Expression of TMEM87B interacting with the human papillomavirus type 18 E6 oncogene in the Hela cDNA library by a yeast two-hybrid system

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Abstract. The high-risk human papillomavirus oncoprotein 18 E6 (HPV18 E6) is associated with cervix cancer. This study was conducted in order to identify the transmembrane protein 87B (TMEM87B) as a novel binding protein interacting with the HPV18 E6 oncoprotein and to perform an initial bioinformatics analysis. The yeast strain AH109 was transformed with pGBKT7-HPV18 E6 and the yeast mating assay was utilized to identify the interaction between TMEM87B and HPV18 E6 in the human Hela cDNA library. *TMEM87B* mRNA was detected in Hela cells by using RT-PCR. The *TMEM87B* gene structure, genomic localization, physical and chemical characteristics, subcellular localization and functional domain were predicted, as well as the systematic evolution analysis on similar proteins among several species. In the yeast two-hybrid assay, HPV18 E6 mRNA was expressed and there was no self-activation and toxicity in the AH109 strain. The special *TMEM87B* mRNA expression was detected in Hela cells and the blue clones were validated by the yeast mating assay. An efficient bioinformatics analysis fundamentally identified that TMEM87B is a secretary protein, containing many phosphorylation sites and functional motifs and possibly involved in signal transduction and transcriptional control in carcinogenesis. It was indicated that the yeast two-hybrid system is efficient for screening interacting proteins. The novel gene *TMEM87B* may interact with HPV18 E6 and may be a potential oncogenesis target according to the bioinformatics analysis.

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Introduction

Gene rearrangement, mutation and infection by certain viruses can contribute to the development of cancer (1). One group of tumor viruses directly implicated in cancer pathogenesis is the 'high-risk' subgroup of human papillomaviruses (HPVs), exemplified by HPV type 18 (HPV 18), which is thought to be closely linked to the development of cervical cancer (2,3). Previously, accumulating evidence indicated that HPV oncogenes E6 and E7 are always expressed in HPV-associated carcinomas (4) and inactivate critical tumor suppressor proteins enabling the viruses to override checkpoints that regulate cell proliferation. E6 binds to p53 via the E6AP and induces its degradation through the ubiquitin-proteasome pathway (5-7), leading to the loss of p53-mediated cell cycle arrest and apoptotic responses to DNA damage (8,9).

To gain a better understanding of the role of HPV18 E6 in cancers, the yeast two-hybrid interaction system was performed in order to identify novel interaction targets of HPV18 E6 in this experiment. The bait gene HPV18 E6 is expressed as a fusion of the GAL4 DNA-binding domain, while the Hela cDNA library is expressed as a fusion to the GAL4 activation domain. When bait and library fusion proteins interact, the DNA-BD and AD are brought into proximity, thus activating the transcription of four reporter genes. Then, from the Hela cDNA library, the novel binding transmembrane protein 87B (TMEM87B) was found.

The gene was first discovered in the genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays (10) and later the TMEM87B cDNA sequence were analysized (11,12). However, thus far, the research on TMEM87B is very limited and its bioinformatic analysis and initial functional validation of the biological properties in cancer is completely unknown. We demonstrate herein that TMEM87B binds to the high-risk (cancer-associated) HPV18 E6 proteins. Given the strong likelihood that human TMEM87B, similar to its yeast counterpart, will function as a coactivator in multiple mammalian transcriptional pathways, the combination of TMEM87B by the high-risk HPV18 E6 and possibly by other viral oncoproteins, may represent a crucial part of the viral oncogenic strategy.

Materials and methods

Plasmid, E. coli and cDNA library. Plasmids pGBKT7-HPV18 E6 and pcDNA-HPV18 E6 were constructed by our laboratory and *E. coli* (DH5α) was purchased from Invitrogen Ltd (USA). *Saccharomyces cerevisiae* AH109 (MATa, trp1-901, leu2-3, 112, ura3-52, his3-200, gal4D, gal80D, LYS2::GAL1UAS-GAL1TATA-HIS3, GAL2UAS-GAL2TATA-ADE2, URA3::MEL1UAS-MEL1 TATA-lacZ) and Y187 (MATa, ura3-52, his3-200, ade2-101, trp1-901, leu2-3, 112, gal4D, met-, gal80D, URA3::GAL1UAS-GAL1TATA-lacZ), as well as other relative agents of the yeast two-hybrid system kit, a serious yeast selective culture medium, YPD medium and X-α-gal were purchased from Clontech Corp. (USA). The Hela MATCHMAKER cDNA library, which was inserted into the downstream of pACT2 (GAL4 AD, LEU2, Ampr) AD, was kindly given by Dr Li Daizong, Clontech Corporation.

The construction of bait plasmid pGBKT7-HPV18 E6 and the assays for toxicity and autonomous activation. The bait plasmid pGBKT7-HPV18 E6 was constructed by using a complete E6 cDNA obtained by PCR from pcDNA-HPV18 E6. The complete cDNA was amplified with the primers, containing the restriction sites *Eco*RI and *Bam*HI (Promega, USA). The upper primer: 5' ggaattcagcatcaagatcaatcgagtagat ccc 3' and the lower primer: 5' ttgcggccgcctgttcacgcatcacagtc agccacag 3'. The primers were synthesized by Sangon Co. (Shanghai, China). The PCR product was digested with the restriction endonucleases and subcloned in a pGBKT7 plasmid (Clontech) with T4DNA ligase (Promege). After the transformation into DH5α, the positive clones, containing the plasmid pGBKT7-HPV18 E6, were obtained by plating with kanamycin resistance and then the plasmid was amplified, isolated and digested with restriction endonucleases. The sequence analysis was performed to ensure the E6 sequence. Then, the *E. coli*-purified bait plasmid was transformed into the AH109 strain, which was incubated on the SD/-Ade/-His/-Leu/-Trp/X-α-gal agar medium for detection.

About 2 mm AH109 colonies, transformed by pGBKT7-HPV18 E6 and pGBKT7 were incubated in 3 ml of a YPDA liquid medium at 30˚C for 16 h with shaking (250 rpm) to a stationary phase, respectively. The absorptance OD_{600} values in different groups were compared. Moreover, the transformants, containing the pGBKT7-HPV18 E6 and pGBKT plasmids were transferred on SD/-Trp/X-α-gal and SD/-Trp/-his/X-α-gal and SD/-Ade/-Trp/X-α-gal at 30˚C for 5 days, respectively. In parallel, AH109 cells transformed by pGBKT7-53 and pGBKT7-Lam as the positive and negative controls were performed.

Screening of positive clones and gene sequencing analysis. The yeast competent cells were prepared according to the MATCHMAKER GAL4 two-hybrid system 3 and the libraries user manual. After the transformation of the pGBKT7-HPV18 E6 plasmid into competent AH109 cells, the Hela MATCHMAKER cDNA library was subsequently transferred to the same cells. Then, 6 ml of a sterile PEG/LiAc solution was added and the co-transformanted AH109 were incubated at 30˚C for 30 min with shaking (200 rpm). Following the mixture of 0.7 ml DMSO, AH109 cells were treated with 15 min heat shock in a 42˚C water bath, then centrifuged and re-suspended with 10 ml 0.5 x YPDA liquid medium, incubated on SD/-Ade/-His/-Leu/-Trp/X-α-gal agar plates at 30˚C for 4 weeks (100 μl per 100 mm plate). Moreover, the candidate colonies were restreaked on SD/-Ade/-His/-Leu/-Trp/X-α-gal agar plates twice, then the positive colonies were collected on SD/-Ade/-His/-Leu/-Trp/X-α-gal master plates in a grid fashion and incubated at 30˚C for 4 weeks.

The positively retested $Ade^+/His^+/Mel1^+$ colonies were transferred into an SD/-Ade/-His/-Leu/-Trp liquid medium at 30˚C and plasmid DNA was isolated from the yeast by using the YEASTMAKER™ yeast plasmid isolation kit (Clontech). Alternatively, the yeast-purified plasmid DNA was directly transferred to competent *E. coli* cells (DH5α) by electroporation (13). The transformants containing only the pGADT7-Hela cDNA library plasmids were selected by plating on an LB agar medium containing ampicillin at 30˚C overnight and were incubated in 3 ml of an LB/Amp liquid medium at 30˚C with shaking (250 rpm) for 16 h. The *E. coli*-purified plasmid DNA fragments were sorted by enzyme-digesting with *Nde*I and *Xho*I (Promega) and 2% agarose gel electrophoresis. A glycerol (Sigma, USA) stock of each unique type was stored at -80˚C. At the same time, the inserts in the positive pGADT7-Hela cDNA library plasmids were sequenced by using the 3' AD and T7 sequencing primers (Sangon). The sequences were then compared to those in the GenBank (http://www.ncbi.nlm.nih.gov). From these candidate genes, the TMEM87B protein was chosen for further research.

Bioinformatic analysis and tools. The TMEM87B referred sequences of the gene and encoded product were sourced from the GenBank of the NCBI. Megablast was used to retrieve the human genome database (http://www.ncbi.nlm.nih.gov/genome/seq/BlastGen/BlastGen.cgi?taxid=9606) for genome mapping. The gene structure, such as exons and introns, analyzed by Wheelan *et al* (14) (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey/) and the mRNA sequence was aligned to its corresponding genome sequence to verificate the gene structure by using the Human Blast search (http://genome.ucsc.edu/cgi-bin/hgBlat). The physical-chemical properties of the encoded protein were examined by ProtParam on the Expasy website (15) (http://www.expasy.org/) and the subcellular localization of the TMEM87B protein was estimated by PSORT II (16) (http://psort.nibb.ac.jp/form2.html). 'InterProScan' (http://www.ebi.ac.uk/InterProScan/) (17) and 'Conserved Domains' (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) of the NCBI were used to estimate protein function as well as structural domains (18). The functional motifs of the TMEM87B protein were estimated by 'Motif Scan' (http://myhits.isb-sib.ch/cgi-bin/motif_scan) and 'ELM' (http://elm.eu.org/). The web server 'TMHMM' (http://www.cbs.dtu.dk/services/TMHMM/) was used to estimate possible transmembrane helices, while 'SignalP3.0' (http://www.cbs.dtu.dk/services/SignalP/) was employed to estimate possible cleavage sites of the signal peptide (19). By consulting 'UniGene' of the NCBI (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=unigene) and 'SOURCE' (http://genome-

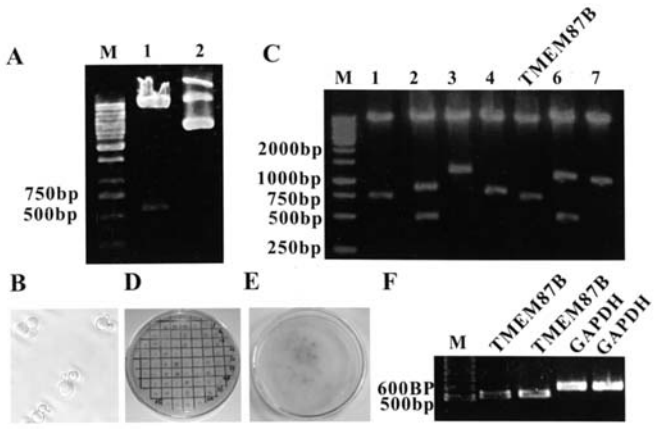

Figure 1. (A) The construction of bait plasmid pGBKT7-HPV18 E6. M, marker; lane 1, the plasmid pGBKT7-HPV18 E6 after enzyme-digesting; lane 2, before enzyme-digesting. (B) The diploids were detected in the yeast mating assay. (C) The insert fragments of the pGADT7-Hela cDNA library plasmids were sorted into seven by enzyme-digesting. (D) The screening of positive clones on SD/-Ade/-His/-Leu/-Trp/X-α-gal master plates in a grid fashion and 428 putative positive yeast colonies were obtained. (E) The verification of protein interactions between TMEM87B and HPV18 E6 in yeast. (F) The verification of TMEM87B mRNA expression in Hela cells by using RT-PCR, and the positive bands of 501 bp were shown by the use of agarose gel electrophoresis.

www5.stanford.edu/cgi-bin/source/source Search), the abundance of the TMEM87B protein comparative expression in different tissues or stages was further investigated. 'Blastp' was employed to explore the NCBI nonredundant-protein database for the homologous proteins existing in other species. The sequence alignment between TMEM87B and the most similar sequence was performed by means of 'ClustalX', while a phylogenetic tree was constructed and 'MEGA 3.1' was used to view the tree structure.

The verification of insert sequence mRNA expression in Hela cells. Hela cells were harvested and total RNA was extracted with the Trizol (Invitrogen) and then an insert sequence mRNA expression was detected by using RT-PCR. The amplification product was separated with 2% agarose gel and stained with ethidium bromide. The upper primer: 5'-GAGA GCAAGGTGACAGAGATTCC-3' and the lower primer: 5'-CCTTTTGGAGAGAATGAATCTT-3'. A housekeeping gene, GAPDH, was used as an internal quantity control. The predicted sizes for insert sequence and GAPDH PCR products were 501 and 598 bp, respectively.

Briefly, cells from one 6 cm dish, about $2.5x10^6$, were harvested and lysed in 500 μl Trizol and total RNA was extracted according to the manufacturer's instructions. The concentrations of total RNA were determined spectrophotometrically at 260 nm. RNA was reversely transcribed into cDNA described 2 μg of total RNA, 500 μmol/l dNTPs, 0.05 μmol/l oligo(dT), 1x first-strand buffer (including mg^{2+}), 10 units RNasin, 200 units thermostable transcriptase (Takara, Japan) and DEPC-water to a final volume of 20 μl and was incubated at 42˚C for 60 min, then quick-chilled on ice. A fixed amount of RT products (one fifth of RT products) and 0.5 μmol/l upper-lower primers were co-amplified at a final concentration of 1x PCR buffer, 50 μmol/l dNTPs, 1 unit Taq DNA polymerase (Ferment, USA) in a total volume of 100 μl. The PCR mixture was amplified with a PCR thermal cycler. The amplification profile involved denaturation at 94˚C for 30 sec, primer annealing at 62˚C for 30 sec and extension at 72˚C for 30 sec. Eight microliters of each PCR reaction mixture was electrophoresed in 2% agarose gel in 1x Tris Borate and EDTA (TBE) buffer. Gels were stained with 5 μg/ml of ethidium bromide for 15 min and photographed by using an imaging system (Biotech, USA).

The verification of protein interactions between TMEM87B and HPV18 E6 in yeast. The pGBKT7-HPV18 E6 plasmid transformed AH109 and TMEM87B transformed Y187 cells were incubated on SD/-Trp and SD/-Leu agar plates for 72 h, respectively. Then ~2 mm AH109-pGBKT7-HPV18 E6 and Y187-pGADT7-TMEM87B colonies were co-cultured in 0. YPDA liquid medium (5 ml) at 30˚C with shaking (250 rpm) for 20 h and the diploids were detected under an inverted microscope. Moreover, 100 μl mating mixture was plated on an SD/-Ade/-His/-Leu/-Trp/X-α-gal agar medium at 30˚C for 5 days. The positive blue colonies were restreaked on SD/-Ade/-His/-Leu/-Trp/X-α-gal agar plates three times at 30˚C for the yeast colonies.

Results

The construction of pGBKT7-HPV18 E6 plasmid. The bait plasmid pGBKT7-HPV18 E6 was constructed by using a complete HPV18 E6 cDNA, which was amplified and obtained with the primers containing the restriction sites *Eco*RI and *Bam*HI. PCR product was subcloned in a pGBKT7 plasmid with T4DNA ligase. The yeast-purified plasmid cDNA was retested with the sequencing analysis and the results suggested that the cDNA was in-frame and no artifacts were added to the HPV18 E6 sequence and that the restriction sites were totally correct (Fig. 1A).

The toxicity and autonomous activation assays of pGBKT7-HPV18 E6 plasmid. The same-sized AH109 colonies, transformed by pGBKT7-HPV18 E6 and pGBKT7, were incubated in 3 ml of a YPDA liquid medium for 16 h, respectively. The absorptance OD_{600} values in AH109-pGBKT7-HPV18 E6 and AH109-pGBKT7 groups were 0.98 and 0.99, which suggested that the pGBKT7-HPV18 E6 plasmid had no toxicity to yeast and had no effects on the yeast growth. Furthermore, AH109-pGBKT7-HPV18 E6 clones were white and were detected on the SD/-Trp/X-α-gal agar medium, however, were absent on SD/-Trp/-his/X-α-gal and SD/-Ade/-Trp/X-α-gal plates. Therefore, the HPV18 E6 protein was considered to have no autonomous activation effect.

Yeast mating assay. The diploids were detected under an inverted microscope 20 h after incubation (Fig. 1B), which indicated that yeast mating was successful. Nine hundred and eighty-six positive colonies on the SD/-Ade/-His/-Leu/-Trp/X-α-gal agar medium were grown and re-streaked three times. Then, 428 putative positive yeast colonies were obtained (Fig. 1D).

Gene sequencing and analysis. Fifty positive yeast colonies were selected randomly and the yeast-purified plasmid DNA

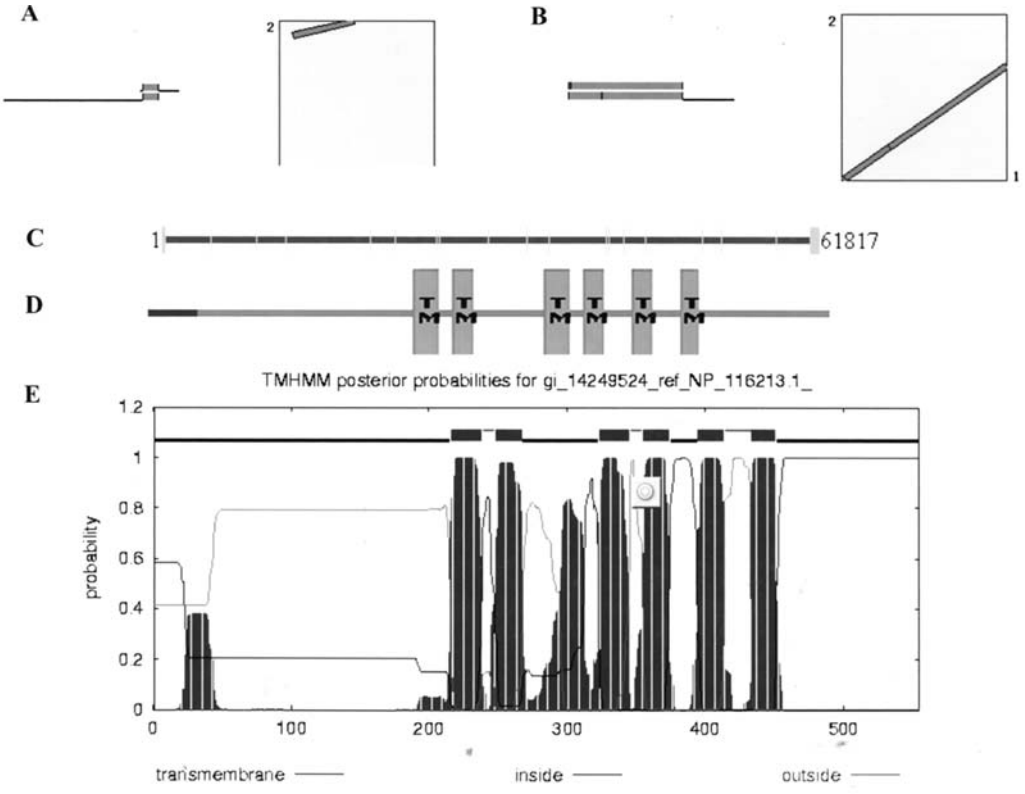

Figure 2. (A) A comparison between the 730 bp insert sequence MYE16-11 and the genome sequence of NM_032824.1 revealed a 289 bp perfect match in the noncoding region of the TMEM87B nucleotide sequence. (B) A 4258 bp contig electron-spliced by MYE16-11 was also aligned with the genome sequence of TMEM87B, the results of the comparison from 1 to 2933 bp showed a 2924 bp near-perfect match. (C) The hypothetical protein named NP_116213.1 possessed 19 exons and 18 introns. (D-E) Six transmembrane structures of the TMEM87B protein were located at 216-238aa, 248-267aa, 323-345aa, 355-374aa, 395-414aa and 434-451aa.

was directly transferred to competent DH5α cells by electroporation. The transformants containing only the pGADT7-Hela cDNA library plasmids were obtained by plating on an LB agar medium containing ampicillin and the insert fragments removing false positive and duplicate ones were sorted into seven by enzyme-digesting and 2% agarose gel electrophoresis (Fig. 1C). The sequencing analysis was employed to confirm these seven insert sequences. From these candidate genes, the TMEM87B protein was chosen for further research.

Results of bioinformatic analysis. The sequence was analyzed by 'Human blast'. A comparison between the 730 bp insert sequence MYE16-11 and the genome sequence of NM_032824.1 revealed a 289 bp perfect match in the noncoding region of the *TMEM87B* nucleotide sequence (Fig. 2A). Alignment results indicated that: Expect = 0.0, Identities = 5 152/5 152 (100), Gaps = 0/5 152 (0). Moreover, a 4258 bp contig electron-spliced by MYE16-11 was also aligned with the genome sequence of *TMEM87B*, the results of the comparison from 1 to 2933 bp showed a 2924 bp near-perfect match (Fig. 2B), Expect = 0.0, Identities = 2924/2933 (99%) and Gaps = 4/2933 (0%). Therefore, the 730 bp insert sequence MYE16-11 and T*MEM87B* genome sequence were presumed to be the same one.

The GeneID of *TMEM87B*, transmembrane protein 87B, is NM_032824.1. To date, there is no related notation about it in many databases such as Gene Ontology and KEGG and furthermore, the literature regarding the structure, function and biological properties of TMEM87B is completely unknown. To gain a better understanding of the role of TMEM87B, we further explored its protein function. Human *TMEM87B* complete cDNA has an overall length of 2933 bp. Its CDS lies in the sites between 355 to 2022 bp and it encodes a 555aa hypothetical protein named NP_116213.1. 'Megablast' analysis was employed to search for a genomic sequence similar to *TMEM87B* in the human genome database and such a genomic sequence was found in chromosome 2q13. It was indicated that NP_116213.1 possessed 19 exons and 18 introns when the *TMEM87B* mRNA referring sequence was aligned to the corresponding genome complete sequence by using 'Spidey'. The results indicated that all exons exactly matched the genome sequence (100%) and all of the 19 exons contained typical acceptor and donor splice sites (Fig. 2C).

The NP_116213.1 protein sequence was submitted to 'Prot Param' tool of Expasy and its molecular weight was estimated to be 63536 Da. The NP_116213.1 protein was classified as a basic macromolecular protein because of its isoelectric point of 7.16. The TMEM87B protein was considered as an unstable protein with a computed instability index of 43.54 and its half-life in reticulocytes was 30 h *in vitro*. The possibility of the TMEM87B protein being located in the endoplasmic reticulum, plasma membrane and nuclear was estimated at 55.6, 33.3 and 11.1% with K-NN (K-nearest neighbor) method of PSORT II, respectively. Therefore, TMEM87B was hypothesized to be an intracytoplasm protein.

The TMEM87B protein appears to be a secretary protein and 1-42aa was estimated to be a potential cleavage site of its

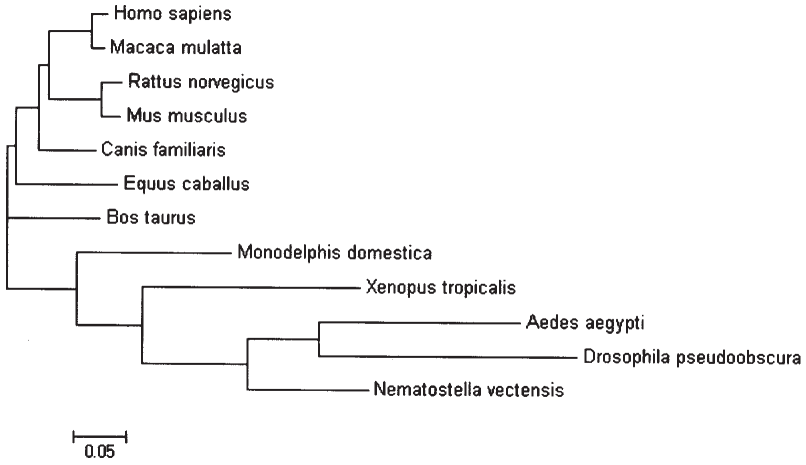

Figure 3. The phylogenetic tree of similar proteins of TMEM87B in several species.

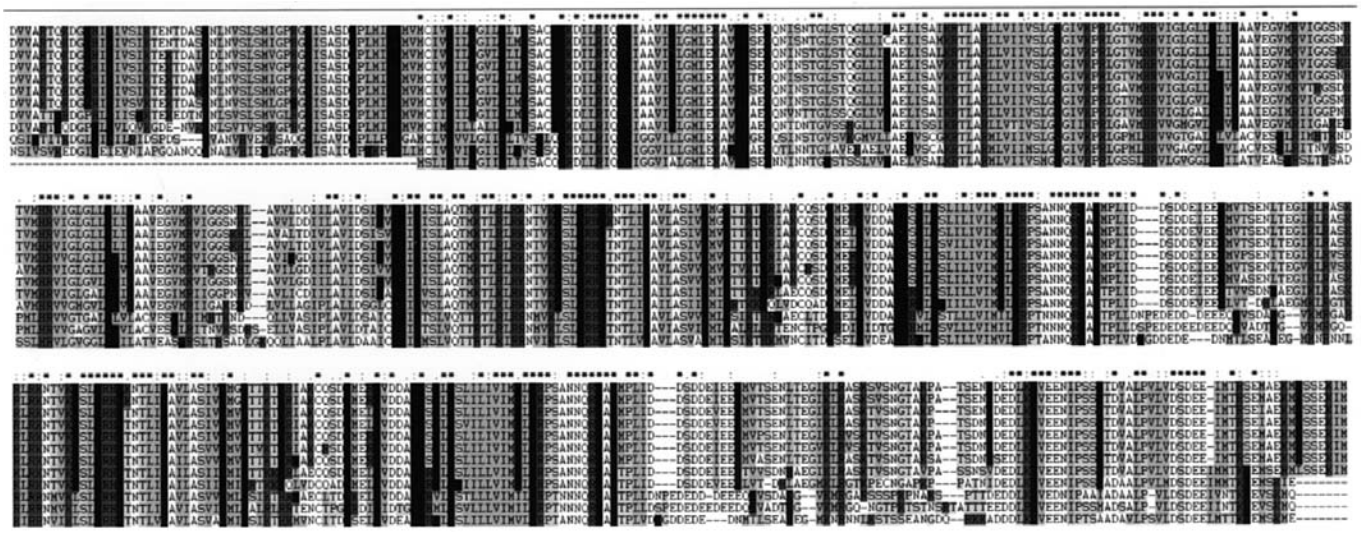

Figure 4. The results of a multi-sequence alignment of TMEM87B.

signal peptide by using SignalP3.0. Six transmembrane structure of the encoded protein was detected by the server 'TMHMM' and the transmembrane sites were located at 216-238aa, 248-267aa, 323-345aa, 355-374aa, 395-414aa and 434-451aa (Fig. 2D-E). We submitted the sequence to 'InterProScan' and 'Motif Scan' and found that it contains a lung seven transmembrane receptor (LSTR) domain at 173-458aa (E-value = 6.2e-142) and a DUF321 domain (E-value = 2e-24) at 248-267aa. The LSTR functional domain was detected by Conserved Domains of NCBI. Much more functional domains, such as cAMP- and cGMP-dependent protein kinase phosphorylation sites, N-glycosylation sites, casein kinase II phosphorylation sites and protein kinase C phosphorylation sites were found by 'Motif Scan'. The above potential functional domains were detected when the sequence was submitted to 'ScanProsite'. Searching the protein sequence by using ELM, it was found that there were many TM helixes and smart/pfam domains, which were consistent with the six transmembranes structure of the TMEM87B protein.

By consulting UniGene in the NCBI, it was found that the EST of the gene sequence is highly expressed in several normal tissues at different developmental stages of human beings and several tumors. The gene is expressed in normal tissues from low to high abundance in the mouth, umbilical cord, lymph node, parathyroid, small intestine and placenta. The most abundant expression of the gene was found in the neonate and embryoid body. There is also a widespread expression of TMEM87B in tumor tissues reported by SOURCE and Unigene, especially in adrenal, head and neck, breast and cervical tumors.

The results of multi-sequence alignment of 'ClustalX' software revealed that the TMEM87B sequence was highly conserved from junior and senior species, which might be associated with an important function (Fig. 4). The conserved sequences mainly consisted in the positive charge region (Arg and Lys), the negative charge region (Asp and Glu) and the hydrophobic region (Phe and Tyr). The phylogenetic tree of similar proteins in several species is depicted in Fig. 3.

The special TMEM87B mRNA expression by RT-PCR. The special *TMEM87B* mRNA expression was detected in Hela cells, while the positive bands of 501 bp were shown by the

use of agarose gel electrophoresis (Fig. 1E). This result indicated that TMEM87B indeed represented the product of a transcribed human gene in cancer.

The verification of protein interactions between TMEM87B and HPV18 E6 in yeast. The diploids were detected under an inverted microscope and blue colonies on the SD/-Ade/-His/-Leu/-Trp/X-α-gal agar medium were grown (Fig. 1F).

Discussion

HPVs are associated with epithelial lesions and high-risk HPVs such as HPV16 and HPV18 are associated with carcinomas (1,2). The transfection of the high-risk HPV DNA into primary human keratinocytes results in their immortalization, indicating that the HPV genome encodes oncogenes that mediate a cellular transformation (5). Increasing evidence supports the concept that many viral transforming proteins, such as the HPV oncoprotein E6 abolishing the function of the p53 tumor suppressor protein, efficiently induce oncogenesis by interacting with and perturbing the function of key cellular proteins involved in the maintenance of normal cellular behavior. Alternatively, many of the viral oncogene targets have been demonstrated to play key roles in human cancer, providing a clear rationale for searching for new targets of viral oncoproteins. Therefore, the interactions among a series of proteins in the organism are closely involved in the intracellular flow of information, the signal conduction and the cell cycle regulation, even cellular function.

The ability of E6 oncoproteins of high-risk HPVs to efficiently immortalize human epithelial cells has led to a considerable interest in identifying their cellular targets. Yeast two-hybrid is an advanced system providing a transcriptional assay *in vivo* in yeast to screen the Hela cDNA library for novel binding proteins, expressed as a fusion to the activation domain, that interact with a bait protein E6, expressed as a fusion to the DNA-binding domain. When bait and library fusion proteins interact, the DNA-BD and AD are brought into proximity, thus activating the transcription of the four reporter genes. This technology may be critical for proper protein functioning of complex biological systems (20,21) and can be used to identify novel protein interactions, confirm suspected interactions and define interacting domains. The results of yeast two-hybrid screening in our study are repeated, sorted and verificated by restreaking positive colonies, nutrition screening with X-α-gal, sequencing and yeast mating.

The studies reported here identify the protein, TMEM87B, as a novel E6 interactor in cellular oncogenic transformation. The only clue for a functional interaction between these two proteins is provided by our observation that E6 serves as a substrate for TMEM87B when the two were co-expressed *in vivo* in yeast. Our interest was aroused and further research was performed. Although the sequence of TMEM87B was discovered years ago from analysis and estimation of the human genome sequence, relatively little is known about the structure and biological properties of TMEM87B. Moreover, the role of E6-TMEM87B interaction must remain speculative at present. Therefore, in order to lead a theoretical direction for the research, the TMEM87B basic properties were roughly estimated with bioinformatic tools and databases.

The gene structure and chromosome localization were analyzed, as well as its transcript, then the biological properties were estimated. According to the predicted results, TMEM87B is hypothesized to be a secretary protein with a six-transmembrane structure and a signal peptide. The encoded protein appears to be classified as an intracellular protein, mainly located in the endoplasmic reticulum. Moreover, 'Interproscan' analysis revealed that the protein contains a LSTR domain at 173-458aa and a DUF321 structural domain at 248-267aa, which may be kept highly conserved in the evolution from low species such as zebrafish and *Xenopus laevis* to higher animals such as the mouse and human being. Many phosphorylation sites and functional motifs such as cAMP- and cGMP-dependent protein kinase phosphorylation sites and N-glycosylation sites were found and these domains play an active part in a variety of biological processes, such as cell cycle regulation, transcriptional control, mitochondrial and peroxisomal protein transport, neurogenesis and protein folding (22,23). Furthermore, the 'ClustalX' results revealed that the TMEM87B sequence was highly conserved from junior and senior species, which might be associated with an important function, while searching the protein sequence by using ELM, it was found that there were many TM helixes and smart/pfam domains, which were consistent with the six-transmembrane structure. In addition, it was reported by 'SOURCE' and 'Unigene' that the TMEM87B protein was hypothesized to be expressed universally in tumor tissues, especially in adrenal, head and neck, breast and cervical tumors. TMEM87B mRNA was then detected in the Hela cell line in this study to confirm the foundation of the hypothesis about the encoded protein.

In conclusion, our findings have important implications for the TMEM87B protein, containing poly-regulatory sites and being adjusted by other regulative proteins, may be a signaling molecule, which plays a role in signal transduction and transcriptional control in carcinogenesis. Notably, the novel oncogene target, TMEM87B, closely interacted with the high-risk transforming protein HPV18 E6, may be a potential oncogenesis target for cancer biotherapy. This investigation provides functional clues for further exploration of the novel TMEM87B binding protein.

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