Human Papillomavirus E7 Protein Deregulates Mitosis via an Association with Nuclear Mitotic Apparatus Protein 1

Christine L. Nguyen and Karl Münger*

Channing Laboratory, Brigham and Women’s Hospital, and Department of Medicine, Harvard Medical School, 181 Longwood Avenue, Boston, Massachusetts 02115

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We previously observed that high-risk human papillomavirus type 16 (HPV16) E7 expression leads to the delocalization of dynein from mitotic spindles (C. L. Nguyen, M. E. McLaughlin-Drubin, and K. Munger, Cancer Res. 68:8715–8722, 2008). Here, we show that HPV16 E7 associates with nuclear mitotic apparatus protein 1 (NuMA) and that NuMA binding and the ability to induce dynein delocalization map to similar carboxyl-terminal sequences of E7. Additionally, we show that the delocalization of dynein from mitotic spindles by HPV16 E7 and the interaction between HPV16 E7 and NuMA correlate with the induction of defects in chromosome alignment during prometaphase even in cells with normal centrosome numbers. Furthermore, low-risk HPV6b and HPV11 E7s also associate with NuMA and also induce a similar mitotic defect. It is possible that the disruption of mitotic events by HPV E7, via targeting of the NuMA/dynein complex and potentially other NuMA-containing complexes, contributes to viral maintenance and propagation potentially through abrogating the differentiation program of the infected epithelium. Furthermore, in concert with activities specific to high-risk HPV E6 and E7, such as the inactivation of the p53 and pRB tumor suppressors, respectively, the disruption of the NuMA/dynein network may result in mitotic errors that would make an infected cell more prone to the accumulation of aneuploidy even in the absence of supernumerary centrosomes.

During mitosis, cells undergo a series of concerted efforts in order to ensure the faithful segregation of chromosomes to daughter cells. At the onset of mitosis, nuclear mitotic apparatus protein 1 (NuMA) is targeted to the spindle poles by the microtubule motor dynein, where it is involved in spindle organization and stabilization (7, 8, 19, 20). Furthermore, opposing motor activities by kinesins, including Eg5, and dyneins were shown to be necessary for the organization of the mitotic spindle (7). NuMA is also involved in chromosome alignment in mitotic cells (17), and ultimately, the cross talk between the motor proteins and their binding partners and/or cargoes allows the proper alignment of chromosomes on the metaphase plate, triggering entry into anaphase.

In addition to having mitotic functions, NuMA and dynein play important roles in other cellular events. NuMA is involved in nuclear reassembly following mitosis and is a structural component of the nuclear matrix during interphase (reviewed in reference 31). Furthermore, several studies show that NuMA is cleaved by caspases early in apoptosis and that this may contribute to nuclear breakdown during apoptosis (1, 34). The dynein motor complex is involved in the positioning of organelles as well as the transportation of cargo from the endoplasmic reticulum, lysosomes, and endosomes (reviewed in references 6 and 24).

Both NuMA and dynein have also been implicated in the establishment of cell polarity. In order for the proper stratification and differentiation of mammalian skin to occur, basal cells must divide upward and in an asymmetric fashion, producing a committed suprabasal cell and a proliferative basal cell. NuMA localizes to the apical surface of the dividing basal cell and may thus importantly contribute to establishing the cell polarity necessary for proper epithelial differentiation (16). Recent reports suggested that dynein may also play an important role in this process, as it was shown that dynein regulates epithelial polarity in Drosophila melanogaster follicle cells (11, 18).

Human papillomaviruses (HPVs) are ubiquitous DNA viruses that infect epithelial cells. Low-risk HPVs generally cause benign warts, while high-risk HPVs are associated with almost all cases of cervical cancer (reviewed in reference 23). The HPV life cycle is tightly linked to the differentiation program of infected epithelial cells, and because HPVs depend upon host cell machinery for genome synthesis, the virus must infect proliferating basal cells in order to establish a persistent infection (reviewed in reference 22). Viral E6 and E7 proteins are necessary for maintaining an environment conducive to DNA replication in differentiating cells, thus allowing for efficient viral production in the upper layers of the epithelium. We previously reported that the expression of HPV type 16 (HPV16) E7 leads to the delocalization of dynein from the mitotic spindle (26). Here, we identify an association between HPV E7 and NuMA that may contribute to the delocalization of dynein and that also correlates with a mitotic delay, potentially due to defects in chromosome alignment. We hypothesize that these events may play an important role in viral persistence but may also contribute to the destabilization of the host genome when cells are infected by high-risk HPVs.

MATERIALS AND METHODS

Cells. HPV-negative cervical cancer C33a cells; HPV-positive cervical cancer CaSkI, SiHa, and HeLa cells; NIH 3T3 mouse embryo fibroblasts; and HEK293 human embryonic kidney cells were obtained from the ATCC and maintained as...
previously described (26), pRBP107/p130−/− mouse embryo fibroblasts were previously described (3) and were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 50 U/ml penicillin, and 50 µg/ml streptomycin. NIH 3T3 cells with the stable expression of empty vector (3T3-poz), C-termally Flag- and hemagglutinin (HA)-tagged HPV16 E7 (3T3-CE7), C-termally Flag- and HA-tagged HPV16 E7Δ21-24 (3T3-Δ21-24), and C-termally Flag- and HA-tagged HPV16 E7Δ29-83 (3T3-Δ29-83) and human foreskin fibroblasts (HFFs) with the stable expression of pBABE-purumycin (vector), pBabe-purumycin HPV16 E7 (wild-type E7), pBabe-purumycin HPV16 E7Δ21-24 (E7Δ21-24), and HPV16 E7Δ79-83 (E7Δ79-83) were made and maintained as previously described (26). Normal oral keratinocytes (NOKs) immortalized by human telomerase (hTERT) with or without HPV16 E7 (28) were cultured as previously described (26).

IF. Extractions and immunofluorescence (IF) experiments were performed and imaged as described previously (26). Antibodies used for IF were anti-dynein (MAB1618; Chemicon International), anti-γ-tubulin (H-183; Santa Cruz Biotechnology), and anti-NuMA (NB500-174 [Novus Biologicals] and C-20 [Santa Cruz Biotechnology]).

For transient transfection assays, cells were transfected with the plasmid of interest (either pBabe-purumycin, pBabe-purumycin HPV16 E7, pBabe-purumycin HPV16 E7Δ21-24, or HPV16 E7Δ79-83 [see above] or pOz-empty, pOz-C6bE7, pOz-C1E7, or pOz-C6bE7) (13) together with pEGFP (transfection marker; Clontech) at a 5:1 ratio. Transfections were performed with FuGENE6 reagent (Roche Applied Science) according to the manufacturer’s protocol. After 48 h, cells were fixed in 4% formaldehyde (in phosphate-buffered saline) and processed for IF.

GST pull-downs and Western blotting. For immunoprecipitations (IPs), cells were cross-linked with 1 mM dithiobis(succinimidyl)propionate (DSP) for 30 min at 4°C, where indicated. Excess DSP was quenched with 10 mM glycine. Cells were then rinsed with phosphate-buffered saline, scraped on ice into ML buffer (20 mM Tris [pH 8], 1 mM EDTA, 300 mM NaCl, 0.5% NP-40, and protease inhibitors [Complete EDTA-free tablets; Roche Diagnostics]), and cleared by centrifugation (20 min at 16,000 × g). Five micrograms anti-HPV16 E7 antibody (ED19, generated by our laboratory), 20 µg anti-NuMA antibody (C-20; Santa Cruz Biotechnology), or 5 µg anti-dynein antibody (MAB1618; Chemicon International) was coupled to 50 µg protein G (Promega) slurry by rotating at 4°C for 30 min. Coupled antibody was incubated with 5 to 20 mg whole-cell lysates for 4 h at 4°C. IPs were washed with ML buffer and resuspended in loading buffer. For room-temperature IPs, cross-linking, scraping of cells for lysate, and incubation with coupled antibody were performed at room temperature.

GST pull-downs were performed as described previously (27). Samples from pull-downs and IPs were analyzed by Western blotting and visualized as described previously (26). Membranes were probed with anti-HPV16 E7 (ED19), anti-GST (3818-1; Clontech), anti-NuMA (NB500-174 [Novus Biologicals] or C-20 [Santa Cruz Biotechnology]), and/or anti-pRB (G3-245 [BD Pharmingen]) or Ab2 (Oncogene Research Products) antibodies.

Live-cell imaging. Cells were fixed using a retrovirus encoding green fluorescent protein-histone 2B (a generous gift from Randall W. King, Harvard Medical School, Boston, MA), and ~2 × 10^6 cells were plated onto poly-L-lysine-coated cover glass-bottom dishes (catalog no. P35SC-1.5-14-C; MatTek, Ashland, MA) 24 h prior to imaging. Images were acquired automatically at 20 locations on the coverslip using a Nikon TE2000E inverted microscope fitted with a Plan Apo 20× Nikon objective, a linearly encoded stage (Proscan; Prior), a Hamamatsu Orca-ER charge-coupled-device camera, and the Perfect Focus System (Nikon, Melville, NY).

Fluorescence illumination was performed with an X-CITE mercury-arc lamp (Exfo, Ontario, Canada) with two neutral-density filters (for a total 32-fold reduction in intensity). The microscope was controlled using NIS-Elements (Nikon, Melville, NY) and was housed in a custom-designed 37°C chamber with a secondary internal chamber that delivered humidified 5% CO2. Fluorescence and differential interference contrast images were obtained every 6 min with 200-ms and 20-ms exposures, respectively, for a total period of 24 h. Twenty-five cells were analyzed at every other location until a total of 200 cells (per cell type) were scored.

RESULTS

HPV16 E7 associates with the NuMA through the C terminus of E7. In order to gain insights into the potential mechanism by which HPV16 E7 alters dynein localization (26), we performed a large-scale GST pull-down experiment using NIH 3T3 cells. We also performed a large-scale GST pull-down using pRB/p107/p130−/− mouse embryo fibroblasts to identify putative pRB/p107/p130-independent HPV16 E7-binding partners since it was clear that dynein delocalization did not depend upon the association between HPV16 E7 and the pRB family of proteins but instead relied upon the C terminus of HPV16 E7 (26).

Among other proteins, NuMA was identified in the GST pull-down experiments, where 20 (using NIH 3T3 cells) and 27 (using pRB/p107/p130−/− mouse embryo fibroblasts) peptides were identified by mass spectrometry. NuMA was a candidate protein because it is necessary for the formation and stabilization of mitotic spindles at least in part through an association with dynein (20, 21), and NuMA levels were shown to affect dynein localization (29). To confirm the HPV16 E7/NuMA association under physiological conditions, IPs were performed using the HPV-negative and HPV-positive cervical cancer cell lines C33a and CaSki, respectively. In the event that the interaction was transient, the cells were cross-linked in vivo with DSP prior to cell lysis and IP. In CaSki cells, an HPV16 E7 antibody coprecipitates NuMA, and conversely, a NuMA antibody coprecipitates E7 (Fig. 1A). The interaction was also detected without cross-linking and when the IP was performed at either room temperature (Fig. 1A) or 4°C (data not shown). The room-temperature immunoprecipitation was performed in case the interaction was dependent on intact spindles, as cold temperatures destabilize unattached microtubules. Therefore, HPV16 E7 interacts with NuMA, a protein that associates with dynein in order to organize mitotic spindles.

To map the association between HPV16 E7 and NuMA, GST pull-downs using GST alone or GST fused to wild-type HPV16 E7 or the HPV16 E7Δ6-10, HPV16 E7Δ21-24, HPV16 E7Δ79-83, or HPV16 E7C91S mutant were performed with HeLa whole-cell lysates. While wild-type HPV16 E7 and the HPV16 E7Δ6-10 and HPV16 E7Δ21-24 mutants associate with NuMA in the GST pull-down assay, the C-terminal HPV16 E7Δ79-83 and HPV16 E7C91S mutants do not (Fig. 1B). These C-terminal mutants were also defective in inducing dynein delocalization from mitotic spindles compared to wild-type HPV16 E7 (26), suggesting that NuMA binding and dynein delocalization may be mechanistically linked.

NuMA migrates as a doublet on sodium dodecyl sulfate-polyacrylamide gels, and the slower-migrating forms have been suggested to represent phosphorylated species (2, 12, 30). GST pull-downs suggest that HPV16 E7 efficiently binds a slower-migrating form(s) of NuMA (Fig. 1C). Consistent with a previous report that dynein associates specifically with phosphorylated NuMA (9), the slower-migrating form(s) of NuMA is also communoprecipitated by a dynein-specific antibody (Fig. 1C). However, our attempts to use treatments with various protein phosphatases to show that the slower-migrating forms of NuMA coprecipitated by E7 or dynein are indeed due to phosphorylation were inconclusive, and it therefore remains possible that the electrophoretic shift of E7- and/or dynein-bound NuMA may be caused by another posttranslational modification.

These results show that HPV16 E7 associates with NuMA through C-terminal sequences of HPV16 E7 that are necessary for the induction of dynein delocalization and suggest that
The stable expression of HPV16 E7 in NIH 3T3 cells or HFF cells does not globally disrupt NuMA localization to mitotic spindles; however, in many of the cells in which dynein was delocalized from mitotic spindles, NuMA also had an abnormal staining pattern. In NIH 3T3 cells, the delocalization of dynein correlated with a decreased NuMA signal as well as a disorganized distribution of NuMA that did not appear to be specific to mitotic spindles (Fig. 2B). In HFF cells, the NuMA antibody stained much brighter, and while in cells with delocalized dynein, NuMA still appeared at mitotic spindle poles, NuMA lacked clear spindle staining (Fig. 2B). When quantified in 3T3-poz and 3T3-CE7 cells, aberrant NuMA localization was seen in 61.0% (61/100 cells) and 62.7% (116/185 cells), respectively, of mitotic cells with delocalized dynein. As described previously, however, there was still an overall increase in the population of mitotic 3T3-CE7 cells with delocalized dynein (21.6% compared to 8.6% in 3T3-poz cells) (26) and, therefore, an increase in the amount of mitotic cells with delocalized NuMA. Ultimately, NuMA is delocalized in cells where dynein does not properly localize to the mitotic spindle, and the implications of the similarity in frequency between control vector and HPV16 E7-expressing cells will be discussed below.

**Dynein delocalization and NuMA interaction correlate with disorganized chromosome alignment during metaphase in HPV16 E7-expressing cells.** Dynein delocalization from mitotic spindles has been associated with an increased incidence of multipolar mitoses in some cancer cells (29), but we did not observe this in our own studies (26). Instead, we detected abnormal dynein localization in cells with normal centrosome numbers undergoing bipolar metaphase and noticed that these cells often displayed a highly abnormal, distorted chromosome alignment. Normally, during metaphase, chromosomes align tightly along a metaphase plate (Fig. 3A). The alignment of chromosomes in cells with delocalized dynein, however, appeared disrupted and disorganized (Fig. 3B). Thus, stable NIH 3T3 cells were analyzed to determine the percentage of disorganized metaphases in the presence or absence of HPV16 E7.

In addition to wild-type HPV16 E7, the HPV16 E7Δ21-24 and HPV16 E7Δ79-83 mutants were also examined, as these represent mutants that differ in both their abilities to induce dynein delocalization and their abilities to associate with NuMA (Fig. 1) (26). The expression of wild-type HPV16 E7 resulted in a 1.8-fold increase in disorganized metaphases compared to the empty vector control (36.2% compared to 20.6%; $P = 0.04$ by a Student’s $t$ test), and the expression of the HPV16 E7Δ21-24 mutant resulted in a 1.7-fold increase (35.4% compared to 20.6%; $P = 0.05$) (Fig. 3C). The Δ79-83 mutant, however, showed only a 1.3-fold increase in abnormal metaphases (26.3% compared to 20.6%; $P = 0.18$). To ensure that this effect is not specific to NIH 3T3 cells, primary human foreskin fibroblasts with a stable expression of empty vector, wild-type HPV16 E7, HPV16 E7Δ21-24, and HPV16 E7Δ79-83 as well as hTERT-immortalized NOKs with or without HPV16 E7 expression (28) were examined. Similar to what was observed in stable NIH 3T3 cells, the stable expression of HPV16 E7 and HPV16 E7Δ21-24 in HFFs resulted in 1.7- and 1.5-fold increases in disorganized metaphases, respectively, compared to the empty vector control (39.7% [$P = 0.03$] and 36.8% [$P = 0.06$], respectively, compared to 24.0%), whereas
HPV16 E7Δ79-83 expression produced only a 1.1-fold increase (27.5% compared to 24.0%; \( P = 0.26 \)) (Fig. 3C). NOK E7 cells showed a 1.4-fold increase in disorganized metaphases compared to control NOK cells (58.3% compared to 43.1%; \( P = 0.0152 \)) (Fig. 3C). To demonstrate that this represents an immediate effect of E7 expression, HEK293 cells were transiently transfected with control vector, wild-type HPV16 E7, HPV16 E7Δ21-24, and HPV16 E7Δ79-83, and the percentages of disorganized metaphases were determined after 48 h. Here, wild-type HPV16 E7 and HPV16 E7Δ21-24 induced 2.0- and 1.9-fold increases, respectively, in disorganized metaphases (42.9% \( P = 0.04 \) and 45.9% \( P = 0.01 \), respectively, compared to 21.9%), whereas the HPV16 E7Δ79-83 mutant caused only a 1.2-fold increase compared to control vector-transfected cells (27.3% compared to 21.9%; \( P = 0.20 \)) (Fig. 3D). Therefore, the HPV16 E7Δ79-83 mutant that has a reduced capacity to cause dynein delocalization (26) and does not associate with NuMA is also defective in inducing disorganized metaphases.

**Low-risk HPV E7s associate with NuMA and increase the incidence of disorganized metaphases.** In order to determine if the association with NuMA and the correlation with altered mitoses were specific to high-risk HPV16 E7, we performed the above-mentioned experiments with low-risk HPV6b E7 and HPV11 E7. GST pull-downs using GST alone or GST fused to wild-type HPV6b E7, HPV11 E7, or HPV16 E7 were performed with HeLa whole-cell lysates. The HPV6b E7, HPV11 E7, and HPV16 E7 fusions each associated with NuMA at levels above background levels (Fig. 4A). Further-
more, the transient expression of HPV6b E7 and HPV11 E7 in HEK293 cells also resulted in a similar increase in the incidence of disorganized metaphases (46.11% \([P = 0.003]\) and 48.12% \([P = 0.002]\), respectively), as seen upon HPV16 E7 expression (41.33%; \(P = 0.026\)) compared to an empty vector control (28.6%) (Fig. 4B).

**HPV E7 expression results in a prometaphase delay during mitosis.** To determine whether the increase in disorganized metaphases was reflective of a prolonged prometaphase that still progressed toward an organized metaphase or whether these cells continued to be disorganized until anaphase occurred, it was important to observe individual cells as they proceeded through mitosis. Thus, we performed live-cell imaging of stable NIH 3T3 cells. After analyzing 3T3-CE7 cells, we did not observe an obvious population of cells with disorganized chromosomes immediately prior to anaphase. When 3T3-poz cells were compared to 3T3-CE7 cells, it became clear that the disorganized metaphases that we previously noted were cells in prometaphase and that the increased incidence of “disorganized metaphases” was a result of an extended prometaphase (Fig. 5A and B). A cumulative frequency plot of the length of mitosis (from nuclear breakdown to anaphase onset) for each cell line indicates that 3T3-CE7 cells have a statistically significant mitotic delay; the average length of mitosis for 3T3-CE7 cells is 23.58 min, compared to 19.83 min for 3T3-poz cells \((P < 0.0001)\) (Fig. 5B). This 19% lengthening accounts for an increased population of prometaphase cells under fixed conditions and therefore suggests that unlike cells expressing HPV6b E7, HPV11 E7, HPV16 E7, or HPV16E7Δ21-24, cells expressing HPV16 E7Δ79-83 do not acquire an appreciable mitotic delay.

**FIG. 4.** Low-risk HPV E7 proteins associate with NuMA and induce disorganized metaphases. (A) Western blot analysis of GST pull-down experiments using the indicated GST fusion proteins and HeLa whole-cell lysates (WCL). One hundred fifty micrograms of HeLa whole-cell lysates represents 5% of the input. The blots were probed for NuMA, pRB (positive control; the binding pattern is as expected), or GST. (B) Bar graph showing the percentage of disorganized (Disorg.) metaphases in HEK293 cells transiently transfected with the indicated E7 expression plasmids. The results represent averages from three independent experiments where >150 cells were counted per experiment. Error bars indicate the standard errors between experiments.

**FIG. 5.** Expression of HPV16 E7 results in a mitotic delay. (A) Time lapse images of a representative cell going through normal mitosis. DNA was visualized via the expression of green fluorescent protein-histone 2B. Each image was taken after a 6-min interval. (B) Time lapse images of a representative cell going through prolonged mitosis. (C) Cumulative frequency plots of the lengths of mitoses in 3T3-poz cells (diamonds) or in 3T3-CE7 cells (squares). The tables to the right show the numerical values of the cumulative frequency plot, where “minutes” signifies the time to reach anaphase.
We previously showed that the expression of HPV16 E7 leads to the delocalization of dynein from mitotic spindles (26). To determine the mechanistic basis for this observation, we performed large-scale GST pull-down experiments followed by mass spectrometry and identified an association between HPV16 E7 and NuMA that was validated by coimmunoprecipitation experiments. NuMA travels to mitotic spindle poles at the onset of mitosis via dynein/dynactin-mediated transport and is responsible for forming and stabilizing spindle microtubules (15). Additionally, NuMA is not merely a cargo protein of the dynein/dynactin complex, but its microtubule binding domains may also make it an accessory to the motor complex (reviewed in reference 31). Accordingly, spindle poles are dramatically altered upon the immunodepletion of NuMA or upon a disruption of either dynein or dynactin individually, and the microinjection of NuMA antibody into cells results in disorganized metaphases that are similar to those which we observed previously in HPV16 E7-expressing cells (8). We thus hypothesized that the association between NuMA and HPV16 E7 was responsible for the increased incidence of dynein de-localization during mitosis. In agreement with this notion, we found that, similar to the dynein delocalization phenotype, the interaction between NuMA and HPV16 E7 mapped to carboxy-terminal HPV16 E7 sequences, which are distinct from the region of HPV16 E7 necessary for HPV16 E7-mediated centrosome overduplication and the disruption of γ-tubulin dynamics (4, 25, 26). Furthermore, it appears that HPV16 E7 and dynein both preferentially associate with the slower-migrating form(s) of NuMA. When a GST-HPV16 E7 pull-down was compared with a dynein IP on a Western blot, similar levels of NuMA were detected, but no dynein signal was detected in the GST-HPV16 E7 pull-down. Hence, we hypothesize that HPV16 E7 associates with a distinct pool of NuMA that is not associated with dynein, although we are admittedly limited by the level of detection that this assay affords us, and that the association between HPV16 E7 and NuMA may interfere with and/or compete with the association between dynein and NuMA. Nevertheless, IPs with NuMA and dynein in HPV16 E7-expressing cells coprecipitated dynein and NuMA, respectively, suggesting that NuMA/dynein complexes are not completely disrupted upon E7 expression cells (data not shown).

Because dynein is responsible for localizing NuMA to microtubules and, conversely, the microtubule binding sites on NuMA may be important in stabilizing dynein on microtubules, it is possible that the disruption of the NuMA/dynein complex could essentially delocalize both proteins. This concept is supported by our observation that in many mitotic cells where dynein is delocalized from mitotic spindles, we also noticed a delocalization of NuMA from mitotic spindles and that, in these cases, NuMA and dynein localization do not overlap. It is important to note that when control mitotic cells with delocalized dynein, of which there is a small population, are compared to HPV16 E7-expressing mitotic cells, the frequencies of delocalized NuMA within each specific population are approximately the same. Nevertheless, the overall incidence of NuMA delocalization is increased, and therefore, it remains unclear whether the increased incidence of delocalized dynein is a result of HPV16 E7 disrupting the localization of NuMA. Ultimately, our observations lead us to propose a model whereby the interaction between HPV16 E7 and a subpopulation of NuMA renders that subpopulation “inactive” and unable to associate with its usual binding partners (Fig. 6). Since NuMA is dynamically exchanged between soluble and microtubule-based pools (15), we believe that the HPV E7/NuMA interaction does not result in a global disruption of NuMA-dependent mitotic processes but rather results in a decreased rate of efficiency, as “inactive” HPV E7-bound NuMA must exchange with a non-HPV16 E7-bound “active” NuMA in order to perform its normal microtubule-based functions. Therefore, during HPV E7 expression, spindle pole formation would occur more slowly, and other processes that involve NuMA, such as chromosome alignment, would be less efficient (Fig. 6). Consistent with such a model, we observed through live-cell imaging that HPV E7-expressing cells acquire a mitotic delay. Importantly, however, our data do not rule out the possibility that E7 may also associate with NuMA during interphase. The biological sequelae of such an association, however, would be difficult to predict, as the nuclear activities of NuMA have not been studied in much detail (reviewed in reference 31).

The ability of low-risk HPV E7 proteins to associate with NuMA and deregulate mitosis as well as the fact that the observed “disorganized metaphases” eventually progress into apparently “normal” metaphases appear to negate the possibility that these events may contribute to genomic instability and HPV-mediated carcinogenesis. Nevertheless, it remains possible that defects in spindle stability and/or chromosome alignment may contribute to aneuploidy when occurring in concert with other activities specific to high-risk HPV oncoproteins. One can envision that abrogated chromosome align-
ment could result in chromosome missegregation if a cell were to have a defective mitotic spindle assembly checkpoint. In fact, high-risk HPV E6 and E7 oncoproteins have been reported to interfere with multiple mitotic cell cycle checkpoints including the spindle assembly checkpoint (32, 33). The spindle assembly checkpoint component Mad2 is aberrantly expressed in cells with inactivated pRb (10), and thus, high-risk HPV E7 expression may compromise the vigilance of this checkpoint through the inactivation of pRb. Supporting this notion, we detected an increased rate of chromosome missegregation in cells expressing HPV16 E7 in the live-cell-imaging experiments, where lagging chromosomes were detected in 28 out of 200 cells containing HPV16 E7, while only 4 out of 200 control cells contained lagging chromosomes (data not shown). Thus, this newly described function of HPV E7 may contribute to the destabilization of the host genome and to malignant progression.

Because low-risk HPV6b E7 and HPV11 E7, in addition to high-risk HPV16 E7, each associate with NuMA and induce a prometaphase delay, it would appear that this association and/or function may be integral to the viral life cycle. One intriguing hypothesis is that the disruption of dynein and NuMA could be beneficial to viral replication via the disruption of cell polarity; in this situation, the observed mitotic delay may represent an obligatory, yet unintended consequence of abrogating the multiple functions of these mitotic proteins. As mentioned above, basal cells must divide in an asymmetric fashion, producing a committed suprabasal cell and a proliferative basal cell. NuMA has been implicated in the establishment of cell polarity that is necessary for the proper differentiation of mammalian skin (16). Dynein may be an important motor for this process, as it was shown that in Drosophila follicle cells, dynein regulates epithelial polarity (11, 18). Therefore, the disruption of the NuMA/dynein network by HPV E7 may cause a defect in cell polarity that supports viral maintenance and reproduction. The production of two proliferative basal cells as a result of deregulated cell polarity would increase the number of cells that could maintain viral episomes and that could further proliferate to yield a larger pool of basal cells capable of producing and maintaining viral genomes. In agreement, it was shown that HPV16 E7 was necessary to perturb the differentiation of keratinocytes in the context of the viral life cycle, and this appeared to be important for viral DNA amplification and efficient viral production (5). Interestingly, several Drosophila proteins involved in the establishment of cell polarity, including Stardust and DmPar-6, contain PDZ domains (14), and the human homologs would therefore likely be targeted by high-risk HPV E6 proteins. Hence, HPV E6 and E7 may cooperate to disrupt cell polarity in order to interfere with cellular differentiation. It will be important to determine whether asymmetric cell division is disrupted in HPV E6- and E7-expressing cells and whether altered cell polarity is necessary for efficient viral persistence. Moreover, we are excited to further investigate the connection between deregulated basal cell polarity and the establishment of HPV-associated “cancer stem cells”.

In short, we have identified an association between HPV E7 and NuMA that correlates with an increased incidence of disqualified metaphases that represent a prometaphase delay. These discoveries are the foundation for future studies regarding mechanisms by which HPVs alter cellular programs in order to persist within the host. Additionally, experiments examining how these events contribute to genomic instability will give us insights into how the mechanisms that benefit the virus may ultimately become a detriment to the host.

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