Recalcitrant Warts, Epidermodysplasia Verruciformis, and the Tree-Man Syndrome: Phenotypic Spectrum of Cutaneous HPV Infections at the Intersection of Genetic Variability of Viral and Human Genomes

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Abstract

Human papilloma virus (HPV) infections can cause common warts, which usually resolve spontaneously or become recalcitrant, resistant to multiple treatments. In rare cases, they

transform into cutaneous giant horns resulting in the tree-man syndrome (TMS). Defective β-HPVs can cause flat warts in epidermodysplasia verruciformis (EV), a genetic disorder. In typical EV, limited to the skin, the mutated genes are critical for keratinocyte-intrinsic immunity, while atypical, syndromic EV involves genes controlling T-cells. Inborn errors of immunity due to mutations in distinct genes underlying recalcitrant warts and the α-HPV2-driven TMS have been identified, all disrupting T-cell immunity. Collectively, these observations attest to the wide phenotypic spectrum of cutaneous infections caused by different HPV types, at the intersection of the genetic diversity of the viral and human genomes.

Keywords

Cutaneous HPV infections; epidermodysplasia verruciformis; recalcitrant warts; the tree-man syndrome; keratinocyte-intrinsic immunity; T cell immunity

INTRODUCTION

Human papilloma viruses (HPVs), with strict tropism to keratinized and non-keratinized squamous epithelia, are common pathogens; for detailed discussion of HPV virology and pathology, see (Beziat *et al.*, 2021; Bzhalava *et al.*, 2015; McBride, 2021). HPVs are double-stranded DNA viruses with over 400 genotypes identified, grouped into five genera (α-, β-, γ-, μ-, and ν-HPV), on the basis of their nucleotide sequence similarities of the open reading frame encoding the capsid protein L1 (Bzhalava *et al.*, 2015; de Villiers *et al.*, 2004; McBride, 2021). While most cutaneous HPV infections are subclinical and persistent in the absence of immunosuppression, they can cause benign common warts (CW), as well as extensive, unusually severe conditions which can be divided into three distinct clinical categories, (a) recalcitrant common warts (RCW), (b) the tree-man syndrome (TMS), and (c) epidermodysplasia verruciformis (EV) (Figure 1; the clinical pictures are presented with written, informed consent of the patients). While HPV may infect a number of extracutaneous sites, this Perspective will focus on recent progress in understanding the genetic and immunologic underpinnings of cutaneous HPV infections, RCW, TMS and EV, and we suggest updates to wart classification regarding the associated HPV types (Table 1).

Recalcitrant warts as paradigm of human papilloma virus infections

Based on their anatomical locations, CWs can be subcategorized, such as generalized, periungual, plantar, and genital warts. The CWs usually resolve spontaneously, but some of these lesions, defined as RCW, persist despite repeated treatments. In exceptional cases, the warts can also transform into exophytic cutaneous lesions, giant horns, resulting in TMS (Beziat *et al.*, 2021). In some cases, there is a familial susceptibility to development of warts, as in EV, a heritable disorder characterized by the presence of numerous flat warts in generalized distribution (de Jong *et al.*, 2018; Vahidnezhad *et al.*, 2019).

The persistence of HPV-related warts suggests the presence of impaired immunity to cutaneous HPVs, although in many cases these patients do not have an obviously impaired immune system. Nevertheless, in some cases the proliferation of HPV, a common pathogen, is not efficiently controlled by the patients' immune system, and it has been suggested

that deficient host cell immunity, including T cell activities, are involved in immune response selectively against HPVs. In support of this notion, inborn errors of immunity (IEI), associated with RCWs, have been identified, highlighting the role of T lymphocytes in the cutaneous anti-HPV immune response (Beziat *et al.*, 2021). In some cases, as illustrated by typical EV, the development of warts is permitted by deficient keratinocyte-intrinsic immunity in otherwise healthy individuals (de Jong *et al.*, 2018; Beziat *et al.*, 2021)

The tree-man syndrome, a disease of exophytic warts and cutaneous horns with aberrant immunity

In the spectrum of cutaneous HPV infections, TMS presents with the most extensive warts developing into cutaneous horns, which can be giant and generalized (Figure 1). These lesions start as CWs slowly spreading over the hands and feet before transforming into cutaneous horns characteristic of the TMS phenotype. This condition is extremely rare, and only a few cases have been reported, essentially all of them being sporadic with no family history. Due to paucity of reported cases, it is unclear if these lesions in TMS have malignant potential. We have recently analyzed an extended multiplex family with three affected individuals, one of them with characteristic TMS phenotype while two others had multiple RCWs (Beziat *et al.*, 2021). Utilizing whole exome sequencing, we identified a homozygous, previously unreported missense mutation (c.52G>A; p.Gly18Arg) in the *CD28* gene. No CD28 protein on their T cells was detected, with the exception of a small contingent of revertant memory CD4^{+} T cells. CD28 has a major costimulatory role of T-cell receptor signaling when engaged by CD80 or CD86 expressed on antigen-presenting cells. The development of T cells was not affected, but the patient's T cells did not respond to CD28 co-stimulation. CD28 deficient mice were also shown to be susceptible to cutaneous infections with mouse papillomavirus MmuPV1. Collectively, these results suggested that the control of HPV2 and HPV4 in keratinocytes is dependent on the T cell CD28 co-activation. However, the patients with CD28 deficiency, besides the cutaneous HPV infection, were otherwise healthy, indicating that human CD28 is largely dispensable for protective immunity against other pathogens.

Careful examination of the TMS pedigree revealed that the autosomal recessive CD28 deficiency resulted in susceptibility to cutaneous α- and γ-HPVs, but only one of the patients had extensive giant horns characteristic of TMS resulting from a long-term α-HPV2 infection, whereas the other two patients had disseminated RCWs caused by γ-HPV4. These findings attest to the diversity of HPV types in causing cutaneous lesions, and they provide new insights into the context of host-HPV interactions. These observations, in combination with the demonstration of CD28 deficiency in the patient with TMS, indicate that IEIs can underlie the development of TMS. In contrast to HPV2^{+} RCWs, TMS lesions were associated with aberrant HPV2 oncogene expression, and deregulated viral cycle (Beziat *et al.*, 2021). It remains unclear, however, why most patients with IEI underlying severe HPV2-driven warts do not develop TMS.

Genetic basis of epidermodysplasia verruciformis (EV)

Early evidence for genetic susceptibility to warts was provided by studies on EV, an autosomal recessive disorder with propensity for development of numerous flat warts,

which usually first appear during the childhood. Later in life, these warts can evolve into protruding hyperproliferative lesions and verrucous tumors which, particularly on the sun-exposed areas, have propensity for non-melanoma skin cancer formation, mostly squamous cell carcinomas (Becerril *et al.*, 2021). The heritable forms EV have been divided into two categories, typical and atypical, and some sporadic cases with EV-like phenotypes are secondarily associated with immune suppression, such as HIV infection. In typical EV, the clinical manifestations are limited to the skin, the affected individuals being otherwise healthy with no evidence of other infections. In atypical forms of inherited EV, the cutaneous findings are invariably associated with other infections and hematological malignancies, such as the Hodgkin's lymphoma (Youssefian *et al.*, 2019b). Next generation sequencing has identified mutations in a number of IEI-associate genes, including *RHOH*, *LCK*, *MST1*, *CORO1A*, *IL7*, *RASGRP1*, *CARMIL2*, *TPP2*, *DCLRE1C*, *GATA2*, and *DOCK8*. These inborn errors often cause early-onset, life threatening T cell primary immunodeficiency, and consequently, the atypical forms of EV are genetically linked to impaired T cell immunity. While the underlying pathomechanisms of EV in typical and atypical cases can be different, the clinical features are similar with respect to cutaneous β-HPV infection (Figure 1). Thus, in the absence of evidence for other infections or overt systemic malignancies, classification of families as having typical *versus* atypical EV can be difficult before appropriate genotyping of viral and host genomes has been a performed.

The early genetic studies on typical forms of EV identified mutations in *TMC6* and *TMC8*, which encode EVER1 and EVER2, two endoplasmic reticulum plasma membrane proteins, respectively (Ramoz *et al.*, 2002; Youssefian *et al.*, 2019a). Loss of function of these proteins results in proliferation of HPV infected keratinocytes and in replication of the virus in terminally differentiating host cells. More recently, we identified another gene, *CIB1*, which can also harbor mutations in approximately one-third of the families with typical EV (de Jong *et al.*, 2018; Vahidnezhad *et al.*, 2019). The mechanism for cell proliferation as a result of mutated EVER1 and EVER2 was initially suggested to involve intracellular zinc transport, however, subsequent to identification of CIB1, it was demonstrated that these three proteins, EVER1, EVER2 and CIB1, form a tertiary complex providing keratinocyte-intrinsic immunity to β-HPVs. Thus, mutations in any of the three genes encoding these subunits can result in aberrant, keratinocyte-intrinsic immunity manifesting with typical cutaneous lesions of EV due to HPVs, and the patients do not display infections due to other pathogens.

Unifying concepts of HPV-associated cutaneous manifestations

Collectively, infections of cutaneous keratinocytes caused by a number of different HPV types can results in a spectrum of phenotypic lesions, including CWs, specifically associated with the 14 α-, 17 γ-, two μ-, and one ν-HPV types (Table 1). In relatively few cases the clinical phenotype can proceed to TMS with extensive protruding lesions with cutaneous horns. This progression to TMS phenotype is apparently predicated on immunological deficiency limited to α-HPV2, as demonstrated in the family with the T cell CD28 deficiency (Figure 1) as well as in other HPV-typed TMS patients reported to date. Another HPV-associated cutaneous disorder, EV, is characteristically associated with β-HPV infections (Table 1). Thus, EV and TMS are clearly distinct entities with characteristic

cutaneous manifestations and different associated HPV types. Overall, the spectrum of cutaneous manifestations due to HPV infections is determined by the type of underlying genetic mutations in different immune-associated genes, expressed either in keratinocytes or in T cells, with distinct consequences on cutaneous immunity, intersecting with the HPV types causing the infection.

Conclusions and therapeutic implications

Precise understanding of the immunological mechanisms allowing pathogenic propagation of the infection by HPVs, which are commonly present at low, subclinical levels in the skin of populations at large, should provide new information on potential targets for therapy development for these, currently intractable disorders. However, immunization with multipotent anti-HPV vaccines, such as Gardasil 9, has been generally unsuccessful in improving the skin lesions in patients with EV or TMS (Donaldson *et al.*, 2019; Maor *et al.*, 2018). It should be noted, however, that these vaccines do not specifically target the HPV types responsible for EV or TMS. In patients with atypical EV or RCWs due to T cell deficiency as a result of mutations in critical immune genes, with hematological malignancies, bone marrow transplantation has been shown to be curative also of some skin lesions (Bruzzese *et al.*, 2020; Aydin *et al.*, 2019).

In summary, recent genetic observations on HPV-associated skin disorders, including RCW, EV and TMS, emphasize the control of proliferation of HPV infected keratinocytes by the underlying immunological factors. Loss of immune control, either T cell-based or keratinocyte-intrinsic, can be elicited by genetic loss-of-function mutations in immune-associated genes, leading to susceptibility to infections by HPV and transformation of resident keratinocytes into clinical lesions in the spectrum of HPV-associated cutaneous disorders at the intersection of genetic variability of viral and human genomes.

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DATA AVAILABILITY STATEMENT

No new datasets were generated for this Perspective article. The data on the authors' original work, including FASTQ files, are available from the corresponding author upon reasonable request.

Abbreviations:

CW	cutaneous warts
EV	epidermodysplasia verruciformis
HPV	human papilloma virus

RCW	recalcitrant cutaneous warts
TMS	the tree-man syndrome

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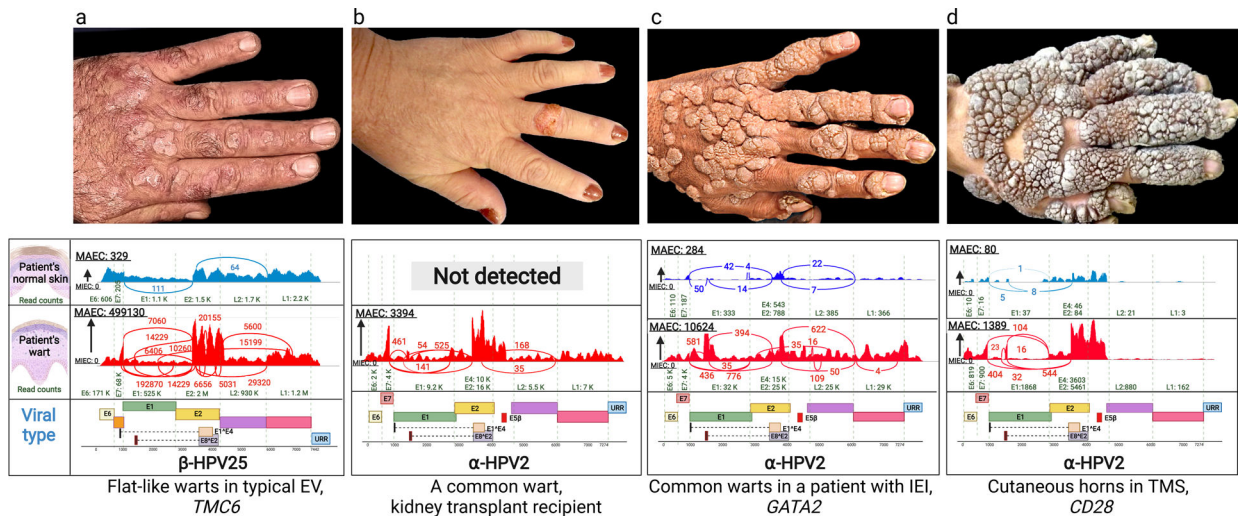
Figure 1. Clinical presentation of warts on the backs of hands of patients with cutaneous HPV infections and identification of HPV types by whole transcriptome sequencing.
(a) Multiple flat warts in a patient with typical EV caused by mutations in the *TMC6* gene. (b) A solitary common wart in the middle finger of a kidney transplant patient. (c) Multiple common warts in a patient with primary inborn error of immunity (IEI) caused by mutations in *GATA2*. (d) Exophytic warts and cutaneous horns in a patient with TMS due to mutations in *CD28*. Below each clinical picture, there are representative Sashimi plots derived from whole transcriptome sequencing by RNA-Seq, identifying the types of HPV associated with the lesions shown above or in the normal appearing skin of the same patients. The corresponding genomic structures of HPVs are shown below; note the presence of Early (E1–8), Late (L1 and L2) and Upstream Regulatory Region. The copy number of viruses is very low (a, c and d), or completely absent (b), in normal appearing skin adjacent to the warts. HPV, human papilloma virus; EV, epidermodysplasia verruciformis; IEI, inborn error of immunity; MAEC, Maximum exon coverage; MIEC, Minimum exon coverage; TMS, the tree-man syndrome.

Table 1:

Nosology of HPV types associated with warts with different clinical presentations[*]

| HPV genera | Cutaneous lesions[**] | | | Mucosal lesions |
	EV	TMS	Recalcitrant warts-Non genital	Recalcitrant warts-Oral/Anogenital
α		2	2, 3, 7, 10, 26, 27, 28, 29, 57, 78, 91, 94, 117	6, 11, 13, 16, 18[#], 27, 30, 31, 32, 33[#], 35, 39[#], 40, 42, 43, 44, 45[#], 51[#], 52[#], 53, 54, 56, 58[#], 59[#], 61, 62, 66[#], 67, 68[#], 70, 71, 72, 73, 74, 81, 83, 84, 85, 86, 87, 89, 90
β	5, 8, 9, 12, 14, 15, 17, 19, 20, 21, 22, 23, 24, 25, 36, 37, 38, 47, 49, 50, 75, 76, 77, 80, 93, 96			
γ		4	4, 48, 60, 65, 88, 95, 126, 128, 129, 130, 131, 132, 133, 134, 142, 147, 148, 149, 154	153, 175, 180
μ			1, 61	
ν			41	

[*] Updated from Orth G., 2006, and Sterling J.C., 2916 (Orth, 2006; Sterling, 2016)

[**] EV, epidermodysplasia verruciformis; HPV, human papilloma virus; TMS, the tree-man syndrome

[#] High-risk oncogenic HPVs (https://www.cancer.gov/about-cancer/causes-prevention/risk/infectious-agents/hpv-and-cancer)