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Beta Genus Papillomaviruses and Skin Cancer

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Abstract

A role for the beta genus HPVs in keratinocyte carcinoma (KC) remains to be established. In this article we examine the potential role of the beta HPVs in cancer revealed by the epidemiology associating these viruses with KC and supported by oncogenic properties of the beta HPV proteins. Unlike the cancer associated alpha genus HPVs, in which transcriptionally active viral genomes are invariably found associated with the cancers, that is not the case for the beta genus HPVs and keratinocyte carcinomas. Thus a role for the beta HPVs in KC would necessarily be in the carcinogenesis initiation and not in the maintenance of the tumor.

Keywords

Papillomavirus; Keratinocyte; Cancer; MAML1; Notch; Epidemiology

Introduction

The papillomaviridae family comprises approximately 200 different human papillomavirus (HPV) types, which are clustered among the five genera alpha, beta, gamma, mu, and nu (www.hpvcenter.se/html/refclones.html). Among the genus alpha HPV types are those that induce benign, non-genital skin warts, the low-risk mucosotropic HPVs that induce genital warts and laryngeal papillomas, and the high-risk mucosotropic HPVs that are associated with cervical cancer, other anogenital cancers and oropharyngeal cancers (Howley, Schiller, and Lowy, 2013).

Genus beta HPV were first identified in flat warts and macular, red, brown, or achromic lesions as well as cutaneous squamous cell carcinomas (SCC) of patients with the rare disease epidermodysplasia verruciformis (EV) and were initially referred to as EV-HPVs. Cutaneous SCC arise in 30% to 60% of EV-patients during the second to fourth decade of life, 10 to 30 years after the onset of benign skin lesions. Cancers are frequently localized in sun-exposed areas of the skin (Orth, 1986). SCC in EV harbor multiple genome copies of

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specific HPV types, especially HPV5 and HPV8 but also HPV14, HPV20, and a few others (de Oliveira et al., 2004; Dell'Oste et al., 2009; Orth, 1986). Viral transcripts have been described in several of these EV-associated SCCs (reviewed in (Pfister and Ter Schegget, 1997)). In 2009, HPV5 and HPV8 were classified by IARC as “possibly carcinogenic” in EV patients (Bouvard et al., 2009).

The oncogenic potential of some of the beta HPV types (described below) and the consistent finding of certain beta genus HPV types in EV-associated SCC make HPV an attractive etiologic agent for at least some SCCs in individuals who do not have EV (Feltkamp et al., 2008). In the general population, the prevalence of beta HPV-DNA in cutaneous SCC is lower than in EV. In contrast to EV, a diverse spectrum of beta HPV types has been detected and no single types predominate. In those positive tumors, the viral load in tumor biopsies is less than one genome per cell (Weissenborn et al., 2005). In addition, transcriptome sequencing failed to identify papillomavirus expression in any of non-EV associated SCCs indicating that beta HPV is not active in cutaneous SCC (Arron et al., 2011). Thus, in contrast to the alpha genus HPV-associated cancers where transcriptionally active HPV DNA is required for tumor maintenance, the presence of a beta HPV is obviously not mandatory for maintenance of the malignant phenotype in SCCs in the general population. Thus if any of the beta HPVs are associated with SCCs in the general population, their role must be in the initiation of the cancer rather than its maintenance. The prevalence of beta HPV-DNA in precancerous actinic keratosis is higher than in SCCs and higher HPV-DNA loads (up to 50 copies/cell) are compatible with a carcinogenic role of the beta HPVs in early phases of skin cancer development (Weissenborn et al., 2005). Active replication and expression of beta HPVs has been demonstrated in actinic keratosis lesions as well as in the adjacent pathological epithelium of SCCs in renal transplant patients, where beta HPV expression is also associated with increased expression of the cellular proliferation marker MCM7 (Borgogna et al., 2014).

Epidemiology of beta HPV infections

The significance of the association of the beta HPVs and skin cancer is challenged somewhat since these HPV types are ubiquitous and infect the skin of all people as a commensal flora. More than 80% of healthy donors were positive for beta HPV DNA in skin swabs or plucked eyebrow hairs where many different beta HPV types are frequently observed (Antonsson et al., 2000; de Koning et al., 2009). HPV positivity can be demonstrated soon after birth (Antonsson et al., 2003; Weissenborn et al., 2009). The hair follicle is regarded as the natural reservoir for the cutaneous beta HPVs. HPV is present in hair follicles from different body sites such as scalp, eyebrow, arm, trunk, leg, and pubic region (Köhler et al., 2007). The beta HPV type spectrum in eyebrow hair follicles is to a significant degree representative of different body sites suggesting generalized infection of the entire skin. Since eyebrow hairs have therefore served as easily collected marker in many recent epidemiological studies (Plasmeijer et al., 2010).

Case control studies of SCC examining beta HPV-DNA and/or serum antibody have revealed moderate odds ratios in the range of 1.5 to 2.8 with lower 95% confidence interval limits between 1.0 and 1.4 (Bouwes Bavinck et al., 2010; Karagas et al., 2006; Struijk et al.,

2003). To assess beta HPV expression serum samples were mostly analyzed for antibodies to the major capsid protein L1. More recently, multiplex serology was used, which allows the simultaneous detection of antibodies against more than 15 beta HPV types. A prospective pilot study based on the prevalence of serum antibodies failed to reveal differences between cases and controls in blood samples taken more than 18 months prior to diagnosis of SCC (Casabonne et al., 2007). This was interpreted to suggest that the antibody response observed in people with SCC is a consequence of tumor formation rather than evidence for previous viral activity and resulted in an IARC working group in 2009 to consider the beta HPVs as not classifiable as to their carcinogenicity in humans (Bouvard et al., 2009). However, this conclusion may not be valid since seroconversion after beta HPV infections has been shown to be extremely slow, occurring years or even decades after initial contact (Michael et al., 2008). Persistent beta HPV infection, as such, may only poorly drive serologic responses without additional “danger” signals such as local inflammation or tumor growth. This could explain that an association with beta HPV seropositivity became only obvious rather late in individuals with prevalent tumors or less than 18 months prior to diagnosis of SCC (Casabonne et al., 2007). Earlier critical viral activities are not excluded by seronegativity. It is highly interesting that beta HPV seropositivity around transplantation implied a hazard ratio of 2.8 to develop KC in organ transplant recipients, who were followed for up to 22 years (Genders et al., 2014).

Recent natural history studies of beta HPV infections have emphasized that the prevalent beta HPVs should be differentiated into types that are transiently detectable and those that persist, with the belief that the beta HPV types that result in persistent infections are more likely to have pathological consequences. Studies of intrafamilial transmission revealed a majority of frequently exchanged beta HPV types and a minority of persisting beta HPV types among individuals (Hsu et al., 2009; Weissenborn et al., 2009). The grouping together of the beta HPV types regardless of whether or not they can cause persistent infections has potentially masked differences among different beta HPV types and has undoubtedly significantly handicapped epidemiologic case control studies. Although more recent epidemiologic studies have tried to focus on the biologically more relevant beta HPV infections, it is still uncertain, which parameters best reflect a biologically relevant beta HPV infection. Different studies have focused on HPV-DNA positivity in the presence of a type specific serological response (Proby et al., 2011), on high viral DNA loads and/or multiple HPV types present (Neale et al., 2013), and/or the presence in the SCC of a patient (Iannacone et al., 2014; Iannacone et al., 2012).

The beta HPV-DNA loads in plucked eyebrow hairs of immunocompetent and immunosuppressed people span 7 orders of magnitude (Weissenborn et al., 2012). Only the beta HPV loads within the highest load decile are close to those observed in EV patients, who are highly predisposed to SCC development (Dell'Oste et al., 2009). The significantly higher proportion of samples from immunosuppressed individuals within the highest load decile correlated with a more than 100-fold increased incidence of cutaneous SCC (Weissenborn et al., 2012). In case control studies, high beta HPV-DNA loads turned out to be associated with cutaneous SCC (Neale et al., 2013). Odds ratios of three were observed for having four or more beta HPV types in the highest load tertile in plucked eyebrow hairs

both in immunocompetent Australians (95% CI: 1.11 – 8.09) and in immunosuppressed organ transplant recipients (95% CI: 1.35 – 7.62).

When the association between beta HPV type-specific seropositivity and SCC was stratified by DNA status of the same HPV type in the SCC, significant odds ratios of 3.4 – 3.8 were observed for HPV5, HPV17, and HPV24 (Iannacone et al., 2012). When the association between beta HPV type-specific DNA-positivity in eyebrow hairs and SCC was stratified by DNA status of the same HPV type in the SCC, the DNA prevalence in eyebrow hairs was significantly higher among cases compared to the controls for 11 of 25 genus beta types tested (HPV5, -12, -19, -21, -24 of species beta 1, HPV22, -23, -38, -80 of species beta 2, HPV75 of species beta 3, and HPV92 of species beta 4) (Iannacone et al., 2014). Striking differences were observed in genus beta type-specific DNA loads in plucked eyebrow hairs between controls and SCC cases with DNA of the same HPV type in the tumor (Fig. 1).

Taken together, recent case control studies have revealed weak beta HPV type-specific associations of seropositivity, DNA prevalence and viral load with SCC. The meaning of the multiplicity of infections and of viral load may provide some suggestion of a dose-response relationship. These observed, although weak associations are consistent with the potential for an etiological role for a subset of the beta HPVs that can establish persistent infections when combined with the growing appreciation for the potential oncogenic activities encoded by the beta HPVs.

Oncogenicity of beta HPVs

The potential oncogenicity of the beta HPVs has been examined using tissue culture transformation and immortalization assays as well as transgenic mice. Several studies have compared various beta HPV types with respect to the ability of their E6 and E7 proteins to transform rodent cells and to immortalize human foreskin keratinocytes (HFKs) (Cornet et al., 2012; Massimi et al., 2008). The oncogenicity of the beta HPVs however is perhaps best demonstrated in transgenic mouse studies. The complete early genome region of HPV8 or the E6 and E7 genes of HPV38 under the control of the human keratin 14 promoter are oncogenic (Schaper et al., 2005; Viarisio et al., 2011). HPV8 mice spontaneously develop papillomas with varying degrees of epidermal dysplasia and SCC develop in 6% of the mice without any additional treatment with physical or chemical carcinogens (Schaper et al., 2005). The HPV38 E6/E7 transgenic mice are highly susceptible to two stage chemical carcinogenesis. Chronic UV irradiation induced actinic keratosis-like lesions and subsequently SCC (Viarisio et al., 2011).

HPV8 E6 (Marcuzzi et al., 2009) and HPV8 E2 (Pfefferle et al., 2008) are independently able to induce skin cancer but with marked differences in kinetics and histology. The HPV8 E6 mice are a phenocopy of mice expressing the complete early genome region, whereas the HPV8 E2 transgenic mice spontaneously develop infundibular hyperplasia and acanthosis and the SCC show an unusually high proportion of spindle cells. The rate of tumor formation in E2 mice critically depends on E2 mRNA levels. In three established lines the E2 mRNA levels differed in the ratio 6:3:1. The mice with the highest E2 mRNA levels developed tumors in their first year of life, only about 60% of mice with medium mRNA

levels developed tumors at the end of their second year of life and only few animals of the line with the lowest mRNA levels developed tumors at an age of more than two years. In the HPV8 transgenic mice, a single UV irradiation or mechanical wounding are highly efficient triggers of papilloma development within three weeks. HPV8 oncogene mRNA is induced about 10-fold one day after UV irradiation (Hufbauer et al., 2010; Marcuzzi et al., 2009). Even about one third of the E2 mice with the lowest mRNA level developed skin lesions within three weeks after UV irradiation and a highly aggressive SCC developed in one case within 13 weeks (Pfefferle et al., 2008). Knocking down HPV8 E6 expression by tattooing gene specific siRNA delayed and partially prevented papilloma development induced by wounding in the course of tattooing (Hufbauer et al., 2010). These studies identify enhanced HPV8 oncogene expression as a necessary prerequisite for tumor development and suggest oncogene expression above a certain threshold can induce SCCs. In certain aspects, the mouse model parallels the natural history of human beta HPV infections. Despite continuous, low level expression of the oncogenic transgene in all skin keratinocytes, it takes months and sometimes more than one year to develop skin tumors without experimental induction. Beta HPV infections in humans are ubiquitous and acquired in early childhood but usually very well controlled, persisting with low viral DNA loads. Extensive sun exposure and impaired control following immunosuppression or late in life due to immunosenescence are likely to induce oncogene expression and to allow transgression of a critical threshold of mRNA levels in line with the increased risk of skin cancer. These transgenic models establish the potential oncogenicity of specific beta HPVs. As potential cancer viruses in humans the role of the beta HPVs must however be at an initiation role since their continued expression would not be required in human SCCs.

Oncogenic characteristics of the beta HPV E6 proteins

The high-risk alpha HPVs encode three oncogenes (E5, E6 and E7) of which E6 and E7 are expressed in the associated cancers. Although the beta HPVs do not encode an E5 gene, the beta E6 and E7 genes are structurally quite similar to their alpha genus counterparts. Recent proteomic studies have highlighted similarities and differences between the alpha and beta genus E6 and E7 proteins.

The E6 proteins have been implicated in the potential carcinogenic activities of the beta HPVs. The skin cancers observed in EV patients mostly occur in sun exposed areas suggesting a synergism between the beta HPV infections and the carcinogenic effects of UV light. An important contribution to SCC development could come from interference of the beta E6 proteins with DNA repair and UV-induced apoptosis (Giampieri and Storey, 2004; Jackson and Storey, 2000; Underbrink et al., 2008), allowing keratinocytes with UV-induced mutations to survive and progress to carcinomas. The E6 proteins of HPV5, -8, -20, -22, -38, -76, -92, and -96 have been reported to target the proapoptotic protein Bak for degradation and thus prevent UV-induced apoptosis (Jackson and Storey, 2000; Underbrink et al., 2008). E6 of HPV5 and HPV8 bind p300 resulting in delayed ATR activation (Muller-Schiffmann, Beckmann, and Steger, 2006; Wallace et al., 2012). The delayed ATR activation leads to delayed accumulation of p53, reduced G1 arrest, the increased likelihood of double strand breaks, and delayed repair of UV-damaged DNA. The beta HPV E6 proteins vary however in their effects on p53 stabilization following DNA damage (White et al., 2014) and several

(HPV5, -8 and -38) have been shown to inhibit its stabilization following DNA damage (Wallace, Robinson, and Galloway, 2014). Several of the beta E6 proteins have been shown to induce telomerase activity resulting in life span extension (Bedard et al., 2008; Gabet et al., 2008). These functions would support a potential role for the beta HPVs in the initiation of SCC, but not be required for tumor maintenance.

Systematic proteomic studies of the HPV E6 proteins from both the alpha and beta genera have revealed that the alpha HPV E6 proteins are bound to the ubiquitin ligase E6AP whereas the beta HPV E6 proteins are bound to the transcriptional co-activator mastermind (MAML1) (White et al., 2012a). MAML1 is a transcriptional regulator that is involved in several cell signaling pathways and the result of the E6-MAML interaction is the inhibition of Notch mediated transcription (Brimer et al., 2012; Meyers, Spangle, and Munger, 2013; Tan et al., 2012). Although Notch functions as an oncogene in some T-cell leukemias, Notch acts as a tumor suppressor in squamous epithelial cells (McElhinny, Li, and Wu, 2008; Wu et al., 2000). Notch has been found often to be mutated in head and neck cancers (Agrawal et al., 2011; Stransky et al., 2011). Notch has a role in keratinocyte differentiation; its activation leads to induction of the cell cycle inhibitor p21 as well as the expression of keratinocyte cellular differentiation markers (Rangarajan et al., 2001; Restivo et al., 2011). In addition to binding MAML1, most of the beta E6 proteins are also found in complex with several MAML1 binding partners including Notch1 and RBPJ (Rozenblatt-Rosen et al., 2012; White et al., 2012a).

The genus-specific interactions of the alpha genus E6s with E6AP and the beta genus E6s with MAML1 respectively involve a very similar alpha helical LXXLL binding motif in each of these cellular proteins (Brimer et al., 2012; Chen et al., 1995; Nuber et al., 1996; Tan et al., 2012). This LXXLL motif is believed to be engaged by a flexible linker region on E6 located between the two globular domains that compose the N- and C-terminal halves of E6s (Nomine et al., 2006).

Aside from MAML1 and other Notch associated components, no other cellular proteins complex with all of the beta E6 proteins (White et al., 2012a). A number of other cellular proteins have been shown to bind the beta E6 proteins, but in each case only a subset of E6s (White and Howley, 2013). For instance, several studies have shown that E6 from HPVs 5, 8, 20 and 25 (all from genus beta, species 1) bind to the acetyltransferases CBP and p300 (Howie et al., 2011; Rozenblatt-Rosen et al., 2012; White et al., 2012a). E6 from HPV38 (genus beta, species 2) binds p300 much weaker than E6 from species 1 HPV types (Howie et al., 2011; Muench et al., 2010). In addition, several of the beta E6s (HPV38 and 92) bind and stabilize p53 (White et al., 2012a), however in response to DNA damage, HPV 38 is reported to attenuate p53 signaling (Wallace, Robinson, and Galloway, 2014). Human keratinocyte cell lines expressing various E6 proteins have been profiled for their transcriptional responses to DNA damage and the beta E6 proteins vary in their ability to block such responses (White et al., 2014). Finally, it HPV5 E6 has been to bind SMAD3 and repress TGF β signaling (Mendoza et al., 2006). These observed differences in biological responses combined with the differences among E6 cellular binding partners indicate that the beta HPVs are not homogeneous with regards to their biologies or the pathologies with which they are associated.

Oncogenic characteristics of the beta HPV E7 proteins

Results from systematic proteomic studies of the HPV E7 proteins revealed patterns of cellular binding proteins that were quite different from those seen with the E6 proteins. Common binding partners were seen for E7 proteins of both the alpha and beta genera suggesting that functions are conserved among the various E7 proteins. The beta E7 proteins, like the alpha E7 proteins, all bind pRB1. The alpha and beta E7 proteins all contain an LXCXE motif responsible for binding to pRB1 and the related 'pocket proteins' pRBL1 (p107) and pRBL2 (p130) (White et al., 2012b). In addition to the pRB family of pocket proteins, the pRB E2F partners were observed binding to many of the E7 proteins. There have also been a number of specific studies of the beta HPV E7 proteins with pRB. For instance, HPV38 E7 has been shown to bind pRB with a similar efficiency as HPV16 E7 and promote pRB destabilization (Caldeira et al., 2003). HPV8 E7 binds to pRB only weakly but is nevertheless able to inactivate pRB to deregulate G1/S transition control (Akgül et al., 2007). It is interesting to note in this context that UV-induced oncogene expression in HPV8 transgenic mice was associated with an upregulation of the oncogenic miRNA 106a and a downregulation of its known cellular target pRB (Hufbauer et al., 2011).

In addition to pRB all the alpha and beta HPV E7 proteins bind UBR4, also known as p600 (White et al., 2012b). UBR4 was initially shown to bind E7s from HPV16, 6b, and 11 as well as from the bovine papillomavirus (BPV1) (DeMasi et al., 2005; Huh et al., 2005). UBR4 contains a UBR box, a motif that is common to proteins involved in the N-end rule-mediated degradation of proteins (Tasaki et al., 2005; Tasaki et al., 2009), and it is suspected that it is a functional E3 ubiquitin ligase that functions in an N-end rule pathway. The conservation of the UBR4 interaction with E7s across several PV genera, suggests this interaction is likely to mediate important PV-related functions that have not yet been discovered. In addition, all E7's complex with KCMF1, another putative E3 ubiquitin ligase that is a binding partner of UBR4 (White et al., 2012b).

Another HPV E7 cellular binding partner shared across the alpha and beta genera is PTPN14, a non-receptor tyrosine phosphatase (White et al., 2012b). PTPN14 has been implicated in density-dependent cell growth (Wang et al., 2012). PTPN14 binds YES1, a regulator of Hippo signaling, and PTPN14 negatively regulates YES1 when cells are at high density. Nothing is known yet about the consequences of E7 binding to PTPN14 and role of this binding in PV biology, but E7/PTPN14 binding suggests the possibility that E7 may affect the Hippo pathway.

Concerning the potential oncogenic role of the beta E7s, it is noteworthy that HPV8 E7 upregulates the expression of the invasion-promoting MT1-matrix metalloproteinase (Akgül et al., 2005; Smola-Hess et al., 2005) and causes invasion of keratinocytes into the dermis of organotypic keratinocyte cultures (Akgül et al., 2005). E7 of HPV5, -8, and -20 increases the number of stem cell-like keratinocytes, defined by high expression of surface markers CD44 and EpCAM, with increased clonogenicity and strongly positive in tumor sphere assays (Hufbauer et al., 2013).

Oncogenic characteristics of the beta HPV E2 proteins

As noted above, the most compelling evidence for the oncogenicity of E2 derives from transgenic mouse experiments in which HPV8 E2 is expressed from the human keratin 14 promoter (Pfefferle et al., 2008). The E2 gene is highly conserved among all papillomaviruses and has key roles in the regulation of virus transcription, viral DNA replication and in plasmid maintenance in dividing cells (Howley, Schiller, and Lowy, 2013). The C-terminal portion of E2 is conserved and consists of a dimerization and sequence specific DNA-binding domain that recognizes cognate sites within the non-coding region upstream of the E6 and E7 genes and adjacent to the origin of DNA replication. The N-terminal portion of E2 is also conserved and binds the viral E1 helicase protein to help recruit it to the origin to initiate viral DNA replication. The conserved N-terminus also binds a number of cellular proteins that are involved in mediating E2's functions. Proteomic studies have defined many binding partners for the alpha and beta genus E2 proteins that are involved in transcription, cell cycle control, RNA processing and apoptosis, among other processes (Muller et al., 2012). The oncogenicity of HPV8 E2 is not yet well understood but could involve the transcriptional regulation of a cellular gene or genes or it could be the consequence of binding to a specific cellular protein or complex.

Summary: A Role for the Beta HPVs in Keratinocyte Carcinoma

The consideration of an etiologic role for the beta HPVs in KCs has been framed largely by the knowledge of the mechanisms by which the alpha genus HPVs cause cancer in which the expression of the viral oncoproteins are required for the maintenance in addition to the initiation of the cancers. Clearly that is not the case for the KCs in which viral transcription is not detected and viral genome load in the cancers is significantly less than one copy per cell. Given the ubiquitous nature of the beta HPVs, epidemiologic studies have proven difficult. The increase of KCs in immunosuppressed patients is highly suggestive of a viral etiology to the cancers (Wieland, Kreuter, and Pfister, 2014). However, a role for these viruses in KCs must be at the early stages of cancer formation; the viruses must participate in cancer initiation.

A number of studies have established the potential oncogenicity of the viruses and their individual genes in transgenic mice as well as in tissue culture models. Furthermore detailed studies of the viral E6 and E7 genes provide mechanistic insights as to how the beta HPVs might participate in initiating carcinogenic processes. The increased risk of KCs in sun exposed areas in patients with EV and in immuno-suppressed patients suggests a synergism between DNA damage from UV light and a mechanistic contribution from the beta HPVs. Several studies with specific beta HPV E6s have shown that they can block DNA damage pathways and inhibit apoptosis in mechanisms that appear to involve E6 binding p300 and /or CBP. The beta E6 proteins also target MAML1, a necessary component of Notch signaling, to inhibit Notch signaling in keratinocytes. Notch plays a role in keratinocyte differentiation and functions as a tumor suppressor gene in epithelial cells. Therefore, inhibiting Notch signaling in keratinocytes can inhibit differentiation and perhaps contribute to the continued proliferation of the HPV infected cell. Through targeting p300 as well as MAML1 and Notch signaling, E6 could function in cancer initiation by allowing a beta HPV

infected cell to accumulate the genomic mutations necessary to progress to cancer. The beta HPV E7s also have oncogenic activities. Its binding partners are similar to those of the alpha genus HPVs, and include the pRB family of pocket proteins that are well established as tumor suppressor genes. They also include binding proteins that have been less well studied with regard to their roles in papillomavirus host cell interactions, including UBR4 and PTPN14 (White et al., 2012b).

A major unanswered question assuming the beta HPVs indeed do have an etiologic role in the initiation of KC is why the presence of the viral genome and its expression are not detectable in the cancers. Is there active selection against the beta HPVs? If so, is this selection immunological, or is continued expression of the virus toxic to the cell? One trivial explanation would be that the beta HPV DNA is lost because its expression is not required for maintenance of the cancer and there is therefore no selection for its persistence. In contrast to the alpha HPV-positive anogenital cancers integration of beta HPV DNA into the cellular genome appears to occur extremely rarely in EV-KC. In one EV-patient HPV5 DNA was not yet integrated in the primary cancer but only in a metastasis (Yabe et al., 1989). Therefore if the major contribution of the beta HPVs to KC is at the initiation stage and viral functions are not required for cancer maintenance since the malignant growth of cancer cells is driven by the accumulated DNA mutations, there would be no selective pressure to maintain the viral DNA.

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The beta HPVs have been associated with skin cancers, but an etiological role remains to be proven.

A role for the beta HPVs in non-melanoma skin cancer would be in initiation, not maintenance.

The beta E2, E6 and E7 genes may each have oncogenic functions.

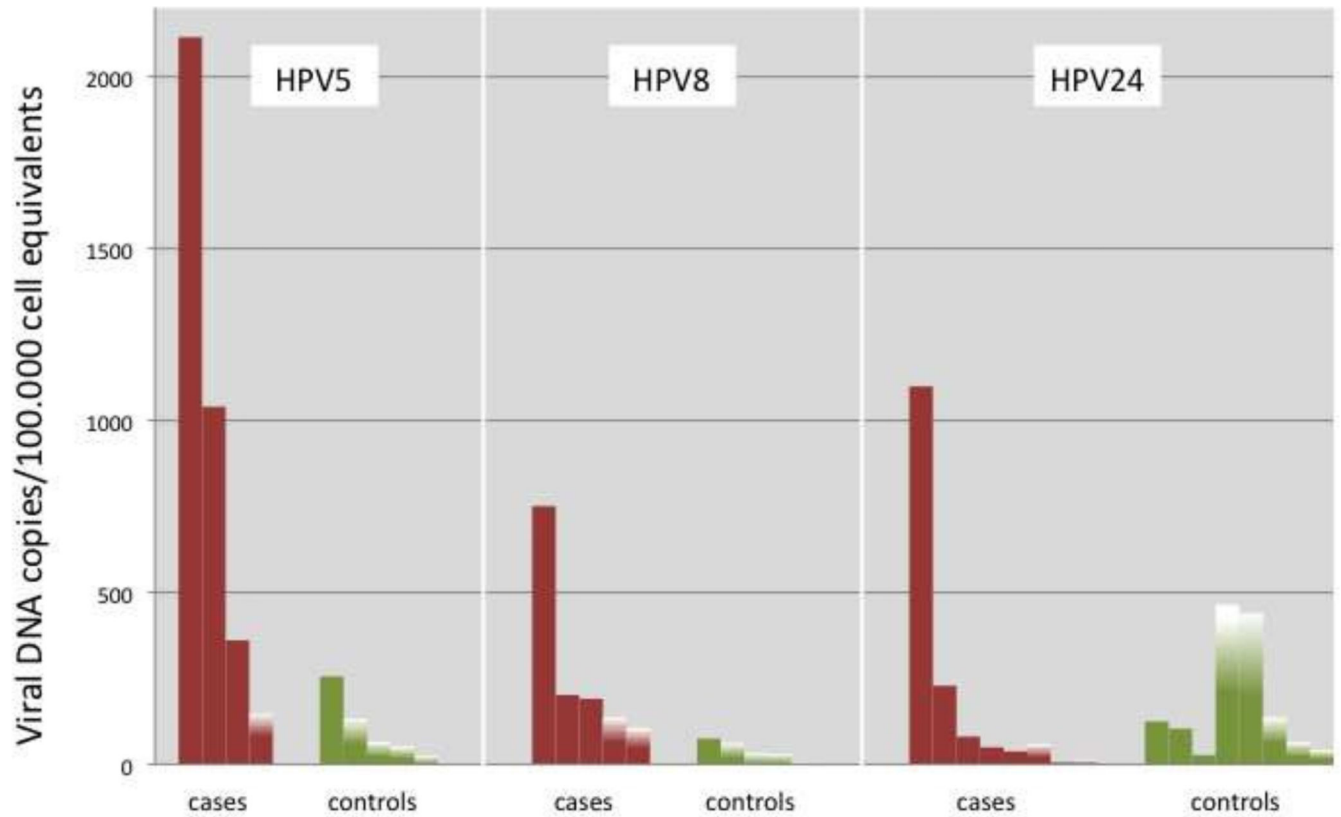


Figure 1.

Genus beta type-specific DNA loads in plucked eyebrow hairs of controls and SCC cases who were concordant for a single HPV type in their eyebrow hair and tumor tissue. Controls had a single HPV type in their eyebrow hair that was the same detected in the SCC cases. Fully colored bars show the result of quantitative PCR. Partially colored bars represent quantitative PCR negative but qualitative PCR positive samples with the height giving the threshold of detection of quantitative PCR (Iannacone et al., 2014).