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INSIGHTS

HPV: CIB1 is for EVER and EVER

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In this issue, de Jong et al. (https://doi.org/10.1084/jem.20170308) identify bi-allelic loss-of-expression, loss-of-function mutations of the calcium- and integrin-binding protein 1 (*CIB1*) gene as a new cause of epidermodysplasia verruciformis (EV) and demonstrate that the CIB1 interacts with the EVER1 and EVER2 proteins to form a complex involved in keratinocyte-intrinsic immune response to human β-papillomaviruses (β-HPVs).

Human papillomaviruses (HPVs) are small, double-stranded DNA viruses with a specific tropism for mucosal and cutaneous epithelium. Over 200 HPVs are known, which are grouped into different genera, species, and types. In particular, cutaneous α-HPVs are responsible for common warts, whereas mucosal α-HPVs are categorized into low-risk and high-risk species, with the latter being associated with ano-genital squamous cell carcinoma (SCC) and head and neck cancer (Cubie, 2013). The μ-, ν-, and γ-HPVs infect the skin epithelium and cause benign papillomas. Finally, β-HPVs also have tropism for the cutaneous epithelium, but typically produce an asymptomatic infection in the general population. By contrast, patients with epidermodysplasia verruciformis (EV) manifest a unique susceptibility to symptomatic and life-long persistent β-HPV infection, which often starts as flat warts but frequently progresses to SCC and other non-melanoma forms of skin cancer, with a high mortality rate.

EV was described by Lewandowsky and Lutz in 1922. Lutz's hypothesis that genetic predisposition may account for the widespread dissemination of warts in these patients was further supported in 1933 by Cockayne, who classified EV among inborn errors of the skin (Cockayne, 1933). This recognition preceded the identification of classical inborn errors of immunity affecting hematopoietic cells, such as Bruton's X-linked agammaglobulinemia. Although the disease was shown to be inherited as an autosomal recessive trait, its molecular basis remained undefined until mutations of the *TMC6* and *TMC8* genes, encoding for the EVER1 and EVER2 proteins, respectively, were identified in 2002 (Ramoz et al., 2002). Yet, the mechanisms through which EVER1 and EVER2 control HPV infection, and why patients with mutations in these genes are uniquely susceptible to persistent and severe β-HPV infection in particular, remained ill defined.

EVER1 and EVER2 are transmembrane channel-like (TMC) proteins that are broadly expressed. No immunological abnormalities have been demonstrated in patients with *TMC6* or *TMC8* mutations, suggesting that the disease reflects impairment of keratinocyte-intrinsic mechanisms of anti-viral response. By homology with other TMC family members, it was hypothesized that EVER1 and EVER2 function as ion channels. Transfection studies suggested that they interact with the zinc transporter ZnT1, which resides in the endoplasmic reticulum, implying a role in controlling zinc homeostasis and free zinc intracellular distribution in keratinocytes, and through this mechanism a function as restriction factors against HPVs (Lazarczyk et al., 2008). In this issue, this hypothesis has been revisited and challenged by de Jong et al.

In particular, de Jong et al. (2018) studied 24 patients with typical EV from six families of various ethnicities. None of the patients carried *TMC6* or *TMC8* mutations, and none of them had signs of T cell immunodeficiency, which may cause atypical forms of EV-like disease. Consanguinity was known for four of these families, and highly suspected for two. Using genome-wide linkage analysis and homozygosity mapping, the authors identified a 2.4-Mb interval on chromosome 15 that showed strong association with the disease. This interval was further

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reduced to 1.74 Mb by searching for common haplotypes. Finally, whole exome sequencing identified nonsynonymous *CIB1* mutations as the only very rare homozygous DNA variants contained in this interval that were common to all patients. Analysis of CIB1 protein expression in lymphoblastoid cell lines (LCLs), peripheral blood mononuclear cells, and primary keratinocytes from the patients showed that in all cases these were loss-of-expression mutants. A strong signal for the CIB1 protein was detected in normal epidermis and hair follicles. Interestingly, very low levels of CIB1 protein were also demonstrated in LCLs from patients with EV due to *TMC6* or *TMC8* mutations, as well as in the skin of newly generated *Tmc6*$^{-/-}$ and *Tmc8*$^{-/-}$ mice. Stable reconstitution of EVER1 and EVER2 expression in *TMC6*- and *TMC8*-mutated LCLs, respectively, also rescued levels of the CIB1 protein without affecting mRNA levels, strongly indicating that expression of both EVER1 and EVER2 is required to maintain CIB1 protein stability. Co-immunoprecipitation studies demonstrated that EVER1, EVER2, and CIB1 form

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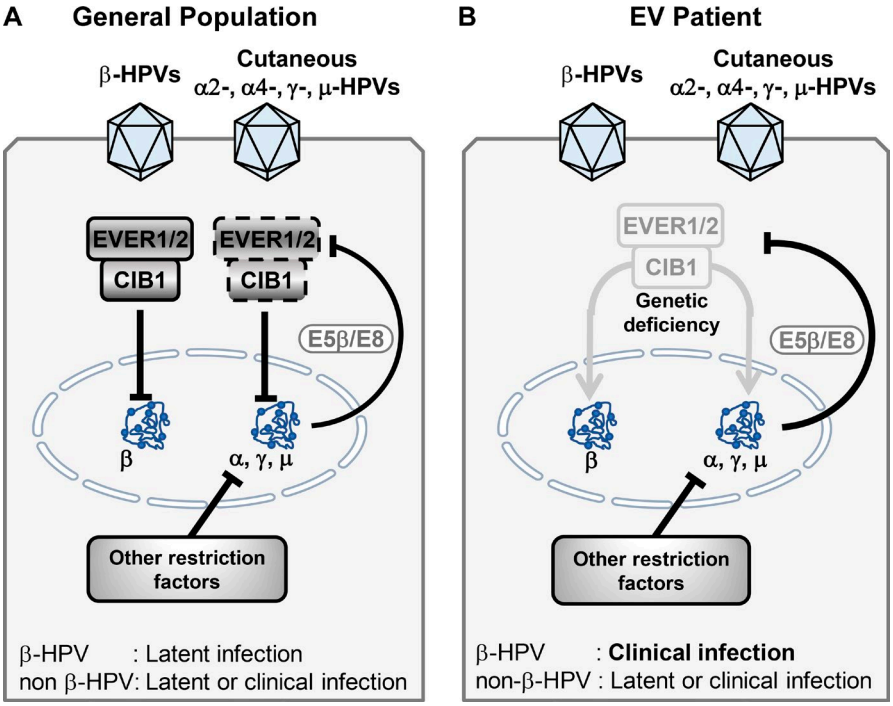

From de Jong et al. (2018). **(A)** In the general population, the CIB1/EVER1/2 complex restricts the transcription of the minichromosome of β-HPV, leading to the absence of clinical manifestation. The proteins E5 and E8 expressed by the other cutaneous HPVs (α2-, α4-, γ-, and μ-HPVs) are able to antagonize the CIB1/EVER1/2 complex. However, additional restriction factors are probably contributing to the absence of HPV lesions in the vast majority of people. **(B)** In EV patients, the lack of CIB1/EVER1/2 permits the transcription of β-HPV minichromosome, which leads to the development of EV lesions on the skin. However, the probable presence of additional restriction factors against cutaneous HPVs other than β-HPVs accounts for their normal control, which does not differ from the general population.

a multimeric complex. Importantly, while the Casanova group confirmed that overexpressed EVER1 and EVER2 interact with ZnT1 and regulate zinc-induced expression of GFP under the control of a metal-responsive element, they failed to demonstrate similar effects for CIB1. Furthermore, both intracellular levels of free zinc and zinc flux kinetics were normal in LCLs from patients with *TMC6*, *TMC8*, and *CIB1* mutations, challenging the hypothesis that EVER1, EVER2, and CIB1 may regulate cellular zinc homeostasis.

The finding that *CIB1* mutations in humans are responsible for EV is surprising for multiple reasons. CIB1 is ubiquitously expressed, and yet no overt phenotype other than EV has been reported in patients. Furthermore, although *Cib1*$^{-/-}$ mice show no obvious developmental defects, they have a decreased ability to respond to ischemic injury (Zayed et al., 2007) and to develop cardiac hypertrophy in response to prolonged hypertension (Heineke et al., 2010). Moreover, male *Cib1*$^{-/-}$ mice are infertile (Yuan et al., 2006). While lack of the first two phenotypes in CIB1-deficient patients is of difficult interpretation, affected males are fertile. Finally, by interacting with α-integrins and with several serine/threonine kinases, CIB1 has been shown to regulate cell adhesion, migration, and intracellular signaling (Leisner et al., 2016), and yet CIB1-deficient keratinocytes showed no defects in focal adhesion and in vitro cell migration.

A clue to a possible mechanism underlying EV in patients with *CIB1*, *TMC6*, or *TMC8* mutations came from transfection experiments in keratinocyte HaCaT cells. In particular, the Casanova group showed that CIB1 interacts with the α-HPV16 E5, γ-HPV4 E8, and the cottontail rabbit papillomavirus (CRPV) E8 protein, which shares structural similarities with HPV16 E5. Interestingly, interaction between the EVER1/EVER2/ZnT1 complex and the CRPV E8 protein had been previously reported (Lazarczyk et al., 2008). Although relatively little is known of the function of HPV E5 proteins, the E5α oncoprotein of HPV16 has been shown to augment signaling from the epidermal growth factor receptor and to induce Met up-regulation, promoting motility of HPV-infected cells (Scott et al., 2018). Furthermore, the CRPV E8 protein is essential for wart formation and growth in vivo (Nonnenmacher et al., 2006). The Casanova group has therefore postulated that the CIB1/EVER1/EVER2 complex acts as a restriction factor for HPV. In the case of infections sustained by α-, γ-, and μ-HPVs, expression of E5 and E8 proteins would allow these HPVs to escape the restriction activity of the CIB1/EVER1/EVER2 complex. On the other hand, because β-HPVs do not express E5 and E8 proteins, they would not be able to induce clinical disease in the general population. By contrast, in patients with *TMC6*, *TMC8*, or *CIB1* mutations, lack of expression of the CIB1/EVER1/EVER2 complex would make these patients susceptible to clinical disease upon infection with β-HPV. To explain unique susceptibility to β-HPV infection in EV, the model proposed also requires the existence of other, yet unknown, CIB1/EVER1/EVER2-independent factors that specifically restrict infection sustained by α-, γ-, and μ-HPVs. Such mechanisms may not necessarily be keratinocyte specific but might also involve other cell types, and their disruption could be involved in the atypical EV associated with severe primary T cell deficiencies.

The article by de Jong et al. (2018) also raises important questions. Several restriction mechanisms have been identified that may operate in keratinocytes to control HPV infection, including activation of the DNA damage response, induction of NF-κB–, TLR-, and c-GAS/STING-mediated signaling, and interferon-mediated responses (Steinbach and Riemer, 2018). While CIB1-deficient keratinocytes have intact NF-κB activation in response to TNF-α and normal intracellular zinc homeostasis, the precise mechanisms through which the CIB1/EVER1/EVER2 complex operates remain unknown. Furthermore, other HPV proteins, including β-HPV E1 and α-HPV16 E2, have also been reported to bind CIB1, and yet the functional implications of these interactions, and their possible relevance to progression of HPV infection, are unknown. Addressing these questions may require development of more adequate tools to analyze expression of EVER1 and EVER2 proteins and confirm their interaction with CIB1 in primary cells and tissues, and of organotypic human skin cultures (or alternative approaches based on differentiation of induced pluripotent stem cells) to model and follow progression of HPV infection,

characterize the biological activity of HPV oncoproteins, and analyze mechanisms of viral evasion that operate at various steps on infection. This may lead to development of novel preventive and therapeutic approaches for severe warts and HPV-related cancer.

Finally, identification of *CIB1* mutations broadens the spectrum of defects affecting extra-hematopoietic, tissue-intrinsic responses against pathogens. Interestingly, many of these defects cause susceptibility to specific viral infections, as in the case of TLR3 signaling defects in central nervous system cells associated with herpes simplex encephalitis, and *IRF7* and *IFIH1* mutations affecting respiratory epithelial cells and causing life-threatening influenza and recurrent rhinovirus infections, respectively. The identification of such defects may help better understand the complex and ever-evolving interaction between viruses and humans.

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