The E7 Protein of Cutaneous Human Papillomavirus Type 8 Causes Invasion of Human Keratinocytes into the Dermis in Organotypic Cultures of Skin

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Abstract

Human papillomaviruses (HPV) have been implicated in the development of nonmelanoma skin cancer (NMSC). The molecular mechanisms by which these viruses contribute towards NMSC are poorly understood. We have used an in vitro skin-equivalent model generated by transducing primary adult human epidermal keratinocytes with retroviruses expressing HPV genes to investigate the mechanisms of viral transformation. In this model, keratinocytes expressing HPV genes are seeded onto a mesenchyme composed of deep-dermalized human dermis that had been repopulated with primary dermal fibroblasts. Expression of the HPV8 E7 gene caused both an enhancement of terminal differentiation and hyperproliferation, but most strikingly, the acquisition of the ability to migrate and invade through the underlying dermis. The basement membrane integrity was disrupted in a time-dependent manner in areas of invading keratinocytes, as evidenced by immunostaining of its protein components collagen types VII, IV, and laminin 5. This was accompanied by the overexpression of extracellular matrix metalloproteinases MMP-1, MMP-8, and MT-1-MMP. These results suggest that the cutaneous HPV type 8 that is frequently found in NMSC of epidermodysplasia verruciformis patients may actively promote an invasive keratinocyte phenotype. These findings also highlight the importance of epithelial-extracellular matrix-mesenchymal interactions that are required to support cell invasion. (Cancer Res 2005; 65(6): 2216-23)

Introduction

Nonmelanoma skin cancer (NMSC) is the most common human cancer in the Caucasian population, with about one million cases in the United States and >40,000 cases occurring annually in the United Kingdom (1, 2). Many epidemiologic studies have shown that the incidence of NMSC has been increasing rapidly over the last decades, and at present, it represents about 30% of all cancer incidence (3). Basal cell carcinoma is the most common NMSC, whereas squamous cell carcinoma (SCC) accounts for 20% of all cutaneous malignancies.

Human papillomaviruses (HPV) are small DNA viruses that induce epithelial hyperproliferation that extends from benign to premalignant and malignant lesions of the cutaneous and mucosal epithelia (4). The association between HPV and skin cancer was first identified in patients with the rare inherited disorder epidermodysplasia verruciformis (EV; ref. 5). These individuals have a predisposition to infection with a specific group of HPV types (EV types) and cancers frequently harbor the oncogenic HPV types 5 or 8 (6). Whereas EV patients are very rare, recent epidemiologic studies have shown that about 30% of NMSC in immunocompetent individuals contain HPV DNA that invariably seems to be EV HPV types. These findings suggest a previously uncharacterized role for EV HPV types in NMSC development in immunocompetent individuals (7, 8).

The two main viral oncoproteins of anogenital HPVs, E6 and E7, display different functions towards the development of cancer, such as cellular transformation, cell death inhibition and immortalization. The major target of the E6 protein on high-risk mucosal HPVs is p53, whose functions are disrupted after its E6-promoted proteolysis (9). The E7 protein functions in cellular transformation by interaction principally with pRb (10) and other cellular targets (for a review, see ref. 11). In vivo, HPV E7 can uncouple cellular differentiation and proliferation and hence, retain differentiating keratinocytes in a DNA replication competent stage. E7 from cervical HPVs can immortalize human foreskin keratinocytes in vitro, alone or in cooperation with E6. However, little is known about the abilities of the cutaneous HPV oncogenes in cellular transformation or immortalization. The E7 genes of cutaneous skin cancer-associated HPV types 8 and 47 failed to induce any detectable transformation of rodent cells (11, 12). In collaboration with the activated Ha-ras gene, however, HPV5 and HPV8 gave rise to transformed cell lines (13). The E7 of the plantar wart-specific HPV1 (a low-risk papillomavirus) fully transformed the mouse fibroblast cell line C127 (14). In the case of cutaneous HPVs, the degree of morphologic transformation by E7 genes therefore seems not correlated to the risk of malignant conversion of the lesions induced by the corresponding HPVs. Little work has been done on cutaneous HPVs with the natural target cell of the virus, the adult epidermal keratinocyte. This may have limited the findings of previous studies investigating the functional activities of early genes. In this sense, it has been recently shown that the E6 and E7 genes of HPV38, a cutaneous EV type, can transform and induce a long-lasting proliferation of primary human keratinocytes (15).

The basement membrane (BM) is a specialized form of extracellular matrix (ECM) that is mainly composed of collagen, nidogen, laminins, and perlecans, that separates epithelial cells from the underlying supporting stroma. In tumor development, epithelial cells disrupt the BM, proliferate, and migrate within the connective tissues. The invasive character is facilitated by the expression of specific extracellular matrix metalloproteinases...
EcoKeratinocytes at passage 1 were seeded out in defined Retroviruses. time retrovirus-containing cellular supernatants were collected. days after transfection, cells were plated into selection media containing 500 transfecting pLXSN-derived DNA into PT67 cells with Superfect reagent keratinocyte serum-free medium (Invitrogen) at a cell density of 9 pLXSN-8E6E7. The E7 open reading frame alone was amplified by PCR by frames was inserted into pLSXN treated with BamI, and the DNA fragment containing the E6 and E7 open reading frames was inserted into plLXSN treated with BamHI and XhoI, to obtain plLXSN-8E6E7. The E7 open reading frame was amplified by PCR by using the primers 5'-AAGCTTTGGATTGAGCTCTGAAC-3' and 5'-GCGACACTGGGACCTGTACATG-3', which contain EcoRI and BamHI restriction endonuclease sites at their 5 ends, respectively. After PCR, the amplicons were digested and subsequently cloned into BamHI/EcoRI digested-pLXSN, thus obtaining plLXSN-8E7. The HPV8 genes were cloned downstream of the Moloney murine leukemia virus 5' long terminal repeat sequence. Recombinant retroviruses were produced by transfecting plLXSN-derived DNA into PT67 cells with Superfect reagent (Qiagen, Hilden, Germany), following the manufacturer's indications. Two days after transfection, cells were plated into selection media containing 300 µg/mL G418 for 3 days. Resistant cells were grown to confluence at which time retrovirus-containing cellular supernatants were collected.

Infection of Human Cutaneous Keratinocytes with Recombinant Retroviruses. Keratinocytes at passage 1 were seeded out in defined keratinocyte serum-free medium (Invitrogen) at a cell density of 9 × 10⁴ cells/cm² in 6 cm dishes. Retroviral supernatants were mixed with an equal volume of DMEM in the presence of 5 µg/mL of hexadimethrine bromide (polybrene, Sigma, Poole, United Kingdom) and added to the keratinocytes. Spin infection was made by centrifugation for 1 hour at 300 × g, and the cells washed with PBS and the cultured in defined keratinocyte-serum-free medium. After 2 days, cells were selected with G418 (500 µg/mL) for 3 days after which time only infected keratinocytes survived. The cultures were trypsinized before reaching confluence and were used immediately in organotypic cultures as described below. The use of pooled stable cell populations minimizes possible variations due to the apparent randomness of the viral integration site in the cellular chromosomes.

Materials and Methods

Cell Culture. Cell cultures were incubated at 37°C in a humidified 10% CO₂ atmosphere. The NIH 3T3 mouse fibroblast line PT67 (Clontech, Heidelberg, Germany), which was used to replicate amphotropic retroviruses, and NIH 3T3 cells were maintained in DMEM, supplemented with 10% FCS and antibiotics. Human dermal fibroblasts and epidermal keratinocytes were isolated from discarded abdominal skin obtained from plastic surgery. Briefly, thin sheets of skin were removed by using a dermatome and digested with trypsin. Primary human keratinocytes were isolated, propagated on lethally irradiated NIH 3T3 feeder cells, and grown in keratinocyte culture media composed of three parts DMEM and one part Ham's F12 with 10% FCS and supplements as described (16). For the liberation of fibroblasts, skin was enzymatically digested in collagenase D (Roche, Lewes, United Kingdom) and cells were passaged in DMEM supplemented with 10% FCS and antibiotics. Before reaching confluency, cells were trypsinized, resuspended in FCS with 10% DMSO and stored in liquid nitrogen.

HPV Expression Vectors and Production of High Titer Retroviruses. The Moloney murine leukemia retrovirus vector pLXSN (17) was used to generate recombinant retroviruses containing HPV8 genes. This vector contains a gene conferring resistance to neomycin, which is transcribed from a SV40 promoter. E6 and E7 open reading frames were amplified together by using the primers 5'-TTACAATGCTGTGACTTGTGCAAT-3' and 5'-GAAGCTTCTTTAGATGTACTACC-3' that contain Bgl II and Xho I restriction endonuclease sites at their 5' ends, respectively. The Moloney murine leukemia retrovirus vector pLXSN (17) was used to replicate amphotropic retroviruses, and NIH 3T3 cells were maintained in DMEM, supplemented with 10% FCS and antibiotics. For the liberation of fibroblasts, skin was enzymatically digested in collagenase D (Roche, Lewes, United Kingdom) and cells were passaged in DMEM supplemented with 10% FCS and antibiotics. Before reaching confluency, cells were trypsinized, resuspended in FCS with 10% DMSO and stored in liquid nitrogen.

Results

The organotypic culture of skin is a useful system for the in vitro analysis of skin biology because it can mimic keratinocyte differentiation more effectively than normal monolayer cultures. Cellular functions that require epithelial differentiation, cell-ECM interactions, or keratinocyte-fibroblast paracrine communications can be observed and analyzed. To study the effect of cutaneous HPV gene expression on epithelial cells, we used the organotypic system of skin with retrovirally transduced normal human epidermal keratinocytes.

HPV8 Early Genes Disrupt the Normal Differentiation and Proliferation Programs of Keratinocytes in Regenerated Epithelium. Human keratinocytes were infected with HPV8 E6E7- and HPV8 E7-containing retrovirus and control empty retrovirus pLXSN and seeded onto deepidermalized dermis repopulated with human fibroblast as described in Materials and Methods. After 14 days in the air-liquid interface, conditions that induce epidermal differentiation, the organotypic cultures were paraffin-embedded and 4-µm sections were H&E-stained for histologic examination. Cells infected with the pLXSN-retrovirus...
generate a normal epithelium with the distinct strata of keratinocyte differentiation over the dermis (Fig. 1). The result shows that the events driving the generation of a normal epithelium are occurring. By contrast, distinct features are observed on E6E7 or E7 cells. These cultures retain the capacity to undergo terminal differentiation. However, when compared with control cultures generated by transducing keratinocytes with empty retrovirus, the organotypic cultures of HPV8 E7–transduced cells show features of hyperkeratinization across the epithelium. The increased cornification was observed not only in the outer epithelial cells, but also within the epithelium itself, leading to the formation of horn pearls (Fig. 1A), features that were commonly detected in the E7-transduced cultures that were not apparent in controls. These structures are similar to concentrically keratinized structures present in well-differentiated spontaneous human skin SCC (20), in severe combined immunodeficient mice transplanted with EV-associated SCC (21), and in skin cancer induced in K14-HPV16 transgenic mice (22).

**Figure 1.** Expression of early HPV8 genes causes invasion of keratinocytes into the dermis. A, normal human epidermal keratinocytes were infected with pLXSN (Control), pLXSN-8E6E7, or pLXSN-8E7 containing retroviruses and used in organotypic cultures as described in Materials and Methods. After 14 days in the air-liquid interface, cultures were paraffin-embedded and sections were H&E stained for histologic examination. H, horn pearl-like structure of highly cornified keratinocytes; D, areas of dermis surrounded by keratinocytes; I, infiltrating keratinocytes; F, fibroblasts. B, vimentin staining indicating that the dermal cells in the HPV8 E7 or E6/E7 cultures are fibroblasts (arrow).
Figure 2. PCNA expression in raft cultures. Sections of organotypic cultures of pLXSN (Control), pLXSN-8E7, or pLXSN-8E6/E7-transduced keratinocytes were stained using an antibody to PCNA (green) as described in Materials and Methods. Note the abundant PCNA staining in the HPV8 cultures in multiple cell layers, PCNA expressing in control cells is limited to basal cells. Propidium iodide staining of nuclei (red). Merged images, nuclei of cells expressing PCNA (yellow).

More importantly, H&E-stained sections of cells transduced either with both E6 and E7 genes, or only with the E7 gene, revealed a striking phenotype, in that the cells seem to have lost their normal polarity and invaded into the dermal matrix (Fig. 1A). We noted that, especially on E7-transduced keratinocytes, instead of keratinocytes migrating upwards as in a normal epithelium, irregular masses of epidermal cells proliferate down into the dermis. Islands of dermis surrounded by keratinocytes and keratinocytes deep within the dermis were also observed. In contrast to the epithelium derived from control cells where the rete ridges are of equal length, in this study we observed that those of the epithelium generated by HPV8-transduced cells were more heterogeneous in length. Together these observations suggest that the HPV8-transduced cells, when compared with controls, have acquired the ability to invade surrounding tissues.

Another feature of the regenerated skin produced from HPV8-transduced keratinocytes in this system, when compared with cultures generated by control retrovirally infected keratinocytes, was the presence of fibroblasts in the dermis (Fig. 1A). The identity of these cells was confirmed by positive staining for vimentin (Fig. 1B). As these fibroblasts were initially seeded onto the reticular side of the deepidermized dermis, their presence in the main body of the dermis indicates an increased migratory capacity, possibly as a result of soluble factors produced by the HPV8-transduced keratinocytes that in turn may also influence keratinocyte proliferation and migration.

In normal epithelium proliferating cells are restricted to basal cell layers. To analyze whether E7 expression resulted in a hyperproliferative phenotype we investigated the expression pattern of proliferating cell nuclear antigen (PCNA) in the organotypic cultures. In control cultures, PCNA expression was, as expected, restricted to basal cells. In marked contrast, increased expression of PCNA was evident in both the HPV8 E7 or HPV8 E6/E7 epithelium. Here PCNA expression was found not only in basal cells but also in suprabasal layers and was most evident in the areas of invading keratinocytes. In addition, HPV-transduced cells also showed some cytoplasmic staining of PCNA suggesting that the expression of the viral genes may alter the cellular localization of the protein (Fig. 2).

The Basement Membrane Integrity Is Disrupted by HPV8 E7–Transduced Keratinocytes in Organotypic Cultures. The BM separates the epithelial and dermal compartments and, as such, represents the initial barrier to tumor cell invasion. Mechanistically, disruption of the BM is a necessary event to allow the migrating cells to invade the dermal compartment. To investigate whether the BM was disrupted in E7-transduced cells, the presence of collagen VII was analyzed by immunohistochemistry. Collagen VII is a component of BM that is still present, structurally intact and forms a continuous layer in the acellular dermis used in the organotypic cultures (23). Degradation of the BM is a marker for early tumor growth (24). In the system used here, the preparation of the dermal substrate leaves the integrity of the BM intact. In contrast to the continuous distribution pattern of the protein along the BM in control cultures (Fig. 3A), collagen VII staining is lost in areas in which E7-transduced keratinocytes are growing downward into the dermis. Interestingly, prolonging the incubation time at the air-liquid interface of the E7-transduced cultures to 21 days, revealed a progressive loss of collagen VII staining of the BM, such that collagen VII-specific staining at the BM was then completely absent. Because collagen VII can normally be synthesized by the keratinocytes under these culture conditions (25), the lack of expression in the HPV-transduced cultures may be due to inhibition of protein synthesis or the active degradation of the protein. We further probed whether the BM function was compromised by investigating the distribution patterns of two other important BM components, collagen IV and laminin V. Both these markers were normally expressed in control cells at both 14 and 21 days. However, as with collagen VII, the intensity of both collagen IV and laminin V staining of the BM zone at the epithelial/dermal juncture was found to be lower at 14 days, and was further reduced by 21 days, in the HPV8 cultures (Fig. 3B and C). In contrast, collagen IV staining of the remnants BM that surrounded dermal blood vessels was evident at both 14 and 21 days (Fig. 3B). Taken together, these findings indicate that expression of HPV8 E7 leads to the progressive loss of BM integrity.
Increased Expression of MMP1, MMP8, and MT1-MMP in HPV8 E7-Transduced Keratinocytes in Skin Cultures. The migration of keratinocytes through the disruption of the BM and ECM are processes that depend on the activity of MMPs and other degrading proteases. Having observed that the E7-transduced keratinocytes lacked collagen VII, collagen IV, and laminin V expression at the BM, we then went to investigate whether the E7-transduced cells had increased expression of specific MMPs that could account for the disrupted BM staining pattern. We were especially interested in the expression of MMP-1, MMP-8, and

![BM disruption by invading keratinocytes.](image)

**Figure 3.** BM disruption by invading keratinocytes. The integrity of BM was analyzed by immunostaining of key components collagen VII, collagen IV, and laminin V. A, control or HPV8-transduced keratinocytes were used in organotypic cultures and incubated in the air-liquid interface for either 14 or 21 days. After 14 days, collagen VII staining is interrupted in the HPV8E7 cultures compared with controls and is completely absent by 21 days in culture. B, progressive loss of collagen IV immunoreactivity at the BM zone. Note that collagen IV expression is retained on the remnants of blood vessels in the dermis. C, laminin V expression is reduced at 14 days and absent in 21-day organotypic cultures of HPV8-transduced keratinocytes.
MT1-MMP, as they are known to be involved in skin diseases (26). Immunostaining of the organotypic cultures with MMP antibodies shows increased expression of specific MMPs in cultures of E7-expressing keratinocytes when compared with controls. Interestingly, MMP-1 (collagenase-1) was present in the dermis and seemed to be concentrated at the dermal-epidermal interphase (Fig. 4). Whether this represents the site of synthesis or results from binding or retention of the protein by other ECM components is at present unknown. The MMP-1 expression was notably increased in dermis and mainly located along a line between the migrating cells and the dermis. MMP-1 staining is also observed in the epithelium of both control and the E7 cultures. In contrast, overexpression of MMP-8 (collagenase-2) is observed also in the dermis (Fig. 4). Finally, membrane-associated MMP (MT1-MMP) was up-regulated on E7 cultures in the epithelial compartment when compared with control cultures (Fig. 4). No expression of MT1-MMP was observed in the dermis of either control or E7-transduced cells. These findings indicate that the E7 protein of HPV8 promotes the expression of different MMPs in separate skin compartments that can co-operate in the E7-induced keratinocyte phenotype and invasion through the dermis. This induction of MMP expression, rather than an inhibition of gene expression, most likely accounts for the lack of BM components of the HPV-transduced keratinocyte cultures.

Discussion
The key molecular changes responsible for the acquisition of an invasive phenotype by tumor cells are not well understood. The investigation and characterization of the cellular changes that result in the ability of the cells to invade surrounding tissues and migrate to distant body sites will yield new insights into tumor metastasis. Our findings that the HPV8 E7 gene alone is capable of generating an invasive phenotype when expressed in epidermal keratinocytes not only provides the first evidence supporting a direct role for the virus in NMSC development but also serves as a general model to study epidermal invasion.

The E6 and E7 oncoproteins of mucosal HPV (such as HPV16 and HPV18) display cellular transformation and immortalization activities in vitro. Molecular interactions between HPV16 E6/E7 and cellular proteins have been described that could explain some of their functions towards cancer development. However, there is a paucity of information about the potential role of cutaneous HPV proteins in skin cancer development. It is very probable that the simple model systems employed to characterize the transforming potential of anogenital viruses are inappropriate for the study of cutaneous HPVs. Different HPV types are associated with the development of lesions at particular body sites. This tropism implies that the viral life cycle has a requirement for specific cellular factors present only in keratinocytes derived from that particular body site, and in addition may require additional cues from mesenchymal cells. The dependence of the viral life cycle on cellular differentiation together with the difficulty of generating a fully differentiated stratified epithelium in vitro has hampered the investigation of cutaneous HPV gene function. Considerable progress has been made in functional analysis of HPVs in organotypic cultures using collagen as matrix (27). Previously, such culture analysis has been made using foreskin keratinocytes expressing the E6 and E7 genes of several EV HPV types and of the high-risk mucosal type HPV16 (28). However, no invasive phenotype of the epithelial cells was described in any of the HPV types analyzed. It should be noted however that organotypic cultures using collagen as matrix do not closely mimic an in vivo environment, as they do not maintain the structural integrity of normal skin together and lack ECM proteins, including functionally important molecules such as glycosaminoglycans. These characteristics are fundamental for in vitro models investigating epithelial-mesenchymal interactions (18). The system used in this paper

Figure 4. HPV8 E7 protein upregulates the expression of MMP-1, MMP-8, and MT1-MMP. Sections of control or HPV8-derived organotypic cultures were immunostained with specific antibodies to MMP-1, MMP-8, and MT1-MMP, as described in Materials and Methods (green).
emulates epidermal regeneration more closely through the in vitro culture of keratinocytes onto an acellular dermal substrate repopulated with dermal fibroblasts, with subsequent differentiation of the culture taking place at the air-liquid interface (18, 29).

We have used this culture system to investigate HPV8 early gene function in primary keratinocytes. Most significantly, we show that HPV8 E7 promotes a tumorigenic phenotype as evidenced by the invasive behavior of the HPV-transduced keratinocytes. Migration of the keratinocytes downward into dermis was facilitated by the degradation of components of the BM and ECM (collagen VII, collagen IV, and laminin V) through the induction of expression of the extracellular proteinases MMP-1, MMP-8, and MT1-MMP. Most interestingly, these in vitro findings are in accord with similar observations in skin cancer, thereby supporting a direct role of the E7 protein of cutaneous HPV8 in NMSC development. We would hypothecize that other viral types closely linked to cancer development may also share the phenotype produced by expression of HPV8 genes. Our studies suggest that both the target keratinocyte, in addition to the cellular and acellular mesenchymal components of the organotypic system itself, are critical in eliciting the invasive behavior. Increased proliferation was shown by increased PCNA expression in suprabasal cell layers, which is in agreement with Boxman et al. (28), who also described PCNA staining in suprabasal keratinocytes in cultures containing E6 and E7 genes of EV HPVs. Additionally, HPV8 E7 alters the normal differentiation program of the cells resulting in hyperkeratosis and horn pearl formation. These features are also described in SCC of EV and immunocompromised patients and further suggest that HPV8 early genes E6 and E7 can alter the normal homeostasis of the keratinocyte.

Invasion of malignantly transformed cells is a complex, sequential multistage process that involves the controlled degra-
dation of structural barriers such as basement membrane and collagenous ECM and migration of cells through the degraded matrix. Collagen VII degradation is an early event observed in NMSC progression and is used as marker for early invasion. Degradation of collagen VII is observed in E7 cultures (Fig. 3A), most strikingly after prolonging the air-liquid interface incubation time from 14 to 21 days. The decreased levels of collagen IV and laminin V in the HPV8 cultures provided further evidence that BM integrity was compromised.

Immunostaining of the organotypic cultures with MMP antibodies showed increased expression of MMP-1 and MMP-8 in the dermis and MT1-MMP in the epidermis, suggesting that the activity of different MMPs in separated compartments can cooperate in the movement of transduced keratinocytes through the dermis. Because the organotypic culture is a biccular system in which normal human dermal fibroblasts seeded on the reticular side of the dermis are cocultivated with the keratinocytes, both cell types can contribute to the expression of MMP-1 and MMP-8 located in the dermis. The apparent increase in the number of fibroblasts in the dermis of the HPV8 cultures may facilitate this process. The observations in distribution of expression of MMP-1, MMP-8, and MT1-MMP are consistent with previous findings in tissue samples of basal cell carcinoma and SCC (ref. 30 and references therein; ref. 31). In addition, up-regulation of these MMPs has also been described in SCC lines (31–33), and their role in keratinocyte invasiveness showed. Interestingly, microarray analyses of other HPV associated cancers, such as cervical cancer (34) that is typically associated with HPV types 16 and 18 and cell lines that express specific HPV16 genes (35), have also shown increased expression of MMPs and ECM remodeling proteins. Enhanced MMP-1 production also occurs in other skin conditions where homeostasis is perturbed, such as psoriasis where EV HPV types have been detected (36, 37), or exposure to UV light (38), a well-known NMSC cocarcinogen. The subset of MMPs induced by HPV gene expression supports a model in which native dermal collagen is degraded by the collagenases MMP-1 and MMP-8, especially collagen VII, the main component of the BM of the skin. Subsequently MT1-MMP can degrade gelatin (product of the enzymatic digestion of collagen), as well as other components of the dermis (39), allowing the epithelial cells to migrate in the dermal stroma (40). Interestingly, MT1-MMP also has a direct role in the activation of MMP-2 (gelatinase-A) in the outer side of the cellular membrane. MMP-2 regulates cell migration and proliferation during cancer cell invasion and is also capable of degrading BM and ECM components (41). It cannot be discounted at present that the possible activation of MMP-2 in the external surface of HPV8 E7-expressing keratinocytes may contribute to the migration of the invading cells.

In summary, our findings suggest that specific HPV types may play a direct role in skin carcinogenesis. The development of this in vitro model of HPV-associated epithelial tumorigenesis will greatly facilitate the investigation and better understanding of the molecular mechanisms that underpin tumor invasion.

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References


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