Human Papillomaviruses Associated with Epidermodysplasia Verruciformis in Non-Melanoma Skin Cancers: Guilty or Innocent?

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Non-melanoma skin cancers (NMSC), namely basal cell carcinomas (BCC) and squamous cell carcinomas (SCC), represent the most common form of malignancy in fair-skinned populations. Solar ultraviolet radiation (UVR), immunosuppression, and genetic constitution of the host are well-known risk factors for the development of NMSC. Epidermodysplasia verruciformis (EV), a rare genetic disease associated with a high risk of skin cancer, provided the first clue to a possible role of viruses in human skin carcinogenesis (Jablonska et al, 1972). Due to mutations in either of the related EVER1 and EVER2 genes, EV patients are abnormally susceptible to a specific group of related human papillomavirus genotypes (known as EV HPV) and to the oncogenic potential of some of these viruses (Orth, 1987; Ramoz et al, 2002). EV HPV cause widespread, inapparent infections in the general population (Antonsson et al, 2003). In EV patients, however, infection leads to persistent wart-like and macular skin lesions, and premalignant lesions and SCC develop on sun-exposed areas of the skin in about half of the patients. EV SCC harbor high copy numbers of episomal HPV genomes (HPV5 or occasionally HPV8, 14, 17, 20, or 47) and abundant transcripts of the E6 and E7 genes. Phenocopies of EV are exceptionally observed in immunosuppressed patients. EV is thus a rare experiment of nature that provides a model for studying the interaction among potentially oncogenic HPV genotypes, UVR, immunity, and genetic factors (Orth, 1987).

EV HPV DNA sequences have only rarely been detected in cutaneous premalignant tumors and NMSC in non-EV patients by Southern blot hybridization, which is sensitive enough to detect one HPV genome per cell. Using highly sensitive PCR approaches, however, HPV DNA sequences were detected in 25%–65% of NMSC in immunocompetent individuals and in up to 90% of SCC in organ transplant recipients (OTR), a population at a greatly increased risk of developing SCC. Similar HPV detection rates were found for actinic keratoses (AK), the precursor lesion of SCC (reviewed in Forslund et al, 2004). These data led researchers to consider a role for HPV, especially EV HPV types, in the development of NMSC (Orth, 2004).

Available epidemiological and seroepidemiological data provide some support to an association between the presence of EV HPV DNA in normal skin or eyebrow hairs or the seroreactivity to some EV HPV and an increased risk of AK and SCC (reviewed in Purdie et al, 2005; Weissenborn et al, 2005). The link between EV HPV and the development of NMSC in immunocompetent and immunosuppressed individuals, however, remains unclear (reviewed in Orth, 2004). EV HPV and non-EV cutaneous HPV types represent impressively diverse, ubiquitous viruses, which are highly prevalent in the normal skin of healthy adults (Antonsson et al, 2003). A great diversity of cutaneous HPV types, most frequently EV HPV or putative novel EV HPV-related types, is detected in AK and NMSC, and mixed infections are frequently observed in tumors of OTR patients. Furthermore, it has recently been shown that cutaneous HPV DNA is common on top of AK, SCC, and BCC but less prevalent (3.2–7.9-fold) in “stripped” tumors, suggesting that HPV DNA positivity may reflect, in part, contamination of the surface of the tumors by viral DNA or particles shed from infected healthy skin (Forslund et al, 2004). It should be also stressed that the tumor HPV DNA loads are usually low and that it remains unknown whether the viral sequences are transcriptionally active. The articles by Weissenborn et al (2005) and by Purdie et al (2005), both published in this issue of the Journal, address these questions about the role of EV HPV in skin carcinogenesis.

Weissenborn and colleagues determined HPV DNA loads by quantitative, type-specific, real-time PCR (Q-PCR) in a series of specimens of AK, NMSC, perilesional tissues, and SCC metastases. The majority of the samples were obtained from immunocompetent patients, and all of them had previously been found by PCR to harbor EV HPV types 5, 8, 15, 20, 24, or 36. For 40% of the 78 samples yielding conclusive results, including almost all metastases and half of the NMSC specimens, viral loads were beyond the detection limit (two HPV DNA copies in the assay). For the other specimens, HPV DNA viral loads ranged from 1 copy per 14,200 cell equivalents to 1 copy per 0.02 cell equivalents. An interesting finding was that the viral loads detected in AK were significantly higher than in SCC, whereas similar viral loads were observed for SCC, BCC, and perilesional tissues. Viral loads of 1 HPV DNA copy per less than 50 cells were found in 40% of 25 AK, but in only 8% of 13 BCC and 15% of 13 SCC. Only a few scattered, positive nuclei were detected by a very sensitive in situ hybridization (ISH) method in sections of NMSC and perilesional skin, in agreement with their similar low HPV DNA loads detected by Q-PCR. The localization of HPV DNA in AK was not investigated.

The aims of the study of Purdie and colleagues were both to search for the presence of EV HPV DNA sequences in tumor cells and to determine whether these sequences...
were transcriptionally active in SCC (mostly OTR tumors) and in OTR warts. Specimens were previously found by PCR to harbor EV HPV types 5, 14, 15, 20, or 23, and further analyzed by DNA–DNA or RNA–RNA ISH. EV HPV DNA sequences were detected in 27.3% of 11 SCC found positive by PCR, whereas the 2 warts tested were found negative. Viral transcripts were detected in 38.5% of 13 SCC and 20% of five warts, mainly in the mid to upper epidermal layers. The four SCC illustrated showed positive areas, or clusters of positive cells, contrasting with the labeling pattern reported by Weissenborn et al. Nest of enlarged keratinocytes with pale stained cytoplasms, reminiscent of the cytopathic effect specific of EV HPV, were observed in the carcinoma in situ region of one OTR SCC. High amounts of HPV20 DNA and both early and late transcripts were detected in these cells. It seems likely that this case represents an EV phenocopy. Transcripts of the E2/E4 region (highly expressed during the late phase of the viral life cycle) were detected in the other specimens (including three SCC and one wart found negative for viral DNA by ISH), whereas no transcripts of the viral E6 and E7 oncogenes or L1 and L2 capsid genes were detected. Unfortunately, HPV DNA loads were not determined and viral transcripts were not characterized. This would have helped to appreciate the sensitivity and specificity of the ISH method.

Their data led Weissenborn and colleagues to several conclusions. Although a passenger state cannot be excluded, the higher viral loads found in AK, compared with SCC, are compatible with a carcinogenic role of EV HPV in the early steps of tumor progression. For NMSC, the presence of HPV does not seem to be required for the maintenance of the malignant state, and the similar low viral loads found in perilosomal skin may suggest that HPV is more a passenger than a driver. Whether the presence of HPV in some tumor cells may have an impact on growth and invasion properties of the tumor, as proposed by the authors, would be worth considering. The study of Purdie and colleagues brings some evidence for EV HPV gene expression in a proportion of SCC, which may suggest an active rather than a latent or passenger status. The detection and characterization of viral E6 and E7 transcripts in tumor cells, however, remain to be achieved. Overall, the data reported in the two articles should stimulate further work on the contribution of EV HPV to the development of NMSC.

When considering the possible implications of their findings, the authors refer to functional studies involving ectopic overexpression of E6 and E7 proteins of EV and non-EV cutaneous HPV genotypes (reviewed in Storey, 2002). For instance, the E6 proteins of several cutaneous HPV have been shown to promote the degradation of the proapoptotic Bak protein induced in keratinocytes by UVR, and this antiapoptotic effect could favor the accumulation of UVR-induced mutations. A crucial question is whether the E6 and E7 proteins of EV HPV or non-EV cutaneous HPV (less frequently detected in NMSC) behave as oncoproteins when expressed in vivo, under the control of their own promoter. It should be kept in mind that infection in EV patients leads to the development of disseminated, widespread, persistent benign lesions of the skin. About 20 specific HPV types may be characterized in these lesions, and patients are usually infected by more than one genotype (up to 11).

The abundant replication of these viruses leads to a specific cytopathic effect, and ISH experiments usually detect a single EV HPV genotype in each lesion. Upon their third or fourth decade, about half of the patients start developing AK and Bowen's carcinoma in situ, which evolve into invasive NMSC (mostly SCC). In contrast with the great diversity of EV HPV observed in benign lesions, EV carcinomas are predominantly associated with HPV5 or, in single cases, with HPV8, 14,17, 20, or 47, and high levels of transcripts of the E6/E7 region are detected. This indicates that only a subset of EV HPV is endowed with an oncogenic potential. EV cancers usually arise on sun-exposed areas of the skin and a synccarcinogenic role of HPV5 and UVR is supported by the frequent mutations of the p53 gene in premalignant and malignant EV tumors (Padlewska et al., 2001).

EV HPV cause widespread asymptomatic infections in the general population (Antonsson et al., 2003). Recent studies have disclosed that EV HPV infections are under the control of two genes of unknown function, EVER1 and EVER2, which are mutated in EV patients (Ramoz et al., 2002). Furthermore, an EV-like syndrome has been found to occur frequently after hematopoietic stem-cell transplantation in patients with a severe combined immune deficiency caused by common γc cytokine receptor subunit or JAK-3 deficiency. This may suggest that γc/JAK-3 signaling in keratinocytes could have a role in EV HPV immunity (Laffort et al., 2004). Focusing not only on HPV but also on the genes controlling these viruses should provide clues to our understanding of the role of EV HPV in the skin carcinogenesis process in non-EV patients.

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References