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Karyopherin $\beta 3$: a new cellular target for the HPV-16 E5 oncoprotein

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Abstract

Epidemiological and experimental studies have shown that high-risk human papillomaviruses (HPVs) are the causative agents of cervical cancer worldwide, and that HPV-16 is associated with more than half of these cases. In addition to the well-characterized E6 and E7 oncoproteins of HPV-16, recent evidence increasingly has implicated the HPV-16 E5 protein (16E5) as an important mediator of oncogenic transformation. Since 16E5 has no known intrinsic enzymatic activity, its effects on infected cells are most likely mediated by interactions with various cellular proteins and/or its documented association with lipid rafts. In the present study, we describe a new cellular target that binds to 16E5 in COS cells and in stable human ectocervical cell lines. This target is karyopherin $\beta 3$, a member of the nuclear import receptor family with critical roles in the nuclear import of ribosomal proteins and in the secretory pathway.

Keywords

HPV-16; E5 oncoprotein; karyopherin $\beta 3$

Introduction

High-risk human papillomaviruses (HPVs), the causative agents of cervical cancer, are considered a major health threat worldwide and represent one of the most common causes of cancer in women [1,2]. Out of 120 different HPV types that have been isolated, more than 40

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infect the genital mucosa [3]. Some of these genital HPVs are low-risk (e.g. HPV-6 and -11) and are associated primarily with non-malignant lesions. However, there are 12–15 high-risk HPVs that have been linked to cervical cancer [1–4]. HPV-16 alone is responsible for approximately half of all cervical cancers [2,5,6].

Three HPV-16 genes possess activities that promote malignant transformation: E6, E7 and E5. 16E6 and 16E7 cooperate to induce cellular immortalization. The main role of 16E6 is to bind to the tumor suppressor protein p53 and facilitate its ubiquitin-mediated degradation. One of the primary functions of 16E7 is to inactivate the retinoblastoma tumor suppressor protein [4,7–9].

In contrast to 16E6 and 16E7, the biological functions of HPV-16 E5 (16E5) are rather poorly understood [7]. 16E5 is a small (83 amino acids) hydrophobic protein that localizes to membranes of the endoplasmic reticulum (ER) [10,11]. It exhibits weak transforming activity *in vitro* [7,12], but can induce malignant tumors in transgenic mice [13]. 16E5 also is believed to play a role in immune evasion by HPV-16-infected cells [14]. Importantly, since 16E5 has no innate enzymatic activity, its ability to alter cellular function most likely relies on its association with numerous proteins and/or lipid rafts [14] of the host cell. Documented intracellular binding targets for 16E5 include the 16kDa pore-forming subunit of the vacuolar H⁺-ATPase [10,15], the heavy chain of HLA type I [16] and ErbB4, a member of the epidermal growth factor (EGF) receptor family [17]. In this study we identify a new cellular target for 16E5, karyopherin β 3 (KN β 3). KN β 3 is a member of the nuclear import receptor superfamily [18–21], which includes more than 20 members in humans and 14 in yeast. These proteins regulate the nuclear import of various cargo, including ribosomal proteins, histones and viral proteins; however, they may also act as cytoplasmic chaperones and as microtubule motor adaptors [20]. KN β 3 localizes mainly to the cytoplasm, but also binds to some nucleoporins and can therefore be visualized in the nuclear envelope by immunostaining [18,22].

Materials and methods

Cells and viruses

Retroviruses encoding either HPV-16 E6 and E7 genes in the vector pBabePuro [23], or encoding codon-optimized 16E5 or a C-terminal deletion mutant of codon-optimized 16E5 lacking the last 10 amino acids (16E5 (–10)) in the vector pLXSN [24], were generated using the Phoenix cell system [25]. The cloning of codon-optimized 16E5 into the pJS55 expression vector has been described previously [11,26]. 16E5 (–10) was cloned into the *Bam*HI restriction site of pJS55. Both 16E5 proteins were N-terminally tagged with the AU1 epitope, DTYRYI [27]. Primary human ectocervical cells (HECs) were derived from cervical tissue after hysterectomy for benign uterine disease as described [28], and were immortalized by infection with an HPV-16 E6/E7-encoding retrovirus and selection in the presence of puromycin (0.5 μ g/ml). Stable 16E5-expressing cell lines were generated from immortalized HECs by infection with retroviruses encoding 16E5, 16E5 (–10) or the empty pLXSN expression vector, and selection in the presence of geneticin G418 (100 μ g/ml).

HEC lines were grown at 37°C and 5% CO₂ in keratinocyte growth medium (KGM; Invitrogen, Carlsbad, CA), supplemented with gentamicin sulfate (10 μ g/ml). To maintain constant levels of 16E5 and 16E5 (–10) expression, G418 was administered to cell cultures at alternating passages. COS cells were grown in Dulbecco's modified Eagle medium (DMEM) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin G and 100 μ g/ml streptomycin sulfate (Invitrogen).

Transfection

Prior to transfection, COS cells were grown to 90% confluence in 10-, 15-cm tissue culture dishes, or on 22×22 mm sterile glass cover slips in six-well tissue culture plates, in antibiotic-free Opti-MEM (Invitrogen) containing 4% FBS. For each 10-cm dish, 15-cm dish, or cover slip, 32 µg, 72 µg, or 4 µg DNA (in pJS55) was mixed with 4 ml, 9 ml, or 0.5 ml serum-free Opti-MEM and 40 µl, 90 µl, or 5 µl Lipofectamine 2000 transfection reagent (Invitrogen) and added to the cells for 4 h.

Isolation and microsequencing of 16E5-associated proteins

15-cm tissue culture dishes of COS cells were transfected with 16E5 or 16E5 (−10) in pJS55, as described above. For each DNA, 8 dishes were placed on ice 24 h after transfection and washed with 25 ml/dish phosphate-buffered saline (PBS). Cells were scraped into 2.0 ml/dish modified RIPA buffer [29] and disrupted by vigorous shaking for 15 min at 4°C. The protein concentration of lysates was determined using the Bio-Rad (Hercules, CA) DC protein assay with bovine IgG as the standard. Aliquots of lysates containing 20 mg protein were mixed with 400 µl of 50% (v/v) protein A-sepharose beads (ImmunoPure Plus; Pierce, Rockford, IL) and 40 µl of mouse anti-AU1 ascites (Covance, Princeton, NJ) and were rotated end-over-end for 90 min at 4°C. Subsequently, the beads were washed 3 times with 5 ml RIPA buffer (5 min rotating end-over-end per wash) and 3 times with 5 ml PBS. Bound proteins were eluted in SDS lysis buffer [30] by incubating 15 min at 37°C and 20 min at 110°C. The SDS eluant was mixed with 0.1 volume of 20% (w/v) Triton X-100, 500 µl of 50% (v/v) protein A-sepharose beads and 400 µg of Pierce unconjugated rabbit anti-mouse IgG (H+L); and was rotated end-over-end overnight at 4°C. 75 µg of *E. coli* RNA was added to the unadsorbed fraction and proteins were precipitated with 10% trichloroacetic acid (TCA) for 1 h on ice. The precipitates were centrifuged for 5 min at 8,000 g at 4°C and extracted with 800 µl of (95% acetone, 0.1 N HCl). Extracted TCA precipitates were solubilized in 50 µl of 2×Laemmli SDS-PAGE sample buffer (125 mM Tris-HCl (pH 6.8), 4% SDS, 20% glycerol, 0.01% bromophenol blue, 10% β-mercaptoethanol) for 20 min at 37°C and 6 min at 110 °C. To maintain pH, 3 µl of 1 M Tris-HCl (pH 8.0) was added. Electrophoresis was performed in 1.5-mm polyacrylamide Tris-glycine mini-gels (Invitrogen). After electrophoresis, gels were washed 3 times with deionized H₂O, stained for 3 h with GelCode Blue Stain Reagent (Pierce) and rinsed with several changes of deionized H₂O over a period of 18 h. Bands of interest were excised for microsequencing by ProfTech, Inc (Eagleville, PA).

Metabolic labeling

10-cm dishes of 80% confluent COS cells were washed with PBS (15 ml per dish) and incubated for 2 h in DMEM without cysteine and methionine (4 ml per dish; BioSource International, Carlsbad, CA). [³⁵S] methionine/[³⁵S] cysteine protein labeling mix (Perkin-Elmer “Easy Tag”, Waltham, MA) was added (200 µCi per dish) for 3 h and the cells lysed in modified RIPA buffer.

Immunoprecipitation and immunoblotting

AU1 epitope-tagged 16E5 proteins were detected in RIPA lysates by immunoprecipitation and Western blotting [29], using 4 µg rabbit anti-AU1 polyclonal antibody for immunoprecipitation, and 1:5,000-diluted mouse anti-AU1 ascites for immunoblotting (Covance). KNβ3 was detected in RIPA lysates using 4 µg Santa Cruz Biotechnology (Santa Cruz, CA) anti-KNβ3 rabbit polyclonal or mouse monoclonal antibodies for immunoprecipitation, and 1:200-diluted rabbit polyclonal or mouse monoclonal antibodies for immunoblotting, as described [30–31].

Confocal microscopy

HECs and COS cells were grown on 22×22 mm sterile glass cover slips in six-well tissue culture plates, fixed in 4% (w/v) paraformaldehyde and labeled as described [29], except that Alexa Fluor-conjugated donkey secondary antibodies (Molecular Probes, Carlsbad, CA) were used at a concentration of 5 µg/ml. The following primary antibodies were used: Covance anti-AU1 mouse monoclonal (1:7,000 dilution of ascites) and Santa Cruz anti-KNβ3 rabbit polyclonal antibody (1:150 dilution). HECs were cultured in keratinocyte serum-free medium (KSFM; Invitrogen) for approximately 24 h prior to fixation to increase flatness. A Zeiss Axiovert confocal microscope was used for visualization and microphotography.

Results

To identify new potential targets for the 16E5 oncoprotein, COS cells were transfected with DNA encoding AU1 epitope-tagged wild-type (wt) 16E5 or a 16E5 mutant lacking the C-terminal 10 amino acids (16E5 (–10)) that is defective for altering endosomal pH [24] and enhancing ligand-dependent EGF receptor activation [12,15]. The cells were then labeled with [³⁵S] methionine and immunoprecipitated with an anti-AU1 antibody to isolate 16E5 and associated proteins. A unique 116 kDa polypeptide was found to be associated only with wt 16E5, and not with 16E5 (–10) (Fig. 1A, upper panel), even though both 16E5 proteins were expressed at similar levels (Fig. 1A, lower panel). To identify the 116 kDa polypeptide, 16E5 was immunoprecipitated from 20 mg of cell lysate, and the associated 116 kDa band excised from Coomassie blue-stained SDS-PAGE for microsequencing (Fig. 1B). As shown in Fig. 1C, 17 proteolytic fragments of the 116 kDa protein exactly match the amino acid sequence of human KNβ3.

To verify the binding interaction between 16E5 and KNβ3, COS cells were transfected with 16E5 or 16E5 (–10) DNA, and were immunoprecipitated with antibodies recognizing KNβ3. The immunoprecipitates were then analyzed on Western blots that were labeled for AU1 or KNβ3. The results clearly demonstrate an association between KNβ3 and wt 16E5, but not between KNβ3 and 16E5 (–10) (Fig. 2A, upper panel), even though equivalent amounts of KNβ3 are present in all immunoprecipitates (Fig. 2A, lower panel). A strong association of KNβ3 and 16E5 was also detected, as well as weak binding of KNβ3 to 16E5 (–10) when the AU1 antibody was used for immunoprecipitation and KNβ3 antibody was used for immunoblotting (Fig. 2B, upper panel). Both wt and mutant 16E5 proteins were present at similar levels in these immunoprecipitates (Fig. 2B, lower panel).

To verify the association of KNβ3 and 16E5 in a natural HPV host cell in the presence of other HPV-16 early genes, we performed similar immunoprecipitation/immunoblot experiments using 16E6/16E7-immortalized human ectocervical cell (HEC) lines that stably express wt and mutant 16E5 proteins [14]. A strong association between wt 16E5 and KNβ3, and slight association between 16E5 (–10) and KNβ3, was observed when the AU1 antibody was used for immunoprecipitation and KNβ3 antibody for immunoblotting (Fig. 2C, upper panel). Again, both 16E5 proteins were expressed at similar levels in the HECs (Fig. 2C, lower panel). However, we were unable to detect 16E5 in anti-KNβ3 immunoprecipitates of HECs, possibly due to the difference of 16E5 protein levels in HECs and in COS cells.

To further characterize the interactions of these proteins, we localized 16E5 and KNβ3 in COS cells and HECs using immunofluorescence confocal microscopy. KNβ3 shows a typical cytoplasmic and perinuclear staining pattern [18,22] in both cell types (Fig. 3, A and B, left column), while 16E5 exhibits staining typical for ER-localized proteins in HECs and staining typical for ER- and Golgi-localized proteins in COS cells [10,11] (Fig. 3, A and B, middle column). Co-localization of KNβ3 and 16E5 was observed in the perinuclear area in both cell

types; with additional 16E5 sandwiched between KN β 3 and the nucleus in HECs (Fig. 3, A and B, right column).

Collectively, these results demonstrate that wt 16E5 associates and co-localizes with KN β 3, and that 16E5 (–10) is largely defective for this association.

Discussion

In contrast to the well-characterized E6 and E7 oncoproteins of HPV-16, biological activities of the 16E5 oncoprotein and its role in HPV-mediated pathogenesis are not well understood. Known functions of 16E5 include stimulating EGF-dependent proliferation in cells that express large numbers of EGF receptors, inhibition of endosome acidification and the activation of several cell signaling pathways downstream of the EGF receptor [12].

In the current study, we identify a new binding target for 16E5: KN β 3, a member of the nuclear import receptor superfamily. The function of KN β 3 is not as well established as that of other members of this family, e.g. karyopherin β 1 [20]; however, KN β 3 is known to play a critical role in ribosomal protein import and the secretory pathway [18,21]. KN β 3 also binds to and mediates nuclear transport of some viral proteins, including the L2 protein of HPV-16 and HPV-11. This interaction occurs via L2 nuclear localization signals, one in the N-terminus and one in the C-terminus [32,33]. We show that the 16E5-KN β 3 interaction primarily is mediated by 10 amino acids at the C-terminus of 16E5, since a C-terminal 16E5 (–10) deletion mutant is largely defective for binding KN β 3. In contrast to L2, however, 16E5 does not contain a nuclear localization signals and is not found in the nucleus. KN β 3 is an abundant cellular protein localized mainly in the cytoplasm; especially near the nuclear envelope. Consistent with the 16E5-KN β 3 binding interaction, we observe co-localization of KN β 3 and 16E5 in the perinuclear area by confocal immunofluorescence microscopy. The membrane topology of 16E5 has not been determined, but preliminary data from our laboratory indicates that the C-terminus of 16E5 is exposed to the cytoplasm. Thus, the region of 16E5 that binds KN β 3 is in the same subcellular compartment as KN β 3.

The significance of 16E5 - KN β 3 binding still needs further examination. Since 16E5 inhibits endocytic vesicle trafficking [34] and KN β 3 may play a role in vesicle transport [20], it is possible that the mechanism by which 16E5 influences vesicle trafficking is dependent upon the interaction between these two proteins. Our unpublished data show that the 16E5/KN β 3 interaction plays an important role in the induction of perinuclear vacuolization, i.e., koilocytosis, a characteristic morphological feature of papillomavirus infection [35]. For example, siRNA-mediated knockdown of KN β 3 causes a 50% decrease in koilocyte formation in 16E5-expressing HEC lines.

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References

1. Walboomers JM, Jacobs MV, Manos MM, Bosch FX, Kummer JA, Shah KV, Snijders PJF, Peto J, Meijer CJLM, Munoz N. Human papillomaviruses is a necessary cause of invasive cervical cancer worldwide. *J. Pathol* 1999;189:12–19. [PubMed: 10451482]
2. zur Hausen H, de Villiers E-M. Human papillomaviruses. *Annu. Rev. Microbiol* 1994;48:424–447.
3. Trottier H, Franco EL. The epidemiology of genital human papillomavirus infection. *Vaccine* 2006;24:4–15.
4. zur Hausen H. Papillomaviruses and cancer: from basic studies to clinical application. *Nature Rev* 2002;2:342–350.

5. Bosch FX, de Sanjosé S. Chapter 1: human papillomavirus and cervical cancer – burden and assessment of causality. *J. Natl. Cancer Inst. Monogr* 2003;31:3–13. [PubMed: 12807939]
6. Baseman JG, Koutsky LA. The epidemiology of human papillomavirus infections. *J. Clin. Virol* 2005;32:16–24.
7. Fehrman F, Laimins LA. Human papillomaviruses: targeting differentiating epithelial cells for malignant transformation. *Oncogene* 2003;22:5201–5207. [PubMed: 12910257]
8. Longworth MS, Laimins LA. Pathogenesis of human papillomaviruses in differentiating epithelia. *Microbiol. Mol. Biol. Rev* 2004;68:362–372. [PubMed: 15187189]
9. Münger K, Howley PM. Human papillomavirus immortalization and transformation functions. 2002;89:213–228.
10. Conrad M, Bubb VJ, Schlegel R. The human papillomavirus type 6 and 16 E5 proteins are membrane-associated proteins which associate with the 16-kilodalton pore-forming protein. *J. Virol* 1993;67:6170–6178. [PubMed: 7690419]
11. Disbrow GL, Sunitha I, Baker CC, Hanover J, Schlegel R. Codon optimization of the HPV-16 E5 gene enhances protein expression. *Virology* 2003;311:105–114. [PubMed: 12832208]
12. Supryniewicz, FA.; Campo, MS.; Schlegel, R. Biological activities of papillomavirus E5 protein. In: Campo, MS., editor. *Papillomavirus research: from natural history to vaccines and beyond*. Caister Academic Press; 2006. p. 97–113.
13. Genther Williams SM, Disbrow GL, Schlegel R, Lee D, Threadgill DW, Lambert PF. Requirement of epidermal growth factor receptor for hyperplasia induced by E5, a high-risk human papillomavirus oncogene. *Cancer Res* 2005;65:6534–6542. [PubMed: 16061632]
14. Supryniewicz FA, Disbrow GL, Krawczyk E, Simic V, Lantzky K, Schlegel R. HPV-16 E5 oncoprotein upregulates lipid raft components caveolin-1 and ganglioside GM1 at the plasma membrane of cervical cells. *Oncogene* 2007;27:1071–1078. [PubMed: 17704805]
15. Rodríguez MI, Finbow ME, Alonso A. Binding of human papillomavirus 16 E5 to the 16kDa subunit c (proteolipid) of the vacuolar H⁺-ATPase can be dissociated from the E5-mediated epidermal growth factor receptor overactivation. *Oncogene* 2000;19:3727–3732. [PubMed: 10949926]
16. Ashrafi GH, Haghshenas M, Marchetti B, Campo MS. E5 protein of human papillomavirus 16 downregulates HLA class I and interacts with the heavy chain *via* its first hydrophobic domain. *Int. J. Cancer* 2006;119:2105–2112. [PubMed: 16823848]
17. Chen SL, Lin ST, Tsai TC, Hsiao WC, Tsao YP. ErbB4 (JM-b/CYT-1)-induced expression and phosphorylation of c-Jun is abrogated by human papillomavirus type 16 E5 protein. *Oncogene* 2006;26:42–53. [PubMed: 16819515]
18. Yaseen NR, Blobel G. Cloning and characterization of human karyopherin β 3. *Proc. Natl. Acad. Sci. USA* 1997;94:4451–4456. [PubMed: 9114010]
19. Wozniak RW, Rout MP, Aitchison JD. Karyopherins and kissing cousins. *Trends Cell Biol* 1998;8:184–188. [PubMed: 9695836]
20. Harel A, Forbes DJ. Importin beta: conducting a much larger cellular symphony. *Mol. Cell* 2004;16:319–330. [PubMed: 15525506]
21. Jäkel S, Görlich D. Importin β , transportin, RanBP5 and RanBP7 mediate nuclear import of ribosomal proteins in mammalian cells. *EMBO J* 1998;17:4491–4502. [PubMed: 9687515]
22. Deane R, Schäfer W, Zimmermann HP, Mueller L, Görlich D, Prehn S, Ponstingl H, Bischoff FR. Ran-binding protein 5 (RanBP5) is related to the nuclear transport factor importin-beta but interacts differently with RanBP1. *Mol. Cell. Biol* 1997;17:5087–5096. [PubMed: 9271386]
23. Morgenstern JP, Land H. Advanced mammalian gene transfer: high titre retroviral vectors with multiple drug selection markers and a complementary helper-free packaging cell line. *Nucleic Acids Res* 1990;18:3587–3596. [PubMed: 2194165]
24. Disbrow GL, Hanover JA, Schlegel R. Endoplasmic reticulum-localized human papillomavirus type 16 E5 protein alters endosomal pH but not trans-Golgi pH. *J. Virol* 2005;79:5839–5846. [PubMed: 15827198]
25. Pear WS, Nolan GP, Scott ML, Baltimore D. Production of high-titer helper-free retroviruses by transient transfection. *Proc. Natl. Acad. Sci. USA* 1993;90:8392–8396. [PubMed: 7690960]

26. Sparkowski J, Anders J, Schlegel R. Mutation of the bovine papillomavirus E5 oncoprotein at amino acid 17 generates both high- and low-transforming variants. *J. Virol* 1994;68:6120–6123. [PubMed: 8057494]
27. Lim PS, Jenson AB, Cowser L, Nakai Y, Lim LY, Jin XW, Sundberg JP. Distribution and specific identification of papillomavirus major capsid protein epitopes by immunocytochemistry and epitope scanning of synthetic peptides. *J. Infect. Dis* 1990;162:1263–1269. [PubMed: 1700026]JP
28. Baeye AC, Berger A, Schlegel R, Veldman T, Schlegel R. Cervical epithelial cells transduced with the papillomavirus E6/E7 oncogenes maintain stable levels of oncoprotein expression but exhibit progressive, major increases in hTERT gene expression and telomerase activity. *Am. J. Pathol* 2002;160:1251–1257. [PubMed: 11943710]
29. Supryniewicz FA, Disbrow GL, Simic V, Schlegel R. Are transforming properties of the bovine papillomavirus E5 protein shared by E5 from high-risk human papillomavirus type 16? *Virology* 2005;332:102–113. [PubMed: 15661144]
30. Supryniewicz FA, Sparkowski J, Baeye A, Schlegel R. E5 oncoprotein mutants activate phosphoinositide 3-kinase independently of platelet-derived growth factor receptor activation. *J. Biol. Chem* 2000;275:5111–5119. [PubMed: 10671555]
31. Supryniewicz FA, Baeye A, Sunitha I, Schlegel R. c-Src activation by the E5 oncoprotein enables transformation independently of PDGF receptor activation. *Oncogene* 2002;21:1695–1706. [PubMed: 11896601]
32. Darshan MS, Lucchi J, Harding E, Moroianu J. The L2 minor capsid protein of human papillomavirus type 16 interacts with a network of nuclear import receptors. *J. Virol* 2004;78:12179–12188. [PubMed: 15507604]
33. Bordeaux J, Forte S, Harding E, Darshan MS, Klucsevsek K, Moroianu J. The L2 minor capsid protein of low-risk human papillomavirus type 11 interacts with host nuclear import receptors and viral DNA. *J. Virol* 2006;80:8259–8262. [PubMed: 16873281]
34. Thomsen P, van Deurs B, Norrild B, Kayser L. The HPV 16 E5 oncogene inhibits endocytic trafficking. *Oncogene* 2000;19:6023–6032. [PubMed: 11146554]
35. Hajdu SI. A note from history: the link between koilocytes and human papillomaviruses. *Ann. Clin. Lab. Sci* 2006;36:485–487. [PubMed: 17127740]

Fig. 1.

Isolation and sequencing of the 16E5-associated 116 kDa protein. (A) wt 16E5 (lane 1), but not mutant 16E5 (-10) (lane 2) associates with a 116 kDa cellular protein (arrow) in anti-AU1 immunoprecipitates of ³⁵S-labeled COS cells. Molecular mass markers (in kDa) are shown on the left. (B) A doublet of Coomassie blue-stained polypeptides at approximately 110 kDa containing 16E5-associated proteins from large-scale anti-AU1 immunoprecipitations (lane 1; arrow) were excised and microsequenced. Molecular mass markers (in kDa) are indicated on the left. (C) Human KNβ3 (116 kDa) is the major protein present in the excised bands. The KNβ3 sequence was copied from NCBI Sequence Viewer v2.0. Tryptic peptides of the excised

protein were generated and sequenced by ProtTech, Inc. (Norristown, PA) and are framed, showing complete sequence homology with KN β 3.

Fig. 2.

Association of 16E5 and KN β 3. (A and B) Association of wt 16E5 and KN β 3 in COS cells and (C) in HEC lines. COS cells were transfected with the indicated DNA and lysed 24 h later. Molecular mass markers (in kDa) are indicated on the left. IP: immunoprecipitation; IB: immunoblotting.

Fig. 3. Confocal immunofluorescence co-localization of 16E5 and KN β 3. Co-localization of wt 16E5 and KN β 3 in (A) COS cells and (B) HEC lines. Immunofluorescence detection of KN β 3 (green), 16E5 (AU1; red) and co-localization of KN β 3 with 16E5 protein (yellow) are shown. Scale bar represents 10 μ m.