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Original Contribution

New biomarkers of human papillomavirus infection in epidermodysplasia verruciformis☆

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ABSTRACT

The cause of epidermodysplasia verruciformis is infection by human papillomavirus, usually types 5 or 8, and it exhibits a high potential for malignant transformation. The diagnostic histologic features of epidermodysplasia verruciformis are not always present and can be mimicked by non-viral diseases. The purpose of this study was to interrogate such lesions for new potential biomarkers to aid in the diagnostic accuracy. HPV DNA was high copy and localized to the upper half of the lesion in cells with cytologic features that included perinuclear halos, blue-grey cytoplasm, and hyper/parakeratosis. Serial section analyses demonstrated that there was increased expression of importin-β, exportin-5, Mcl1, p16, Ki67 and PDL1 in 13/13 epidermodysplasia verruciformis lesions. Each of these proteins localized primarily to the less differentiated cells in the parabasal aspect of the lesion. Only Ki67 and exportin-5 were expressed in the normal epithelia, though much less so, in 13/13 aged matched controls. It is concluded that the host response to HPV 5/8 infection in epidermodysplasia verruciformis includes the up regulation of several proteins including p16, Ki67, importin-β, exportin-5, Mcl1, and PDL1. Thus, these proteins may serve as new biomarkers of this disease that can aid in cases that are equivocal for epidermodysplasia verruciformis on histologic examination.

1. Introduction

Epidermodysplasia verruciformis is a skin disease charactertized by increased susceptibility to infection by distinct types of human papillomaviruses (HPV) [1-6], especially HPVs 5 and 8 [7-15]. Thus, it has many similarities with the much more common cervical intraepithelial neoplasia (CIN) which is invariably associated with HPV infection [16-19]. Both epidermodysplasia verruciformis and CIN are precursor lesions to squamous cell carcinoma, although malignant transformation is much more common in the former. Both CIN and epidermodysplasia verruciformis are associated with histologic features such as disorganized cell growth, varying sized perinuclear halos, and nuclear atypia that in their unequivocal form are diagnostic of the diseases. The HPV types associated with epidermodysplasia verruciformis and CIN are, however, different. HPVs 5 and 8 cause over 90% of epidermodysplasia verruciformis whereas CIN can be caused by over twenty HPV types, notably HPVs 16, 18, 31, 33, 35, and 51; HPVs 5 or 8 are very rarely evident in CIN [1-6,16-19]. HPVs 5 /8 and the CIN-associated HPVs each contain eight open reading frames including the capsid proteins L1 and L2 and E6/E7 that have been associated with malignant transformation [7-19].

Epidermodysplasia verruciformis has two main clinical correlates. First, there is the rare genetic form, usually autosomal recessive, in which multiple verruca plana appear mostly on the extremities and face by the age of 20 [1-6]. This type may be associated with an inactivating mutation in the transmembrane channel-like protein 6. Second, there is the more common type associated with acquired immunosuppression, most commonly from organ transplantation or AIDS [1-6]. In both clinical scenarios the histologic features are equivalent and HPVs 5 and 8 are the most common types. This suggests that most people are likely exposed to HPVs 5 and 8 and unless they are immunocompromised, or have a rare genetic mutation such as inactivation of transmembrane channel-like protein 6, they do not develop epidermodysplasia verruciformis.

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Another feature epidermodysplasia verruciformis has in common with CIN is that in many cases the surgical pathology is not diagnostic of the disease. Additionally, there are non-viral conditions that can mimic these premalignant lesions. Hence, both false positive and false negative diagnoses are relatively common in each condition [3,6,16-19]. In epidermodysplasia verruciformis cells with blue-grey pallor and perinuclear halos in the granular layer are not always evident and other histologic correlates such as hyper/parakeratosis and nuclear atypia are nonspecific. It has been shown that in such cases a disrupted granular layer may correlate with HPV 5/8 detection [3,6]. Still, in borderline cases the detection of HPVs 5 or 8 may be needed to make an unequivocal diagnosis. Since these viral probes are not readily available, and other types of HPV can cause the disease, other more readily available biomarkers would be helpful to the surgical pathologist in making the correct diagnosis in epidermodysplasia verruciformis.

The purpose of this manuscript was to analyze the host response to the HPVs present in epidermodysplasia verruciformis in the hopes of finding new biomarkers of the disease. The epidermodysplasia verruciformis lesions were interrogated for a series of proteins that have been associated with CIN due to high risk types [20].

2. Materials and methods

2.1. Formalin fixed, paraffin embedded tissues

Formalin fixed, paraffin embedded tissues were available from the files of Cornell Medical Center and one of us (GJN). Thirteen skin biopsies diagnosed as epidermodysplasia verruciformis were available for study with thirteen aged matched controls that were histologically normal skin biopsies from the lower and upper extremities that were adjacent to benign lesions such as intradermal nevi. The age range/mean age for the epidermodysplasia verruciformis cases was 18 to 63 with a mean of 49 years old as compared to controls where the age range was 17 to 65 with a mean of 51 years old. Ten of the epidermodysplasia verruciformis cases were from immunocompromised patients (renal transplant = 7, AIDS = 3) and the other three were considered to be genetic in origin whereas four of the controls were from transplant patients. In each case serial (subjacent) sections four microns apart were numbered to allow in situ based analyses from the same groups of cells.

2.2. In situ hybridization

HPV DNA in situ hybridization was done with a variety of individual probes that can detect HPV 1,2,5,7,8, 13, and 57 as previously reported [20-23] that are all types associated with skin and not genital tract lesions. Also used was the HPV “consensus probe” from Enzo Life Sciences that can detect over 20 different HPV types that are mostly genital tract HPVs but also includes some non-genital types [20,22]. In brief, after protease digestion, the genomic HPV probes labeled with biotin using the Enzo Life Sciences (Farmingdale, NY) random primer kit were co-denatured with the tissue DNA, hybridized for 2–15 h, washed at intermediate stringency, and then detected with using the HPV in situ hybridization kit from Enzo. All cases were also tested with the ultrasensitive HPV in situ hybridization kit from Enzo Life Sciences, in which the HPVs 5 and 8 probes are “hyperbiotinylated”; these are the PATHO-GENE PLUS HPV probes [20]. The chromogens nitro-blue tetrazolium and 5-bromo-4-chloro-3′-indolyphosphate yields a blue signal with nuclear fast red as the counterstain.

2.3. Immunohistochemistry

Our immunohistochemistry protocol has been previously published [20-23]. The skin biopsies were tested for the following antigens: Ki67, p16, importin-β, exportin-5 (ABCAM, Cambridge MA), Mcl1 (Enzo Life Sciences, Farmingdale, NY), and HPV consensus capsid protein L1 (Biocare, Pacheco, CA). The analyses were done on the automated Leica Bond platform with the modification that we used the Enzo Life Sciences peroxidase anti-mouse/rabbit conjugate (catalogue # ADI-950-113-0100) as this reduced background [24].

2.4. Co-expression analysis

Co-expression analyses were done using the Nuance system (CRI) as previously published [20-23]. In brief, a given tissue was tested for two different antigens using fast red as the chromogen for one target followed by immunohistochemistry using DAB (brown) as the second chromogen with hematoxylin as the counterstain. For co-expression with HPV 5/8, the in situ hybridization was done first with NBT/BCIP as the chromogen followed by the immunohistochemistry using fast red. The results were then analyzed by the Nuance and InForm systems in which each chromogenic signal is separated, converted to a fluorescence based signal, then mixed to determine what percentage of cells were expressing the two targets of interest. The associated InForm system can quantify the percentage of cells of a given type positive for the target of interest.

3. Results

3.1. H&E results

The 26 biopsies (13 epidermodysplasia verruciformis and 13 aged-matched controls) were first examined by hematoxylin and eosin stain using sections that were adjacent to those used for in situ hybridization and immunohistochemistry. Blinded pathology analysis confirmed the diagnosis in 9/13 (69%) epidermodysplasia verruciformis cases since the classic histologic features of a thickened granular layer that contained cells with perinuclear halos and blue-grey pallor with associated hyper/parakeratosis was present. As seen in Fig. 1, these histologic changes abruptly merged with the adjacent normal tissue. Fig. 1 also highlights the variable histologic presentation of epidermodysplasia verruciformis. For simplicity, one can divide the epidermis in epidermodysplasia verruciformis into three zones: the parabasal, the granular layer, and the hyper/parakeratotic zone. The parabasal zone shows variable acanthosis of relatively undifferentiated cells. The granular layer, as defined by keratohyaline granules, is the main area for perinuclear halos and blue-grey pallor of cells. As seen in Fig. 1, the granular layer usually shows the cells with the highest HPV copy number. Also note that the parabasal and granular zones, though often distinct, can merge into each other. As evident from Fig. 1, lesions from different patients and even within a given biopsy can vary considerably with regards to the prominence of the parabasal and granular layer and the amount of hyper and/or parakeratosis.

3.2. HPV in situ hybridization

The 26 biopsies were each tested for HPV DNA blinded to the immunohistochemistry data. None of the 13 controls had detectable HPV DNA by in situ hybridization. Each of the thirteen cases was positive for HPV DNA using in situ hybridization. The HPV type distribution of the viral positive cases was: HPV 5 (5/13 cases), HPV 8 (6/13 cases), unknown HPV type detected by consensus probe (2/13 cases). An HPV 5 positive tissue is shown in Fig. 1B, an HPV consensus probe positive case in Fig. 1H, and an HPV 8 positive tissue in Fig. 2B. Note the strong propensity of the HPV DNA to be in very high copy number in cells in the granular layer. Although there was some cross hybridization, the signal with either HPV 5 or 8 was much more intense than the other probe which allowed for typing of the lesions as did the loss of the signal seen with the weaker probe with a high stringency wash (data not shown).

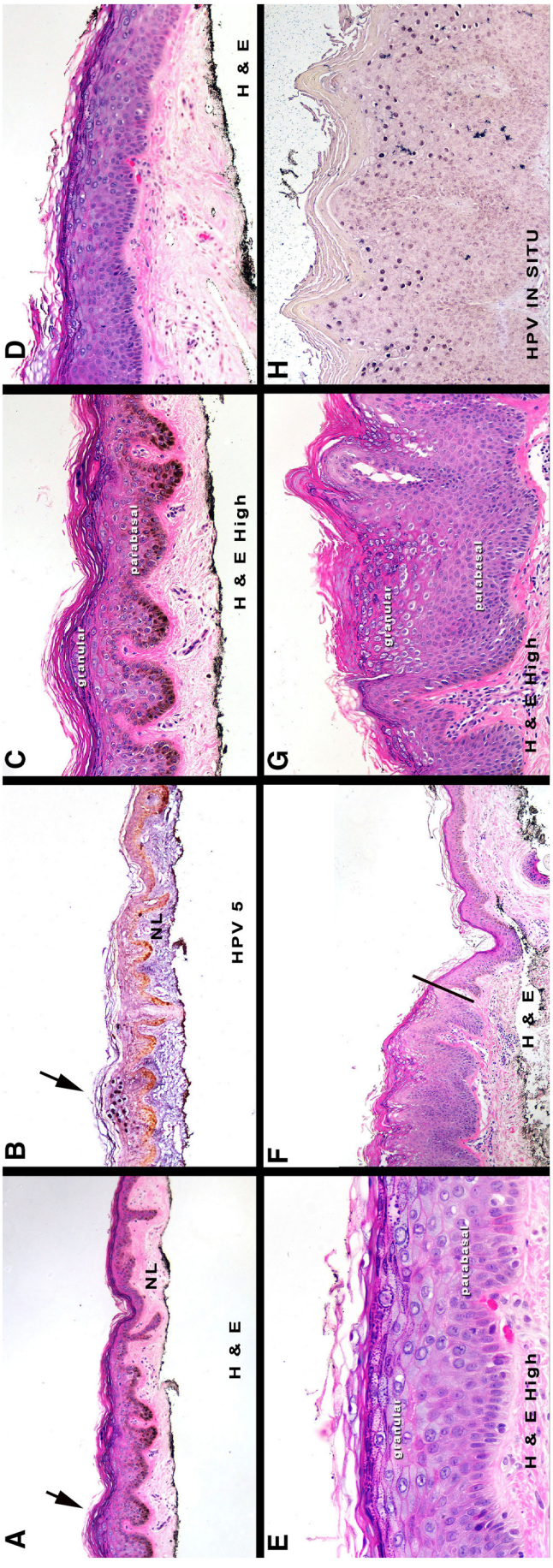

Fig. 1. The variability of the histologic presentation of epidermodysplasia verruciformis. Panel A shows the transition of normal squamous epithelium (NL) to hyperplasia evidenced by increased numbers of cells in the parabasal zone and a more prominent granular layer (arrow). At higher magnification, the dominance of acanthosis in the parabasal layer and paucity of cells with halos and blue grey pallor is evident (panel C). In situ hybridization confirmed the presence of high copy HPV DNA 5 in the hyperplastic area (arrow) but not in the histologically normal area (Panel B). Panels D/E show another case where the blue-grey pallor of cells is more prominent; note that each case shows hyper and parakeratosis. Panel F is a different case where the abrupt transition from normal epithelia is similar to the case in Panel A (line) but where acanthosis and perinuclear halos are more prominent (panel G, higher magnification). In situ hybridization was strongly positive for HPV DNA using a consensus probe primarily in cells with the perinuclear halos (panel H). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

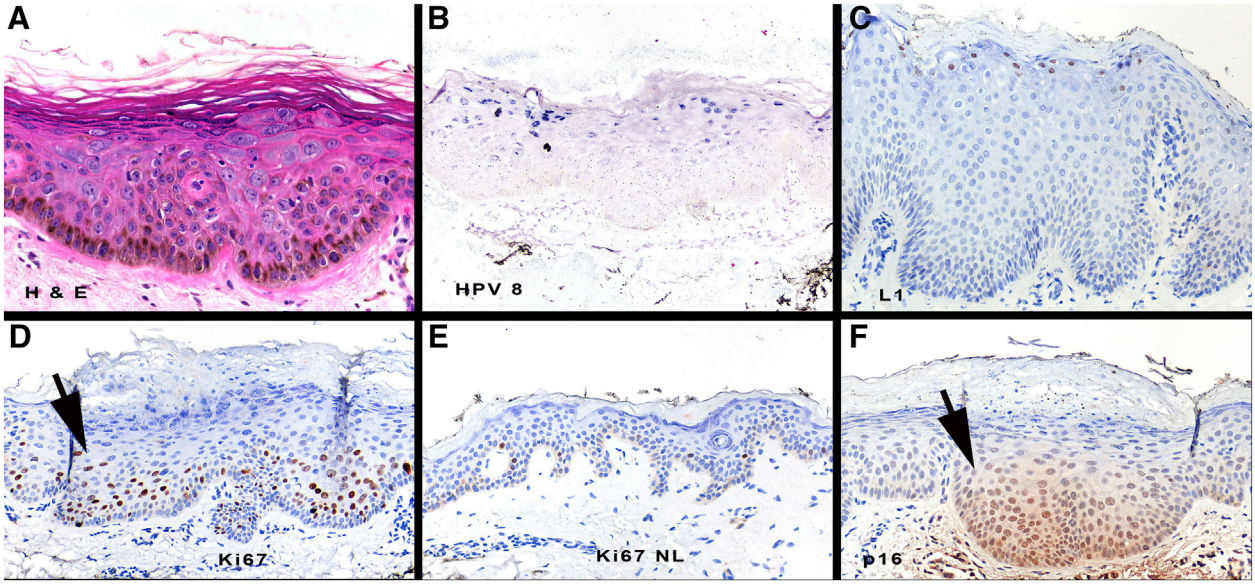

Fig. 2. Expression of Ki67 and p16 in epidermodysplasia verruciformis. Panel A shows the histologic findings of a lesion in a renal transplant patient suspicious for epidermodysplasia verruciformis. The lesion was positive for HPV 8 DNA where most of the cells with detectable HPV DNA were located in the expanded granular layer (panel B). The infection was productive as marked by HPV L1 expression (panel C). Ki67 expression was increased in the lesion, and localized mostly to cells in the expanded parabasal area (arrow, panel D; panel E shows the scattered basal cells with Ki67 in the adjacent normal skin). p16 expression likewise was increased in the lesion and also localized mostly to cells in the expanded parabasal area (arrow, panel F).

3.3. Correlation of viral DNA with immunohistochemistry detection of host proteins

We had previously shown that the host response to the high risk HPV productive infection in CIN lesions included a marked over-expression of Ki67 and p16, as reported by others [16-19], as well as other novel biomarkers that included Mcl1, PDL1, importin-β, and exportin-5 [20]. Given the high viral copy number of HPV in epidermodysplasia verruciformis, it is probable that this represents productive infection. This was documented by the detection of the L1 capsid protein in the cells towards the surface of the lesion, either in cells in the keratin layer or in the upper granular layer (Fig. 2). Thus, since epidermodysplasia verruciformis is a productive HPV infection that is also high risk since from 30 to 60% of such cases will progress to squamous cell cancer [1-6], we next analyzed the 26 cases for importin-β, exportin-5, Mcl1, p16 and PDL1 in a blinded fashion. Of these biomarkers, only exportin-5 and Ki67 were present in the epithelia in the normal controls. However, they were each present in scattered basal cells (Fig. 2). Importin-β was evident in smooth muscle cells that were adjacent to hair follicles and Mcl1 was present in rare inflammatory cells, but neither was present in the normal epidermis. In contrast, each biomarker was strongly increased in the dysplastic epithelia in 13/13 of the epidermodysplasia verruciformis cases.

Representative data for Ki67 and p16 is presented in Fig. 2. Note that these up regulated proteins compartmentalize to the parabasal zone and are rarely evident in the granular or keratin layers. Thus, there is a strong inverse relationship between the HPV DNA in epidermodysplasia verruciformis and Ki67 as well as p16. This same pattern was observed for importin-β, exportin-5, Mcl1, and PDL1 as depicted in Fig. 3Fig. 3 also documents that co-expression analysis confirmed the strong inverse relationship between HPV DNA and the different biomarkers.

In CIN 1 lesions it has been demonstrated that the parabasal zone contains low copy HPV DNA whereas high copy viral DNA/protein/RNA is present towards the apical cells, which would facilitate sexual transmission. To address this question for epidermodysplasia verruciformis, serial sections of the epidermodysplasia verruciformis cases that were HPV 5 or 8 positive were then tested by in situ hybridization using the ultrasensitive PATHO-GENE PLUS polybiotin HPV 5/8 probes according to the manufacturer's recommendations. Prior work has shown that this assay has a sensitivity equivalent to PCR in situ hybridization [20,25]. Each of the 11/13 epidermodysplasia verruciformis cases that contained HPV 5 or 8 was positive with the ultrasensitive probes and the controls were negative. As seen in Fig. 3, viral signal intensity tended to be stronger and the number of positive cells more numerous with the HPV PLUS probes. Interestingly, the HPV DNA 5/8 ultrasensitive probes did not show any signal in the less differentiated cells in the basal and suprabasal part of the lesion (Fig. 3). Finally, to document that the signal was primarily from HPV DNA and not viral RNA, pre-digestion in DNAse eliminated the signal for both HPV 5 and 8 (data not shown).

There were 4/13 cases (31%) in which the histologic findings were considered to be suggestive but not diagnostic for epidermodysplasia verruciformis. In each case, HPV DNA was detected by in situ hybridization that was used to make the definitive diagnosis. Fig. 4 shows a representative example of one of the cases deemed equivocal for a diagnosis of epidermodysplasia verruciformis on histologic examination. Note in Fig. 4 that each of the biomarkers was up regulated in the more basal aspect of the lesion and that HPV 8 DNA was indeed detected by in situ hybridization in the cells in the granular layer, each confirming the diagnosis.

4. Discussion

Epidermodysplasia verruciformis is a classic example of a premalignant lesion caused by a viral infection that has a high propensity to develop into cancer. Although the genetic form of the disease is rare, the disease itself has become relatively common due to its association with acquired immunosuppression that is typically either manifested as AIDS or status post allograft transplant [1-6]. In its classic form epidermodysplasia verruciformis can be diagnosed on histologic examination from the viral induced changes in the cells in the granular layer that include perinuclear halos, blue-grey pallor, as well as hyper/parakeratosis [3,6]. However, as seen in this study where 31% of cases were deemed equivocal for epidermodysplasia verruciformis on histologic examination, and other studies [3,6], these diagnostic features are

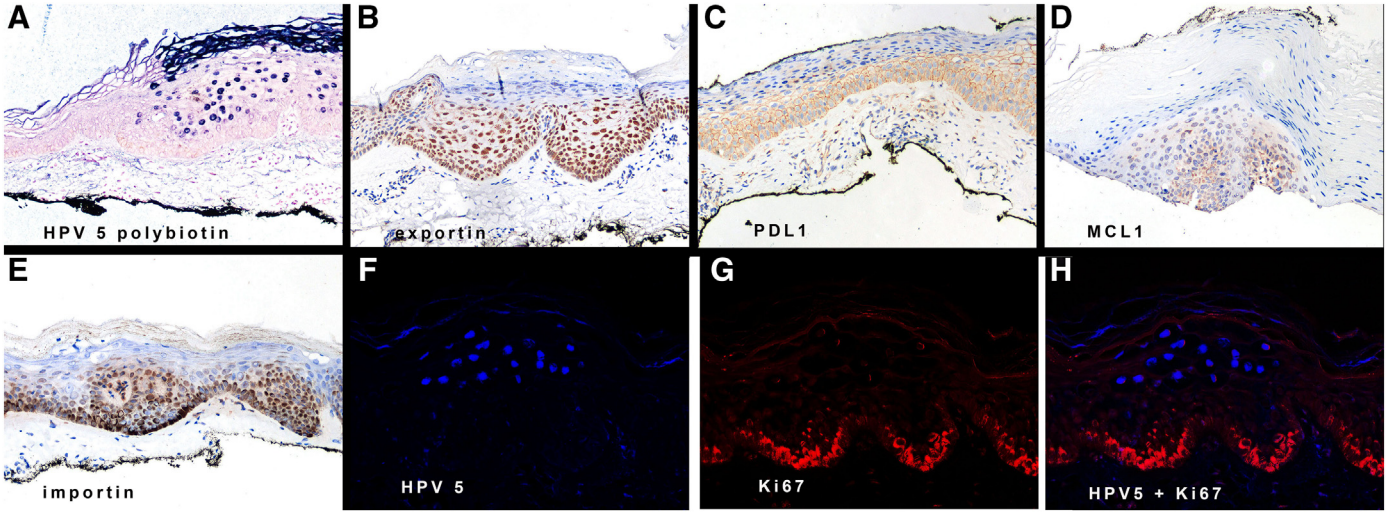

Fig. 3. Expression of MCL1, exportin-5, importin-β, and PDL1 in epidermodysplasia verruciformis. Panel A shows the strong signal for HPV 8 DNA when using the ultrasensitive polybiotin probe. Note that the signal is evident mostly in cells in the expanded granular zone and in the area of hyperkeratosis and that no signal is evident in the less differentiated cells in the parabasal zone. Exportin-5 (panel B), PDL1 (panel C), MCL1 (panel D) and importin-b (panel E) each showed increased expression relative to the normal epidermis and localized to cells in the parabasal zone with some cross over to cells in the granular layer. Panels F–H show the data after co-expression of Ki67 (fluorescent red) and HPV 5 (fluorescent blue). Note the lack of co-expression of these targets as evidenced by the lack of a fluorescent yellow signal. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

not always evident. In such cases, in situ hybridization for HPVs 5 or 8 can be used for a definitive diagnosis. A main reason that in situ hybridization for HPVs 5/8 is so sensitive a test for epidermodysplasia verruciformis is that the viral copy number tends to be very high, often in the hundreds/cell, as seen in this study. However, probes for HPVs 5 and 8 are not readily available. Also, other HPV types including HPVs 10, 47, 20, 21, and 25 can be associated with epidermodysplasia verruciformis [1-6]. In this study 11/13 (85%) of the epidermodysplasia verruciformis cases were due to HPVs 5 or 8. The two other cases were detected with a “consensus HPV probe” that can detect over 25 different HPV types, though not HPVs 5 or 8.

The main diagnostic finding of this study was that by using immunohistochemistry one can detect several different biomarkers that can aid in the diagnosis of epidermodysplasia verruciformis. These novel biomarkers include Ki67 and p16, each often used to diagnose CIN due to high-risk HPV types [16-20], as well as PDL1, Mcl1, importin-β, and exportin-5 and were evident in all 13 cases. None of these biomarkers are expressed in normal skin epidermis except for exportin-5 and Ki67 that are restricted to scattered basal epithelial cells. PDL1, importin-β, and exportin-5 can be induced by other viral infections [22,26-28] but viruses that can infect the skin in immunocompromised people, such as cytomegalovirus, varicella-zoster, and herpes simplex,

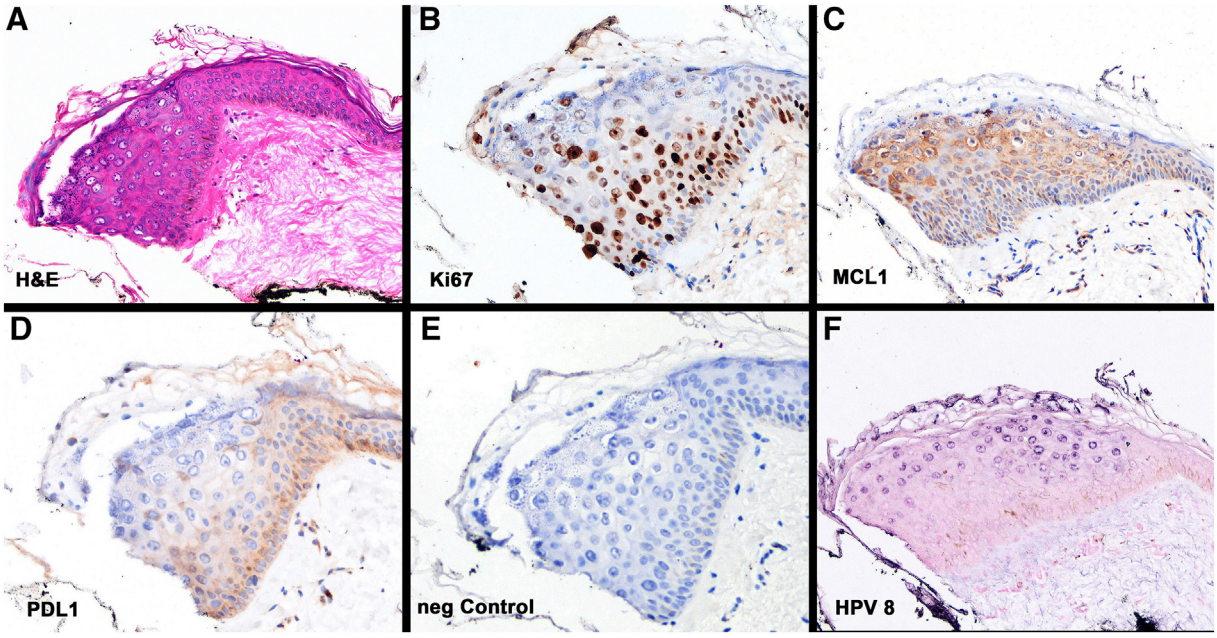

Fig. 4. Detection of the novel biomarkers in a case equivocal for epidermodysplasia verruciformis on histologic examination. Panel A shows the H&E findings of a case deemed suggestive but not diagnostic of epidermodysplasia verruciformis where expanded granular and parabasal layers were evident but without halos or blue-grey pallor. The area shows increased expression of Ki67 (panel B), MCL1 (panel C), and PDL1 (panel D); panel E is the negative control using mouse IgG. HPV in situ hybridization was positive for HPV 8 (panel F) corroborating the diagnosis of epidermodysplasia verruciformis. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

would induce inclusions and cause histologic changes that would not be confused with epidermodysplasia verruciformis. It is also important to stress that these biomarkers show a specific localization pattern in epidermodysplasia verruciformis as they are evident in cells towards the basal aspect of the lesion. This distribution pattern reflects the loss of expression of each biomarker when HPV 5 or 8 become productive, with marked viral DNA/RNA synthesis and L1/L2 protein production. Thus, it is both the expression of these novel biomarkers and compartmentalization in the lesion that is the signature of epidermodysplasia verruciformis.

Much research has focused on the molecular differences between the oncogenic genital HPV types, such as HPVs 16 and 18, and the benign HPV types associated with condyloma acuminatum, such as HPVs 6 and 11 [16-19]. It is clear from many studies that the ORFs E6 and E7 are the main molecular correlates of oncogenesis in the high-risk genital HPV types due, in part, to their ability to inactivate the tumor suppressor proteins retinoblastoma protein and p53, respectively. Although HPVs 5 and 8 appear to have even more malignant potential than HPVs 16 and 18, the molecular basis for this is not clear [7-15]. It has been demonstrated that E6 of the epidermodysplasia verruciformis associated HPVs 5 and 8 can inhibit the tumor suppressor NOTCH [9] and can transform benign cells such as rat fibroblasts whereas in most studies their E7 is not transforming. E6/E7 of HPVs 5/8 at most weakly inactivate Rb protein. ORF E6 and not E7 in the epidermodysplasia verruciformis associated HPV types is most likely responsible for the epithelial hyperplasia, due in part to its ability to hyperphosphorylate EGFR in conjunction with UV light, and the hyper/parakeratosis, due in part to inhibition of caspase-14 mediated apoptosis [7-15]. With regards to similar mechanisms, HPVs 5/8 E6 as well as HPVs 16/18 E6 are each able to interact with the highly conserved PDZ proteins that play a key role in anchoring receptor proteins to the cell membrane, which has been theorized to play a role in transformation [10]. In sum, E6 appears to play a more important role than E7 in transforming cells by HPVs 5/8 and there appears to be some mechanistic differences between these two ORFs proteins when compared to the corresponding proteins in HPV 16/18.

Another interesting comparison between epidermodysplasia verruciformis and CIN 1 was that when using ultrasensitive in situ techniques that low copy viral DNA was found in the parabasal cells of CIN1 [20] but not in the equivalent cells in epidermodysplasia verruciformis. It can be hypothesized that this difference may reflect that HPV 16/18 initially infect the basal cells in areas of squamous metaplasia in CIN while HPV 5/8 probably initiate infection in the granular layer. This data also suggests that the expression of the novel biomarkers in the parabasal zones of CIN 1 and epidermodysplasia verruciformis may represent a response to the productive infection in the cells towards the apical part of the lesion.

The main implication of this work for the diagnostic surgical pathologist is that there is a panel of novel biomarkers that may allow one to differentiate epidermodysplasia verruciformis lesions from their clinical and pathologic mimics, which include tinea versicolor, and clinically atypical verruca vulgaris where the lesion is relatively flat. The latter lesions are usually associated with HPV 2 and have very low malignant potential [21]. A more common problem for the surgical pathologist is making an unequivocal diagnosis in epidermodysplasia verruciformis cases that are equivocal on histologic examination. The importance of this diagnosis is underscored by the observation that from 30 to 50% of epidermodysplasia verruciformis cases will progress to squamous cell carcinoma [1-6]. It is in these cases where immunohistochemistry for these novel biomarkers can be of great utility to the surgical pathologist as they should be positive regardless of the HPV type. Further, if HPV 5 and 8 probes are available, a negative result does not necessarily rule out epidermodysplasia verruciformis since, as seen in this and other studies, other distinct HPV types can be present in around 15–25% of cases [1-6]. However, if the epidermodysplasia verruciformis is associated with squamous cell cancer, then over 90% of such lesions will be HPV 5 or 8 positive.

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Disclosure

There is no duality of interests to declare.

References

[1] Ovits CG, Amin BD, Halverstam C. Acquired epidermodysplasia verruciformis and its relationship to immunosuppressive therapy: report of a case and review of the literature. J Drugs Dermatol 2017 Jul 01;16(7):701–4.
[2] Przybyszewska J, Zlotogorski A, Ramot Y. Re-evaluation of epidermodysplasia verruciformis: reconciling more than 90 years of debate. J Am Acad Dermatol 2017 Jun;76(6):1161–75.
[3] Nuovo GJ, Ishag M. The histologic spectrum of epidermodysplasia verruciformis. Am J Surg Pathol 2000 Oct;24(10):1400–6.
[4] Carlson JA, Cribier B, Nuovo G, Rohwedder A. Epidermodysplasia verruciformis-associated and genital-mucosal high- risk human papillomavirus DNA are prevalent in nevus sebaceus of Jadassohn. J Am Acad Dermatol 2008 Aug;59(2):279–94.
[5] Hu W, Nuovo G, Willen M, Somach S. Epidermodysplasia verruciformis in two half brothers with HIV infection. J Cutan Med Surg 2004 Sep-Oct;8(5):357–60.
[6] Morrison C, Eliezri Y, Magro C, Nuovo GJ. The histologic spectrum of epidermodysplasia verruciformis in transplant and AIDS patients. J Cutan Pathol 2002 Sep;29(8):480–9.
[7] Akgul B, Garcia-Escudero R, Ghali L, Pfister HJ, Fuchs PG, Navsaria H, et al. The E7 protein of cutaneous human papillomavirus type 8 causes invasion of human keratinocytes into the dermis in organotypic cultures of skin. Cancer Res 2005 Mar 15;65(6):2216–23.
[8] Taute S, Pfister HJ, Steger G. Induction of tyrosine phosphorylation of UV-activated EGFR by the Beta-human papillomavirus type 8 E6 leads to papillomatosis. Front Microbiol 2017;8:2197.
[9] Meyers JM, Spangle JM, Munger K. The human papillomavirus type 8 E6 protein interferes with NOTCH activation during keratinocyte differentiation. J Virol 2013 Apr;87(8):4762–7.
[10] Lazić D, Hufbauer M, Zigrino P, Buchholz S, Kazem S, Feltkamp MC, et al. Human papillomavirus type 8 E6 oncoprotein inhibits transcription of the PDZ protein syntenin-2. J Virol 2012 Aug;86(15):7943–52.
[11] Akgul B, Karle P, Adam M, Fuchs PG, Pfister HJ. Dual role of tumor suppressor p53 in regulation of DNA replication and oncogene E6-promoter activity of epidermodysplasia verruciformis- associated human papillomavirus type 8. Virology 2003 Apr 10;308(2):279–90.
[12] Iftner T, Sagner G, Pfister H, Wettstein FO. The E7 protein of human papillomavirus 8 is a nonphosphorylated protein of 17 kDa and can be generated by two different mechanisms. Virology 1990 Nov;179(1):428–36.
[13] Oswald E, Reinz E, Voit R, Aubin F, Alonso A, Auvinen E. Human papillomavirus type 8 E7 protein binds nuclear myosin 1c and downregulates the expression of pre-rRNA. Virus Genes 2017 Dec;53(6):807–13.
[14] Kazem S, van der Meijden E, Struijk L, de Gruijl FR, Feltkamp MC. Human papillomavirus 8 E6 disrupts terminal skin differentiation and prevents pro-Caspase-14 cleavage. Virus Res 2012 Feb;163(2):609–16.
[15] Yamashita T, Segawa K, Fujinaga Y, Nishikawa T, Fujinaga K Biological and biochemical activity of E7 genes of the cutaneous human papillomavirus type 5 and 8. Oncogene (1993 Sep) 8(9):2433–41.
[16] Richart RM, Wright TC, Wright TC Jr. Controversies in the management of low-grade cervical intraepithelial neoplasia. Cancer 1993;71(4 Suppl):1413–21.
[17] Nuovo GJ. Cytopathology of the lower female genital tract: An integrated approach, Baltimore, USA. Williams and Wilkins; 1994.
[18] Crum CP, Nuovo GJ, Friedman D, et al. Accumulation of RNA homologous to human papillomavirus type 16 open reading frames in genital precancers. J Virol 1988;62:84–90.
[19] Stoler MH. The virology of cervical neoplasia: an HPV-associated malignancy. Cancer J 2003;9(5):360–7.
[20] Nuovo GJ, de Andrade CV, Wells SI, Brusadelli M, Nicol AF. New biomarkers of human papillomavirus infection in acute cervical intraepithelial neoplasia. Ann Diagn Pathol 2018 Oct;36:21–7.
[21] Nuovo GJ. In situ Molecular Pathology and Co-expression analyses. 2013; Elsevier/Academic Press, San Diego, CA.
[22] Nuovo GJ, de Andrade CV, Thirukkumaran C, Nicol AF. Importin-β and exportin-5 are indicators of acute viral infection: correlation of their detection with commercially available detection kits. Ann Diagn Pathol 2018 Jan 10;34:36–41.
[23] Mezache L, Magro C, Hofmeister C, et al. Modulation of PD-L1 and CD8 activity in idiopathic and infectious chronic inflammatory conditions. Appl Immunohistochem Mol Morphol 2017;25(2):100–9.
[24] Nuovo GJ. False-positive results in diagnostic immunohistochemistry are related to horseradish peroxidase conjugates in commercially available assays. Ann Diagn Pathol 2016;25:54–9.
[25] Nuovo GJ, MacConnell P, Forde A, et al. Detection of human papillomavirus DNA in

formalin fixed tissues by in situ hybridization after amplification by the polymerase chain reaction. Am J Pathol 1991;139:847–54.
[26] Demart S, Ceccherini-Silberstein F, Schlicht S, et al. Analysis of nuclear targeting activities of transport signals in the human immunodeficiency virus rev protein. Exp Cell Res 2003;291(2):484–501.
[27] Malik P, Tabarraei A, Kehlenbach RH, et al. Herpes simplex virus ICP27 protein directly interacts with the nuclear pore complex through Nup62, inhibiting host nucleocytoplasmic transport pathways. J Biol Chem 2012;287(15):12277–122292.
[28] Freedman DA, Levine AJ. Nuclear export is required for degradation of endogenous p53 and MDM2 and human papillomavirus E6. Mol Cell Biol 1998;18:7288–93.