CUTANEOUS MALIGNANCY

The Viral Etiology of Skin Cancer

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The concept that viruses may be etiological agents of cancers is as old as the discovery of viruses themselves. In 1908, 3 years before Peyton Rous was passaging what later became to be known as Rous Sarcoma Virus in chickens, two Danish scientists, Ellerman and Bang, were characterizing a transmissible filtrate that reproducibly caused leukemia in chickens. These findings were received with harsh skepticism, and the scientific community did not universally accept the concept that tumors could be caused by transmissible agents. Richard Shope, a colleague of Peyton Rous at the Rockefeller Institute, identified an infectious agent that infected cottontail rabbits. It caused cutaneous papillomas that could grow to be quite large and which may be the basis of sightings of the mystical and ravenous ''Jackelope'' of southwestern American lore. Shope later collaborated with Rous to demonstrate that exposure of these papillomas to coal tar or infection of a host that does not support viral replication caused malignant progression to skin cancers. This infectious agent, the cottontail rabbit papillomavirus or *Sylvilagus floridanus* Papillomavirus 1, was the first virus linked to a cancer in a mammalian host (Javier and Butel, 2008; Moore and Chang, 2010) (Figure 1).

PAPILLOMAVIRUSES

Papillomaviruses are small, non-enveloped viruses with double-stranded circular DNA genomes of approximately 8,000 bp in size. Transcription is unidirectional, i.e., only one of the two strands is known to encode genetic information. Papillomavirus genomes consist of three major regions: an early region that encodes five to seven non-structural, regulatory ''E'' open reading frames, the late region encoding the major and minor capsid proteins, L1 and L2, respectively, and a non-coding region referred to as the ''long control region'', which contains sequences that regulate viral gene transcription and genome replication. Papillomaviruses have been detected throughout the animal kingdom. They are highly species specific and infect squamous epithelia. Papillomaviruses have been classified based on the degree of sequence identity and are referred to as genotypes. More than 170 human papillomavirus types have been characterized and most of them fall within the alpha, beta, gamma, and, mu genera (Bernard *et al.*, 2010).

BETA HUMAN PAPILLOMAVIRUSES AND NON-MELANOMA SKIN CANCERS

Around the same time that Shope and Rous discovered that cottontail rabbit papillomavirus caused skin cancers in rabbits, Felix Lewandowsky and William Lutz described a rare skin disorder that would be known as epidermodysplasia verruciformis (EV; Lewandowski and Lutz, 1922). EV patients develop widespread wart-like lesions that can cover entire portions of their skin and frequently develop malignant skin tumors, particularly at sun-exposed areas. Seminal work by Stefania Jablonska and Gerard Orth linked human papillomavirus (HPV) infections with skin lesions and cancers in EV patients (Orth *et al.*, 1978). This work predates Harald zur Hausen's discovery of the mucosal-specific alpha-type HPVs, HPV16, and HPV18, as etiological agents of cervical carcinoma. EV patients suffer from a deficiency that prevents effective clearance of beta HPV infections. Interestingly, however, EV patients do not seem to be at a higher risk for bacterial or other viral infections, including alpha HPV infections (Gewirtzman *et al.*, 2008).

The genetic basis of EV was discovered in 2002 when Favre and colleagues discovered that EV patients harbored mutations in either one of two adjacent genes, *TMC6* or *TMC8*, on chromosome 17 (Ramoz *et al.*, 2002). These genes encode the transmembrane proteins, EVER1 and EVER2, which localize to endoplasmic reticulum membranes and may be involved in intracellular zinc transport. How this relates to susceptibility to persistent cutaneous HPV infections remains to be fully delineated.

Beta HPV genomes can readily be detected in tumor cells of EV patients and also are likely etiologic agents of non-melanoma skin cancers (NMSCs) that arise in chronically immune-suppressed patients (Majewski and Jablonska, 2002; Proby *et al.*, 2011; Iannacone *et al.*, 2013; Neale *et al.*, 2013). Whether or not beta HPV infections also contribute to NMSCs in other patients has been a matter of debate, mostly because subclinical beta HPV infections are very widespread and not every tumor cell is HPV positive in these patients (Arron *et al.*, 2011). As detailed below, this does not rule out, however, that infections with some beta HPVs may be drivers of NMSC initiation in the general population.

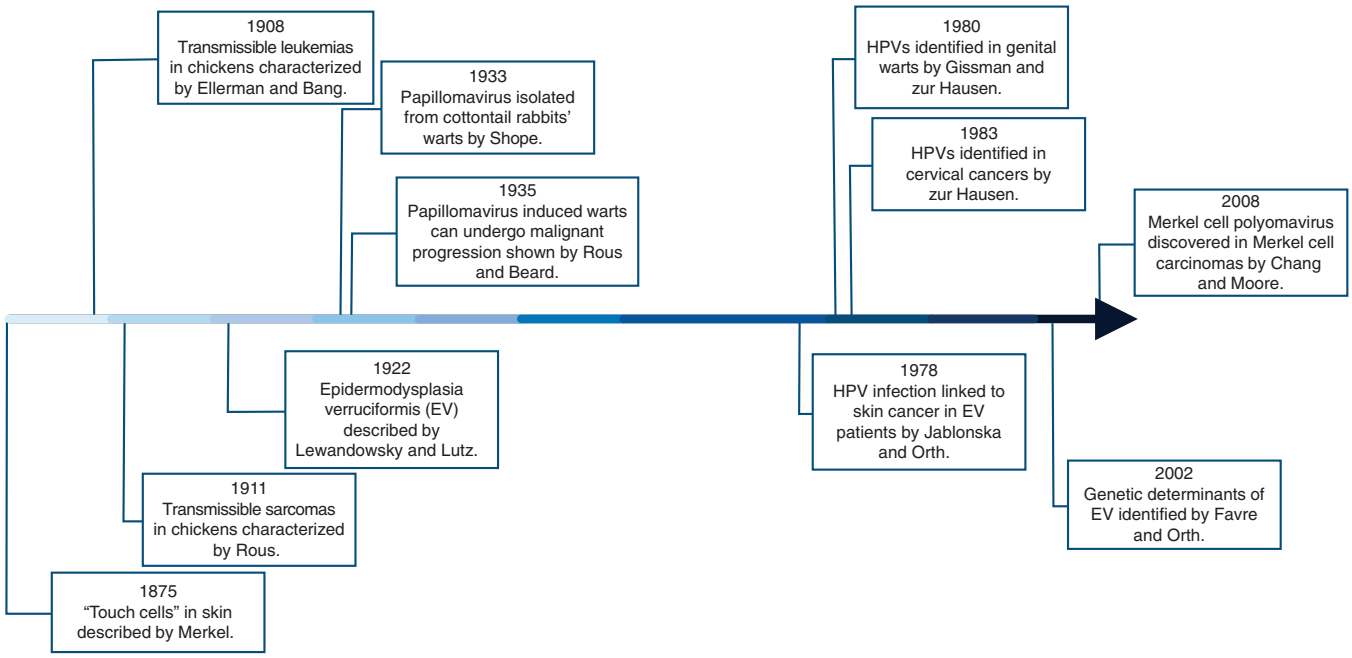

Figure 1. Milestones in the viral etiology of skin cancer. Major discoveries in the field are indicated on a time scale. See text for details and references.

MECHANISTIC CONTRIBUTIONS OF BETA HPVS TO NMSC DEVELOPMENT

Most of the fundamental concepts of how HPVs contribute to human cancer formation have been established by studies with alpha HPVs, which preferentially infect mucosal epithelia. These HPVs have been studied extensively and they fall into "high-risk" and "low-risk" groups based on their propensities to cause lesions that can undergo malignant progression. Notably, high-risk alpha HPV infections cause almost all cases of cervical carcinomas, a significant fraction of other anogenital tract tumors as well as oropharyngeal cancers. Overall, approximately, 5% of all human cancers are caused by high-risk alpha HPV infections. These cancers regularly maintain viral gene expression; every tumor cell generally contains and expresses HPV sequences, and they remain "addicted" to expression of the E6 and E7 oncogenes. The high-risk alpha HPV E6 and E7 proteins target and functionally compromise the p53 and retinoblastoma (pRB) tumor suppressors, respectively, which are frequently mutated in non-HPV-associated cancers.

It has been proposed that beta HPVs may be similarly classified into "high-risk" and "low-risk" groups. HPV5 and the phylogenetically related HPV8 have been originally isolated from NMSCs arising in EV patients (Fuchs *et al.*, 1986; Zachow *et al.*, 1987). Hence, these viruses may be considered "high-risk" for NMSC development in EV patients. Experiments with transgenic mice are consistent with this model. Expression of the early coding region of HPV8 from the basal keratinocyte-specific keratin 14 promoter causes spontaneous development of malignant skin tumors in transgenic mice (Schaper *et al.*, 2005). Additional studies revealed that HPV8 E6 and, surprisingly, E2, scored as the major transforming proteins in this model (Pfefferle *et al.*, 2008; Marcuzzi *et al.*, 2009). While these tumors will arise spontaneously, UV irradiation dramatically accelerates carcinogenesis, thereby recapitulating a key risk factor of EV-associated cancers.

Unlike what has been reported for high-risk alpha HPVs, the HPV5 and HPV8 E7 proteins only weakly associate with and do not destabilize pRB, and similarly the E6 proteins do directly inhibit p53 activity (Caldeira *et al.*, 2003; Rozenblatt-Rosen *et al.*, 2012; White *et al.*, 2012a, b). HPV5 and HPV8 E6 proteins, however, have been reported to inhibit proapoptotic factors activated during UV damage and impair DNA damage response pathways. Several groups have reported that beta HPV E6 proteins can trigger the degradation of the proapoptotic BCL2 family member BAK through a proteasome-dependent pathway (Jackson *et al.*, 2000; Underbrink *et al.*, 2008). BAK is normally retained in the mitochondria but is released and induces apoptosis following UV exposure. BAK degradation in beta HPV-infected cells may, therefore, blunt the apoptotic response to UV irradiation and allow survival of cells that have suffered extensive DNA damage and possibly acquired oncogenic mutations.

There is evidence that the repair of UV-induced DNA damage is inhibited in HPV8 E6 expressing cells (Simmonds and Storey, 2008; Underbrink *et al.*, 2008). HPV5 and HPV8 E6 proteins also inhibit double-strand DNA break repair by associating with and destabilizing the histone acetyl transferase, p300 (Howie *et al.*, 2011; Wallace *et al.*, 2012), which can regulate activity of the ATM/ATR kinases by acetylation. Similar to subverting