM-AGGAGCGACCCGGAAAGTTACCACAGTT-BHQ

HPV-16 E7 (nt 739–816)

Forward primer: 5' AAGTGTGACTCTACGCTTCGGTT 3'
Reverse primer : 5' GCCCATTAACAGGTCTTCCAAA 3'
Probe:FAM-TGCGTACAAAGCACACACGTAGACATTCGTA-BHQ

E6 forward primer (nt 83–103)

5' CTCTGAATTCGCCACCATGCACCAAAAGAGAACTGCA 3' (*EcoR*I site underlined)

E6 reverse primer (nt 575–555)

5' CCCTCGAGGTATCTCCATGCATGATTACA 3' (*Xho*I site underlined)

E7 forward primer (nt 562–582)

5' CTCTGAATTCGCCACCATGCATGGAGATACACCTACA 3' (EcoRI site underlined)

E7 reverse primer (nt 874–853)

5' CCCTCGAGGATCAGCCATGGTACATTATGG 3' (*Xho*I site underlined)

Note : HPV-16: human papillomavirus 16; nt: nucleotide; RT-PCR: reverse transcriptase-polymerase chain reaction. A all primer sequences are written from 5' to 3'. Open reading frames product of the E6 gene (492 bp; nt 83–575) and HPV-16 E7 gene (312 base pairs [bp]; nt 562–874) ware used as a template for in vitro amplification , that was derived from a patient with cervical carcinoma, was amplified using primers specific to HPV-16 as provided above. The PCR reaction conditions were optimized by changing the annealing temperature in the interval from 55 to 59°C and by altering the cycle number at which the phase change is initiated in the interval from 25 to 35 cycles. The highest amplification sensitivity and efficiency was obtained by an initial annealing temperature of 55°C for 34 cycles. The amplified product was electrophorized in 1% Agarose gel electrophoresis.

Cloning of the HPV16 (E6 and E7) genes and transformation

The eluted PCR products were ligated separately into the pEGMTZ cloning vector (Fermentas, USA). A 30µl ligation reaction was setup in 3:1 molar ratio of insert and vector DNA. Ligation mixture was incubated at room temperature (25°C) for one hour after a short spin. The ligated product was later kept on ice until the transformation experiment started. The ligated product was mixed with 200µl of prepared competent cells and incubated on ice for 30 minutes without disturbing. Heat shock was given to the ligation and competent cell mixture at 42°C for 2 minutes. The tubes were transferred quickly onto ice and incubated for 2-3minutes. To the mix, 1ml of LB broth was added and the tubes were placed in an orbital shaker at 37°C for 1 hour with an agitation of ~200rpm. During the incubation period, 50ml of LB agar was melted and allowed to cool to 40°C. To the 50ml of molten LB agar, 50 µl of Ampicillin (50mg/ml) was added to a final concentration of 50 µg/ml, 200 µl of X- gal, to a final concentration of 80µg/ml and 20 µl of IPTG to a final concentration of 80 µg/ ml. The molten agar was mixed properly without forming air bubbles and poured on to the sterile Petri plates. The plates were allowed to solidify for 10-15 minutes and were incubated at 37°C until plating. After 1 h incubation in orbital shaker, the tubes containing cells were centrifuged at 1000rpm for 10 minutes at room temperature and resuspended the pellet in 100 µl of fresh LB broth. From the suspension, 100 µl was spread on LB agar plate using a bent sterile glass rod. The plates were incubated at 37°C overnight.

Screening of positive clones

White colonies having recombinant plasmids due to the insertional inactivation of the *lacZ*gene were selected and streaked on a fresh LB plate containing ampicillin and incubated overnight and facilitated as a master plate for each transformants. All colonies from the master plate were subjected to plasmid DNA isolation and restriction analysis to identify the positive recombinants.

Confirmation of clones by restriction digestion

The purified plasmid was subjected to restriction digestion using restriction endonucleases (Bam H1 and EcoR1 (Merck,

India) Restriction digestion was performed in 10 µl reaction volumes with recommended units of enzyme and appropriate buffers at 37°C for 4h. The products of restriction digestion were resolved in 1% agarose gel for confirming the release of the insert by the restriction endonucleases. The released gene insert was eluted from the Agarose gel using gel extraction kit (Bioline USA).

Cloning of E6 gene

The amplified PCR products were cloned into the CMV expression vector pcDNA3.1, according to the protocol provided by manufacturer. (For ligation reaction see table 1)

Phylogenetic analysis

Phylogenetic tree were generated on the basis of sequences of gene sequences using Clustal W 1.8. The variable and incomplete sites at both the 5' and 3′ ends of the gene sequences were omitted from the alignment. Sites awarding alignment gaps were excluded from analysis. A rooted phylogenetic tree was constructed using the sequences reported here with diverse sequences previously deposited in the GenBank database.

RESULTS

Genomic DNA isolation and quantification

The DNA extracted from cervical cancer tissue biopsies as well as normal tissues, by standard Phenol/ Chloroform method were checked for their quality and quantity in solution by electrophoresis on an ethidium bromide-stained 1% agarose gel. The DNA showed presence of good quality high molecular weight DNA. The concentration of the DNA was estimated either by comparing the band intensities of sample DNA with that of standard 1µg Hind III digested λ DNA molecular weight marker or by UV visible spectrophotometer. The concentration of DNA in different samples was found to be ~ 100-250 ng/µl when compared to intensities of bands of the λ DNA marker. The isolated DNA was then electrophorized in 1% Agarose gel as shown in the Figure 1.

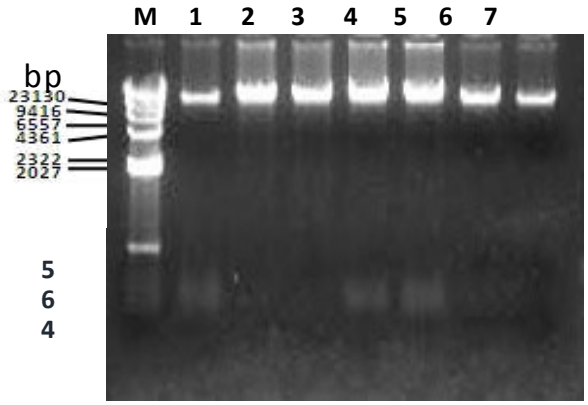

Figure 1. 1% agarose gel stained with EtBr showing the presence of genomic DNA

Lane M: λ DNA *Hind*ɯdigest marker.
Lane 1-7: Genomic DNA sample extracted from cervical biopsies.
DNA Quality assessed by β-globin PCR

PCR amplification of E6 and E7 genes

The strong association found between infection with HPV and the development of cervical neoplasia has been established in numerous case control studies. A prerequisite for studying the presence and variation of HPV in cervical lesions is a sensitive and high-resolution HPV detection system. The inherent sensitivity of PCR makes this method suitable for the analysis of even the most demanding clinical samples. Species specific primers were designed for the *Helicobacter pylori* using the sequences of E2 gene available in NCBI GenBank using Primer 3 Software. The predicted primers were validated initially *in silico* and subsequently in wet lab. The primers could yield an amplicon of the expected size specific to E6 and E7 oncoproteins. The PCR product was electrophoresed and visualised by 1% agarose gel. The primers were found to produce ~492 bp; nt 83–575 for the E6 gene and 312 base pairs [bp] of E7 genebp amplicons of HPV-16.

Cloning of PCR product

As shown the previous studies, the viral oncogenes E6 and E7 are connected with cancer; authors are modified the cell cycle in order to favor the viral genome replication and consequent late gene expression. Most HPV maintain the expression of E6 and E7. A higher HPV16- E6 seropositivity was observed in patients with cervical cancer compared to healthy or with subjects Displaying early lesions. Hence, E6 oncoprotein might be used to monitor infected woman at the very late stage of the infection. PCR yielded a specific amplicon of ~492 bp.purified PCR product was quantified and ligated with cloning vector T vector pEGMTZ (Fermentas, Germany) using T4 DNA ligase enzyme. The ligated plasmid was transformed into E.Coli bacterial strain DH5α. The transformation was done by heat shock method and transformed cell was cultured in the X gal- IPTG-Ampicillin-LB. Agar plate at 37oC for overnight. The white colonies were picked up from the plates and cultured In Ampicillin containing LB broth as shown in the Figure 4.

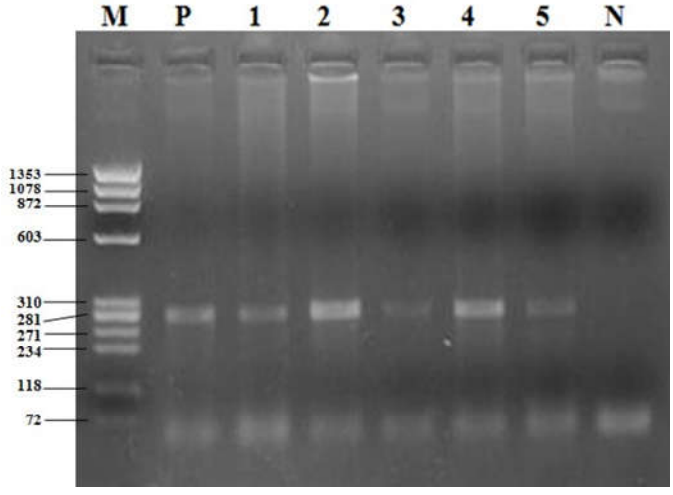

Figure 2. 2% agarose gel showing the amplification of β-globin gene

Lane M: ϕx 174 DNA/ *Hae*III digested marker,
Lane P: Positive control; Lanes 1-5: 268 bp PCR product; Lane N: Negative control.

Plasmid Isolation and Confirmation of clone by restriction digestion

The cloned E6 gene in pcDNA3.1 were screened by digestion with EcoRI restriction enzyme. Plasmid was isolated from the transformed cells by using alkaline analysis method. The isolated plasmid was electrophorized on 1% Agarose gel. The purified plasmid was subjected to restriction digestion using *Bam* H1 and *Eco*R 1 (Merck, India). After incubation at 37oC for 4 hours the restricted product was electrophorized on 1% Agarose gel. The release of the gene product was visualized in the gel as shown in the Figure 6.

Amplification of E6

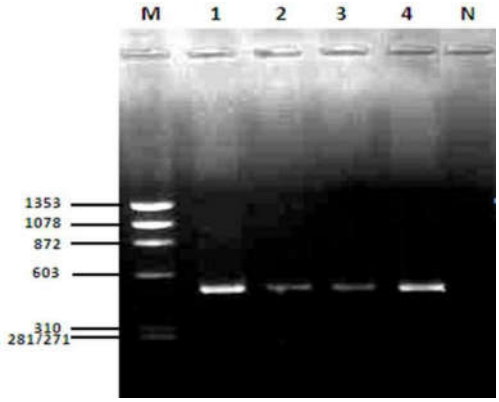

Figure 3. 2% agarose gel stained with EtBr showing the amplification of full length HPV 16 E6 gene. Lane M is λ *Hind* III- digest marker, Lane 1-positive, 2-4 cervical cancer samples; N is negative control

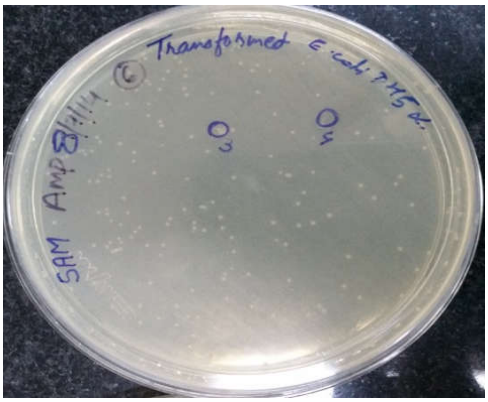

Figure 4. Selection of the transformed bacterial cells containing E6 gene

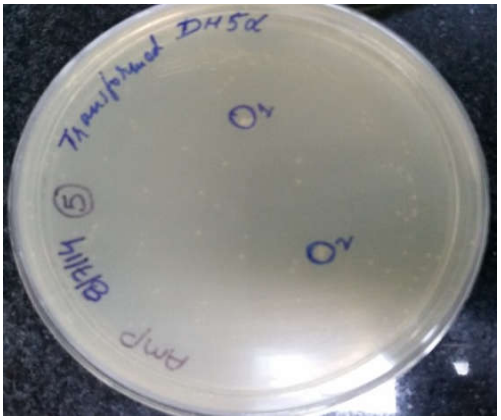

Figure 5. Selection of the transformed bacterial cells containing E7 gene

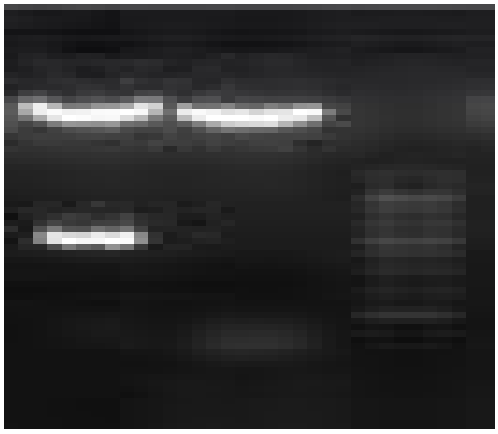

Figure 6. 1% Agrose shows E6 insert after restriction

Table 1. Ligation reaction

Reaction Component	Ligation Reaction
2X Rapid Ligation Buffer, T4 DNA Ligase	5µl
pcDNA3.1	1µl
PCR product	3µl
T4 DNA Ligase (3 Weiss units/µl)	1µl
nuclease-free water to a final volume	10µl

Incubate at 4°C overnight.

Table 2. This protein-only option colours the residues according to their physicochemical properties

Residue	Colour	Property
Avfpmilw	Red	SMALL (small+hydrophobic (incl.aromatic -Y))
DE	Blue	ACIDIC
RK	Magenta	Basic - H
Styhcngq	Green	HYROXYL +sulfhydryl+ amine+ G
Others	Grey	Unusual amino/imino acids etc

Sequence alignment by clustalW

The gene was identified by sequencing of plasmid. An approximately ~492 bp region of the E6 and E7 genes was sequenced at SciGenome Kochi. Nucleotide sequence analysis of gene was used to to investigate the identity HPV E6, E7 genes. Todemonstrate the quality and accuracy of results provided from a public database, we compared sequences to their corresponding GenBank sequences. The sequence had "perfect" match (similarity, 99%) with sequences of their corresponding gene (E6 and E7 both) from GenBank as determined by using BLAST (version 2.7). Cervical cancer detection worldwide and assess the geographic distribution of HPV genotypes, extensive epidemiological studies are required. Given the substantial genetic heterogeneity of HPVs and the possible clinical relevance of specific subtypes, specific molecular tools will be required. The E6 and E7 oncogenes sequences of HPV is also available in GenBank database, which is more similar to this sequence (99% similarity). The N-J tree with branch length was plotted using ClustalW sequence alignment (http://align.genome.jp/), showing the relationship of *E6* and *E7* genes among the closest HPV in the NCBI database.

E2 [Human papillomavirus type 16]

<FASTA FORMAT>

GenBank: AAD33255.1

GenPept Graphics
>gi|4927723|gb|AAD33255.1|AF125673_4
E2 [Human papillomavirus type 16]
METLCQRLNVCQDKILTHYENDSTDLRDHIDYWKHMR
LECAIYYKAREMGFKHINHQVVPTLAVSKNKAL
QAIELQLTLETIYNSQYSNEKWTLQDVSLEVYLTAPTGC
IKKHGYTVEVQFDGDICNTMHYTNWTHI IC
EEASVTVVEGQVDYYGLYYVHEGIRTYFVQFKDDAEK
YSKNKVWEVHAGGQVILCPTSVFSSNEVSSPEI
IRQHLANHSAATHTKAVALGTEETQTTIQRPRSEPDTGN
PCHTTKLLHRDSVDSAPILTAFNSSHKGRIN
CNSNTTPIVHLKGDANTLKCLRYRFKKHCTLYTAVSST
WHWTGHNVKHKSAIVTLTYDSEWQRDQFLSQV
KIPKTITVSTGFMSI

E6 [HPV 16] protein sequence <FASTA FORMAT>

>EMBOSS_001_1
DPQERPRKLPHLCTELQTTIHDIILECVYCKQQLLRREV
YDFAFRDLCIVYRDGNPYAVC
DKCLKFYSKISEYRYYCYSVYGTTLEQQYNKPLCDLLIR
CINCQKPLCPEEKQRHLDKKQ
RFHNIRGRWTGRCMSCCRSSRTRX

E7 [HPV 16] protein sequence <FASTA FORMAT>

>EMBOSS_001_1
DPQVATQKVTTFMHRAANNYT*YNIRMCVYCKQQLLR
REVYDFAFRDLCIVYRDGNPYAV
CDKCLKFYSKISEYRYYCYSVYGTTLEQQYNKPLCDLLI
RCINCQKPLCPEEKQRHLDKK
QRFHNIRGRWTGRCMSCCRSS

CLUSTAL 2.1 multiple sequence alignment of E2 vs E6 oncoproteins

CLUSTAL 2.1 multiple sequence alignment
gi|4927723|gb|AAD33255.1|AF125
METLCQRLNVCQDKILTHYENDSTDLRDHIDYWKHMR
LECAIYYKAREMG
50
gi|325070940|gb|ADY75573.1|
----------MHQKRTAMFQDPQDPPRKLPQLCTELQTT
29
::: :: ::* * ::: *: .:
gi|4927723|gb|AAD33255.1|AF125
FKHINHQVVPTLAVSKNKALQAIELQLTLETIYNSQYSN
EKWTLQDVSLE
100
gi|325070940|gb|ADY75573.1|
IHDIILECVYCKQQLLRREVYDFAFRDLCIVYRDGNPYA
VCDKCLK
75
::: . .:: *: :::: . .:: ::: * .*:
gi|4927723|gb|AAD33255.1|AF125
VYLTAPTG---
CIKKHGYTVEVQFDGDICNTMHYTNWTHIYICEEASVT
V
147
gi|325070940|gb|ADY75573.1|
FYSKISEYRHYCYSVYGTTLEQQYNKPLCDLLIRCINCQ
KPLCPEEKQRH
125
.* . . * . .* *:* *:: :*: : : .* * .
gi|4927723|gb|AAD33255.1|AF125
VEGQVDYYGLYYVHEGIRTYFVQFKDDAEKYSKNKV
WEVHAGGQVILCPT
197
gi|325070940|gb|ADY75573.1| LDKKQRFHNIRGRWTGR---
-------------------------CMS
145
:: : ::.: * * :
gi|4927723|gb|AAD33255.1|AF125
SVFSSNEVSSPEIIRQHLANHSAATHTKAVALGTEETQT
TIQRPRSEPDT
247
gi|325070940|gb|ADY75573.1| CCRSSRTRRETQL----------

158
. **. ..::
gi|4927723|gb|AAD33255.1|AF125
GNPCHTTKLLHRDSVDSAPILTAFNSSHKGRINCNSNTT
PIVHLKGDANT
297
gi|325070940|gb|ADY75573.1| ----------
gi|4927723|gb|AAD33255.1|AF125
LKCLRYRFKKHCTLYTAVSSTWHWTGHNVKHKSAIVT
LTYDSEWQRDQFL
347
gi|325070940|gb|ADY75573.1| ----------
gi|4927723|gb|AAD33255.1|AF125 SQVKIPKTITVSTGFMSI
365
gi|325070940|gb|ADY75573.1| ----------

Phylogenetic analysis between E2 vs E6 protein

This is a Neighbour-joining tree without distance corrections.
(gi|4927723|gb|AAD33255.1|AF125:0.86452,gi|325070940|gb|
ADY75573.1|:0.86452);

Branch length: Cladogram

gi|4927723|gb|AAD33255.1|AF125 0.86452
gi|325070940|gb|ADY75573.1| 0.86452

Branch length: Real

gi|4927723|gb|AAD33255.1|AF125 0.87097
gi|325070944|gb|ADY75575.1| 0.87097

CLUSTAL 2.1 Multiple Sequence Alignments FILE

Sequence type explicitly set to Protein
Sequence format is Pearson
Sequence 1: gi|4927723|gb|AAD33255.1|AF125673_4 365 aa
Sequence 2: gi|325070940|gb|ADY75573.1| 158 aa
Start of Pairwise alignments
Aligning...
Sequences (1:2) Aligned. Score: 10.76
Guide tree file created: [clustalw2-I20140723-175043-0589-6661604-oy.dnd]
There are 1 groups
Start of Multiple Alignment
Aligning...
Group 1: Delayed
Alignment Score 34

CLUSTAL-Alignment file created [clustalw2-I20140723-175043-0589-6661604-oy.aln]

CLUSTAL 2.1 multiple sequence alignment of E2 vs E7 oncoproteins

gi|4927723|gb|AAD33255.1|AF125 METLCQRLNVCQDKILTHYENDSTDLRDHIDYWKHMR LECAIYYKAREMG
50
gi|325070950|gb|ADY75578.1| ----------MHG----------DTP-TLHEYMLD
14
gi|325070940|gb|ADY75573.1| ----------MHQKRTAMFQDPQDPPRKLPQLCTE
25
:: : .
gi|4927723|gb|AAD33255.1|AF125 FKHINHQVVPTLAVSKNKALQAIELQLTLETIYNSQYSN EKWTLQDVSLE
100
gi|325070950|gb|ADY75578.1| LQPETTD-----LYCYEQLNDSSEEEDEI--------DG-PAGQAEPDRA
50
gi|325070940|gb|ADY75573.1| LQTTIHDIILECVYCKQQLLRREVYDFAFRDLCIVYRDG NPYAVCDKCLK
75
:: : . :: : : .. :
gi|4927723|gb|AAD33255.1|AF125 VYLTAPTG--- CIKKHGYTVEVQFDGDICNTMHYTNWTHIYICEEASVT V
147
gi|325070950|gb|ADY75578.1| HYNIVT----FCCK----------CDSTLR-------LCVQS----
71
gi|325070940|gb|ADY75573.1| FYSKISEYRHYCYSVYGTTLEQQYNKPLCDLLIRCINCQ KPLCPEEKQ--
123
* . * . *: :* :
gi|4927723|gb|AAD33255.1|AF125 VEGQVDYYGLYYVHEGIRTYFVQFKDDAEKYSKNKV WEVHAGGQVILCPT
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93
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: . . . * *
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98
gi|325070940|gb|ADY75573.1| CCRSSRTRRETQL----------
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. ..
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gi|325070950|gb|ADY75578.1| ----------
gi|325070940|gb|ADY75573.1| ----------
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365
gi|325070950|gb|ADY75578.1| ----------
gi|325070940|gb|ADY75573.1| ----------

Phylogenetic tree of E2 vs E7 oncoproteins
This is a Neighbour-joining tree without distance corrections.

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gi|325070950|gb|ADY75578.1|:0.37199,
gi|325070940|gb|ADY75573.1|:0.35250);

Branch length: Real

gi|4927723|gb|AAD33255.1|AF125 0.52492
gi|325070950|gb|ADY75578.1| 0.37199
gi|325070940|gb|ADY75573.1| 0.3525

Phylogram

(gi|4927723|gb|AAD33255.1|AF125:0.52492,
gi|325070950|gb|ADY75578.1|:0.37199,
gi|325070940|gb|ADY75573.1|:0.35250);

Branch length: Cladogram

This is a Neighbour-joining tree without distance corrections.

Branch length: Real

gi|4927723|gb|AAD33255.1|AF125 0.52492
gi|325070950|gb|ADY75578.1| 0.37199
gi|325070940|gb|ADY75573.1| 0.3525

The sequence of HPV-E6 and E7 genes amplified by the specific primer is closely matching (99%) with a HPV strain but it is clustered differently Sequence analysis of the E6 and E7 genes from 12 strains was also carried out to obtain new insight into the genetic differences between the peptic ulcer group and the gastritis group. It is well known that local DNA sequences containing repeat sequences (direct repeats or inverted repeats) may cause deletion by misalignment during DNA replication or recombination. (See Table 2)

DISCUSSION

So far, a variety of specific HPV variants have been considered to be related with the cervical neoplasia (Davies *et al*., 2005; Kjaer *et al.,* 1996). It is well-recognized that the sequence

variation in HPV E2 protein possibly affects HPV virus potential to be carcinogenic. Similarly, the study of HPV-16 E5 protein sequence has revealed that the change in amino acid sequence could modify transforming activity of the protein by influencing the interactions with the EGFR, the 16 kDa subunit of the H+-ATPase or, other cellular proteins (Eriksson *et al.,* 1999; Straight *et al.,* 1995). Moreover, a recent study by Jumaah *et al*. 2014, which cloned and characterized the E6 oncogene of HPV 16 isolated from cervical cancer patients, has found that the E6 sequence was found to be 99% similar to the existing sequence in GenBank (JumaahKa *et al*., 2014). Consistently, the current study has also found that HPV antigen E6 and E7 genes isolated from cervical cancer tissue using the specific primer is 99% comparable to the sequence available in the database.

Conclusion

As the characterization of sequence variation within high-risk HPV types are potentially vital in the investigation of epidemiological association of various risk factors and cervical cancer, the current study harmonizes and widens HPV-16 genome sequence data as reported by previous studies (Zhang GA, 1990; Schiffman *et al.,* 1993). The present study, which cloned and characterized the E6 and E7 genes of HPV 16 virus isolated from cervical cancer tissues, demonstrated that the E6 and E7 sequence is almost as comparable to the sequence in the NCBI database. The researchers also concluded that further studies are necessary to assess HPV-16 variant associations with cervical cancer risk and also, to characterize functional differences.

Future Scope

Further research is required to express the gene to get the protein antigen for the production antibodies or effective vaccine against high risk Papilloma virus.

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