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CASE REPORT

A Solitary Cutaneous Tumor with Distinct Areas of Verruca and Seborrheic Keratosis-like Lesion

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A single, exophytic, cutaneous tumor on the thigh of a 52-year-old man was examined by light microscopy, in situ hybridization and immunohistochemistry. It demonstrated distinct areas of verruca and of seborrheic keratosis-like morphology simultaneously. Focally, architectural abnormalities were noted in some deeper parts of the tumor, but there was no morphological evidence of malignancy. The patient has remained disease-free for two and a half years after surgery. Biotinylated full genomic DNA probes of HPV confirmed the presence of types 6/11 exclusively in the verrucous portion of the neoplasm. In the verrucous component p53 protein was overexpressed and, additionally, increased Ki-67 immunopositive signals were detected, being localized below the HPV-DNA-expressing spinous cells. (Pathology Oncology Research Vol 5, No 4, 320–323, 1999)

Keywords: HPV, seborrheic keratosis, skin, in situ hybridization

Introduction

Human papillomavirus (HPV) is the aetiologic agent of verrucae vulgaris (warts) and condylomata acuminata. The histologic features of HPV infection include pronounced epidermal hyperplasia, papillomatosis, acanthosis, parakeratosis, hypergranulosis and koilocytosis. We must bear in mind that koilocytes are inconsistent features even of anogenital warts and it is often necessary to detect HPV-DNA by current molecular techniques in order to verify the diagnosis. Recently, improvements in visualisation and localisation of HPV-DNA have been reported.[6] A diagnosis of an HPV-induced lesion can be made in the absence of koilocytes if other requisite features are present. As a result of a productive viral infection, epithelial cell proliferation is induced; so, markers of cell kinetics are of particular interest in such lesions. Moreover, the E6 oncoprotein of HPV is known to inactivate the control function on cell cycle exerted by p53 tumor suppressor protein in vitro by binding to p53 and thus facilitating the degradation of p53.[3] A limited number of HPV types has been associated with development of malignancy but the HPV types of typical cutaneous warts have no considerable oncogenic potential.

Seborrheic keratoses (SKs) are extremely common benign neoplasms and whether they can be concurrent with HPV-related lesions has given rise to much controversy. In several SKs, focal koilocytosis, a key finding in HPV-induced lesions has been supposed to be observed on meticulous histological examination and so, it has been claimed that a considerable percentage of SKs may be related to an HPV infection.[9] However, it is currently accepted that SKs (as well as benign verrucous acanthomas) are not associated with HPV-DNA even though certain of these lesions may share some clinicopathologic characteristics with warts. According to the constellation of findings proposed by Li and Ackerman,[5] SKs and HPV-lesions are distinguishable even by mere morphology. In any case, the availability of molecular biologic methods that permit probing of routinely processed lesions for specific DNA sequences, suggests a definite approach to the differentiation of a viral-associated from a nonviral lesion.

In this study we describe an uncommonly large benign solitary cutaneous neoplasm which simultaneously dis-

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Abbreviations: HPV: human papillomavirus, SK: seborrheic keratosis, ISH: in situ hybridization, ab: antibody

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played features of both a wart and a SK-like lesion, but in separate areas. To the best of our knowledge, such a coexistence within a single tumor mass has not so far been reported in the literature. Any possible relation between SK and HPV infection in a same lesion and whether or not the HPV infection could irritate or influence the SK are worthy of discussion.

Clinical History

A 52-year-old man presented with a four year history of a single cutaneous tumor located at the outer surface of the thigh, not near the anogenital region. After complete surgical excision, no recurrence or lymph node metastases were reported over the following two and a half years.

Material and Methods

The specimen was fixed in buffered formalin. For electron microscopy, tissues were obtained, minced into small pieces and postfixed in buffered 2% OsO_4. The samples were embedded in Epon. Ultrathin sections, post-stained with uranyl ace-tate and lead citrate, were examined by transmission electron microscope (Philips 300). Sections from paraffin-embedded material were routinely stained and examined by light microscopy.

In situ hybridization (ISH) was carried out on deparaffinized 4-µm sections mounted on organosilanized slides. After rehydration, digestion of the sections was performed with 0.1% protease type XXIV for 30 minutes at room temperature. Denaturation of the cellular DNA was carried out at 95°C for 10 minutes and hybridization at 37°C overnight. The detection system for visualization of the probe target hybrid involved antibiotin and the alkaline phosphatase anti-alkaline phosphatase complex. For HPV typing biotinylated DNA probes for types 1,2,4, 6/11, 7, 16/18 and 31/33/35 (Enzo Diagnostics) had been applied. As positive controls we used HPV-positive sections of condylomata acuminata and, as substitute controls, sections were treated similarly except that the probe was omitted in the hybridization mixture. Only strongly stained nuclei were interpreted as positive.

Additionally, the avidin-biotin-peroxidase complex system (Dako, Denmark) was used in a conventional immunohistochemical assay with microwave pretreatment. The DAKO primary antibodies (Abs) used were the rabbit anti-human Ki-67 antigen Ab and the DO-7 monoclonal mouse anti-human p53 protein Ab.The former antibody is useful for evaluating the growth fraction in tumors as it labels proliferating cells: in fact, it reacts with cells at all stages of the cell cycle (late G_1, S, M and G_2 phases) but not with cells in G_0 phase. The latter antibody reacts with the wild type and mutant type of the p53 protein and is useful for demonstrating accumulation of p53 protein.

Pathological findings

Grossly, the tumor was sessile, well-circumscribed, cauliflower-like and skin-coloured. It measured 9.5x8x3.5 cm and its surface was mainly verrucous and partially smooth.

Microscopically, two distinct morphological patterns were separately observed from area to area *(Figure 1).* In verrucous areas, epidermal papillomatous proliferations consisted predominantly of pale spinous cells *(Figure 1)* some of which were rarely spindle shaped and possessed pink cytoplasm; basaloid cells were rarely observed and, when observed, they were confined to the lower portion of the epidermis. Near the epidermis, dilated tortuous capillaries were noticed. A considerable number of cells of the stratum malpighii appeared vacuolated and had round, hyperchromatic nuclei *(Figure 1).* It is noteworthy that these koilocytes focally extended into the deeper portions of the stratum malpighii. Ultrastructurally, the number of nuclear bodies was increased and so was the number of perichromatin and interchromatin granules, suggesting the possibility of viral infection.[10] Unfortunately, clusters of viral particles could not be found in the examined material; electron microscopy actually seems to have limited value for the diagnosis of HPV since it detects viral particles in only a small proportion of what are typical HPV-related lesions by light microscopy.

In addition to the exophytic growth pattern, the tumor focally showed a tendency toward deep, penetrating growth, resulting in downward proliferations that compressed collagen bundles and pushed them aside. These few deep crypts were rarely filled with horny material and pus. The above findings raised the question of early malignant transformation. However, nuclear atypia and

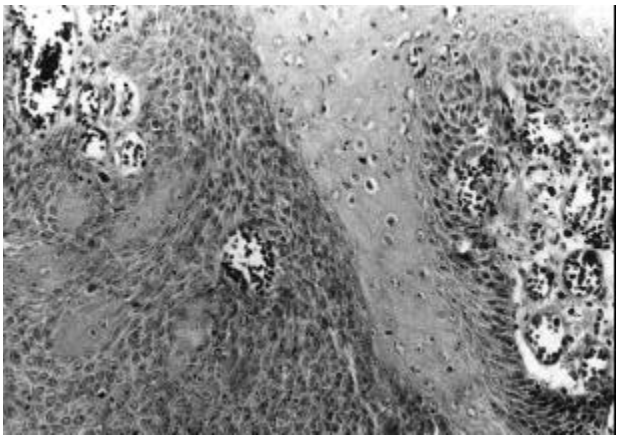

Figure 1. Transitional area with coexistence of both lesions' distinct patterns. On the right, koilocytosis is obvious and spinous cells clearly predominate (verrucous area). On the left, the predominance of basaloid cells and the formation of "squamous eddies" are evident (area of seborrheic keratosis-like lesion) (HE; x100).

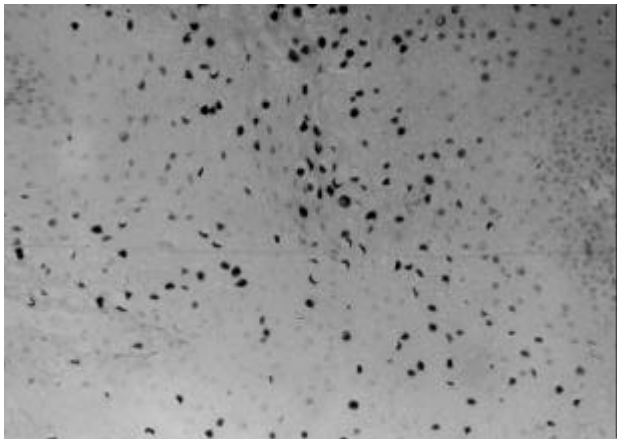

Figure 2. HPV 6/11 DNA sequences in nuclei of keratinocytes in the verrucous area of the tumor (antibiotin-alkaline phosphatase; nuclear fast red counterstain; x200).

individual cell keratinization were absent. Well differentiated invasive squamous cell carcinoma was ruled out because the cells showed an orderly arrangement and the border between the squamous proliferations and the dermis was sharp. The possibility of a verrucous carcinoma was also considered since the occurence of such carcinomas has been occasionally described in areas other than the genitoanal region.[2] Nevertheless, the present tumor lacked broad strands of invasion and, generally, there was not enough evidence for the diagnosis of a verrucous carcinoma.

Superficially, in verrucous areas, compact orthokeratosis was evident across the breadth of this portion of the tumor and subtle mounds of parakeratosis were observable at summits of papillations.

In areas of SK-like morphology, the epithelial proliferation consisted predominantly of basaloid cells *(Figure 1)* which seldom contained melanin and clearly outnumbered spinous cells; the latter were confined to positions beneath the cornified layer and around infundibular tunnels where "squamous eddies" were occasionally formed *(Figure 1).* Koilocytes were totally absent from these areas. Orthokeratosis was delicate and laminated. Dilated capillaries could be observed in the thickened papillary dermis. A mononuclear inflammatory infiltrate was seen in the dermis underlying both parts of the tumor.

ISH succeeded in proving the presence of HPV types 6/11 *(Figure 2).* Labelled nuclei were always restricted to the verrucous areas of the neoplasm and belonged to keratinocytes located mainly in the upper and partially in the lower epidermal layers. The former keratinocytes revealed either an advanced stage of differentiation or koilocytic features. Normal skin at the resection margins and the areas of SK-like morphology within the tumor were negative for the applied HPV-DNA probe mixture.

In verrucous areas, p53 immunolabelling was increased, at least by comparison with areas of SK and it was generally localized more basally in the epidermis than HPV-DNA, although p53-positive and HPV-positive keratinocytes tended to be located closely. In HPV-DNA positive areas, Ki-67 immunoexpression was found in the basal, parabasal and spinous cell layers; in the latter layer it was comparatively increased right below the levels where abundant HPV-DNA was present. In areas of SK-like morphology, no specific observations concerning the above immunomarkers' topographical or quantitative expression were made. Ki-67 immunopositivity was homogeneously distributed among basaloid cells and although p53 immunoreactive nuclei were detectable, their number was not enough to speak of p53 overexpression in these particular tumor areas.

Discussion

It has been argued that a large proportion of genital SK-like lesions, which do not fulfill the morphologic criteria of SKs completely, contain HPV-DNA sequencies[4] and that SKs of black South African patients with epidermodysplasia verruciformis -an uncommon presentation of HPV infection- also contain HPV-DNA sequences.[1] On the other hand, Zhu et al found no evidence of HPV-DNA in SKs obtained from nongenital regions.[4]

It is a fact that the present tumor is too large for conventional SK or verruca vulgaris. Nevertheless, some characteristic features of SK, as described by Li and Ackerman,[5] coexisted with typical features of cutaneous verruca at separate areas of this neoplasm; the term "collision tumor" might thus be attributed to this case. The two coexisting lesions might therefore be either incidental findings or related to each other. This coexistence of two morphologically distinct entities in one single mass could tempt us to speculate a common pathogenic process, perhaps occuring at early stages as concerns the evolution of SK. In any case, in the present tumor, viral cytopathic alterations were certainly absent from areas of SK-like morphology and HPV-DNA was undetectable by ISH in these areas. HPV-DNA was also absent in the tumor neighbouring normal epidermis: this negative finding is probably relevant to the absence of recurrence of the present tumor. HPV presence was strictly limited to verrucous areas of the tumor; HPV6/11 infection at the non-anogenital (non-condyloma) skin region (as observed in the present lesion) is actually very rare. Furthermore, HPV may have acted as an irritating factor to adjacent SK-like areas and this may be the reason for the formation of "squamous eddies" within the areas of the SK-like lesion. Based on its deep penetrating growth pattern, this SK-like lesion could be categorised in the inverted follicular type rather than in the acanthotic one. Interestingly, the former type is supposed to be a verruca vulgaris of the infundibulum caused by HPV. How-

ever, the lack of detection of HPV-DNA sequencies in areas of SK-like morphology might be due to the fact that HPV-DNA replication and transcription are accomplished almost exclusively in epithelial cells at an advanced stage of squamous differentiation, and such cells are generally rare in SKs. The above negative finding is in contrast with the hypothesis that genital or even nongenital SKs may be partially associated with an HPV infection.9 If strict morphological criteria are followed so that correct diagnoses of SKs are made, HPV will probably not be found present within cells of SKs of the anogenital or any other region;[5] in other words, verrucae (and condylomata acuminata) are the lesions that house HPV and not SKs. In summary, SK-like lesions containing HPV must be considered viral warts, pure and simple.[5] SK-like features may be seen in a variety of lesions including viral warts (and squamous cell carcinoma). The present case report potentially reinforces the above view.

In addition, our observations imply some degree of abnormal p53 overexpression focused on HPV-infected areas: p53 protein might thus be susceptible to aberrations in cells in the vicinity of productive HPV infection.[3] The E6 HPV oncoprotein functions by inactivation of cell cycle regulators and potentially provides the initial step in progression to malignancy. However, HPV types 6 and 11, detected in our specimen, are typically associated with little oncogenic potential, especially when found in lesions of the thigh[8] or other nongenital and nonperiungual cutaneous sites; so, malignant transformation of such HPV-related lesions must be considered as a rare event in immunocompetent human subjects. Nevertheless, since double and even triple infection is not uncommon for HPV infection, the possibility of the superimposition of other, high risk types, of HPV infection had to be excluded.

In HPV-positive areas, Ki-67 immunoexpression was evidently detectable but it was always located below the areas where HPV-DNA was found by ISH. In accordance with some previous data,[7] our finding supports the concept that HPV gene products may induce cell proliferation to facilitate the replication of the virus itself. We know that for HPV infection to take place, the virus must first gain access to the basal cells of the epidermis, which are the targets of the virus. HPV-DNA remains latent in the basal cells. Replication of HPV-DNA must rely on the DNA replication machinery of the host cells. HPV-DNA amplification tends to take place in the uppermost, spinous cell layers of the epidermis which have long since exited the cell cycle and therefore are logically expected to be Ki-67 immunonegative. The observed reactivation of Ki-67 synthesis in cells below and near those with high copy numbers of HPV-DNA implies that there is a mechanism in HPV to reactivate the host genes controlling proliferation in order to facilitate the replication of its own viral DNA. However, this interrelation seems to be independent of the oncogenic risk potential of the infecting HPV genotype.[7]

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