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Papillomavirus associated diseases of the horse

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ABSTRACT

The *Papillomaviridae* family comprises a large number of viruses that can infect a broad range of hosts including mammals, birds and reptiles giving rise to benign lesions of the skin or mucosal membranes. They are characterized by great genetic diversity yet adhere to common biological principles. In this review, we first describe the structure and function of the viral proteins encoded by papillomaviruses (PVs), with a particular emphasis on bovine papillomaviruses (BPV). We then discuss the role of BPV types 1 and 2 in the pathogenesis of equine sarcoids and present recent evidence implicating a novel equine papillomavirus (EcPV-2) in the pathogenesis of equine genital cancers.

1. Introduction

Papillomaviruses (PVs) are the causative agents of certain types of cancers in human and animals. These viruses infect either cutaneous or mucosal epithelia and give rise to benign lesions that usually regress spontaneously, but occasionally can progress to form cancer (Campo, 2006). Normally PVs are strictly species-specific, however some viruses are able to infect cross-species and the best known examples are BPV types 1 and 2 (BPV-1, BPV-2), the causative agents of common skin tumours of equids termed equine sarcoids (Nasir and Campo, 2008). Interestingly cross-species infection of BPV has also been reported in buffaloes and bisons (Silvestre et al., 2009; Tomita et al., 2007). Furthermore in recent years strong evidence has emerged for the involvement of PVs in equine genital cancers (Scase et al., 2010; Kainzbauer et al., 2011; Sykora et al., 2012). This review will first describe the structure and function of PVs and their proteins. We then discuss the role of BPV-1 and -2 in the pathogenesis of equine sarcoids and conclude with recent evidence implicating a novel equine PV (EcPV-2) in the development and progression of equine genital cancers.

2. Papillomavirus structure and function

Papillomaviruses (PVs) are small non-enveloped viruses that consist of an icosahedral capsid harbouring a circular double-stranded (ds) DNA genome of approximately 8 kb in length. The viral capsid consists of 72 L1 protein pentamers termed capsomeres, and 12 L2 protein monomers (Howley and Lowy, 2001). The viral genome contains up to eight open reading frames (ORF), which are transcribed as polycistronic mRNAs from one DNA strand. The viral transcripts are subsequently processed via alternative splicing and translated into proteins via ribosomal scanning (Hebner and Laimins, 2006). Based on the point in time of viral protein expression during infection, the viral genome can be grossly divided into an early (E) and a late (L) coding region. The early region codes for up to six proteins, i.e. the regulatory proteins E1, E2 and E4, and the transforming proteins E5, E6, and E7, whilst the late region encodes the major capsid protein L1 and the minor capsid protein L2 (Campo, 2006). The L1 and E6 ORFs enframe a ~1 kbp non-coding long control region (LCR),

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which contains E1 and E2 binding sites required for viral replication and transcription. In addition, the LCR harbours a series of binding sites for cellular transcriptional factors for positive and negative regulation of viral transcription (Hebner and Laimins, 2006).

2.1. Functions of papillomaviral early regulatory proteins

The E1 and E2 proteins function as regulators of viral replication. E1 is an initiator protein with a size ranging between 600 and 650 amino acids (aa) depending on the PV type. It is expressed throughout the viral life cycle and contains several functional domains including a nuclear localization signal (NLS) in the N-terminal region, which controls the localization of the protein within the cell. The N-terminus also displays recognition sequences for cyclin-dependent kinases and is highly protease-sensitive (Stenlund, 2010). As a dimer, the upstream end of the N terminus acts as an E1 DNA-binding domain (DBD) in viral DNA replication (Enemark et al., 2000; Schuck and Stenlund, 2005). Upstream of the DBD lies a helicase domain, which is required for the binding and hydrolysis of ATP (Stenlund, 2010). E2 regulates the transcription of the virus by directly interacting with the LCR, which contains several E2 binding sites. Binding of E2 to consensus sites distal to the LCR's TATA box results in activation of transcription, whereas binding to proximal sites leads to transcriptional repression (Jackson and Campo, 1995). E2 is also critically involved in viral DNA replication, as it accounts for specific binding of E1 protein to the origin of replication initiation (Stenlund, 2003).

During viral replication, E4 is expressed as an E1^E4 fusion protein at high copy numbers. It is essential for efficient amplification of the viral genome, suppression of suprabasal cell differentiation, and maintenance of viral episomes in basal cells (Fang et al., 2006). There is evidence for E4 altering the host cell cycle by interacting with cyclin A/cdk2 and thereby hampering progression through the S phase. This interaction, which is reflected by a sequestration of cyclin A from its cellular substrate, is possibly achieved via a potential cdk phosphorylation site and a short proline-rich region of E4 (Davy et al., 2002). Recent findings also point to an E4 protein region displaying an RXL-cyclin-interacting motif, which is conserved across many PV-types (Ding et al., 2013).

2.2. Functions of papillomaviral early transforming proteins

The viral proteins E6 and E7 have been recognized as the main transforming proteins in the pathogenesis of HPV-induced cervical cancer. PV E6 and E7 are expressed throughout tumour development and act in a combined manner. Importantly, they assure survival of infected host cells by inducing their immortalization. With an average length of ~150 aa, E6 is a relatively short protein, which commonly localizes to the nucleus and the cytoplasm of infected keratinocytes. The E6 protein is a transcriptional activator, which abrogates the apoptotic and cell cycle arrest functions of p53 by repressing the transcription of co-activator CBP/p300 (reviewed in Campo, 2006). For bovine papillomaviruses (BPV) it has been shown that E6 competitively binds to the focal adhesion protein paxillin, and by this prevents the latter from interacting with other focal adhesion proteins, which in turn enables infected cells to grow in an anchorage-independent manner (reviewed in Campo, 2006b). HPV E7 is a small acidic polypeptide of approximately 100 aa and the major HPV oncoprotein. The primary target for HPV E7 is the retinoblastoma protein (pRb) and its associated proteins p107 and p130. E7 oncoprotein in high-risk HPVs is necessary for viral pathogenesis and cellular transformation (McLaughlin-Drubin and Munger, 2009). BPV-1 E7 is a 127 aa zinc-binding protein whose exact role remains to be elucidated. There is evidence for BPV E7 acting as an enhancer of E5- and E6-mediated transformation, which may be at least partially due to its ability to bind to p600 (Borzacchiello and Roperto, 2008). In addition, BPV E7 protein has been shown to inhibit anoikis, and by this, possibly contributes to E6-mediated anchorage-independent growth of infected cells (DeMasi et al., 2007).

E5 is a short hydrophobic transmembrane protein, which is expressed by most but not all PVs. It typically resides within the endoplasmatic reticulum (ER) and the Golgi apparatus (GA) of infected cells. It represents the major oncoprotein of BPV-1 and BPV-2 because of its intrinsic ability to induce neoplastic transformation of infected cells via activation of various kinases (Borzacchiello and Roperto, 2008). In contrast, HPV E5 has weak transforming activity but enhances the immortalization potential of E6 and E7 proteins (Stöppler et al., 1996). In fact BPV E5 is the best characterized of all PV E5 proteins and has served as a model system for HPV E5 studies (Venuti et al., 2011). BPV-1 E5 induces transformation by binding to the Platelet Derived Growth Factor Receptor β (PDGFβ-R) tyrosine kinase in a ligand-dependent manner (Petti et al., 1991; Borzacchiello et al., 2006; Tsirimonaki et al., 2006). BPV E5 proteins also down-regulate MHC I transcription, translation and presentation on the cell surface, thus helping the virus to evade immune clearance (Ashrafi et al., 2005; Marchetti et al., 2006; Ashrafi et al., 2006a,b; Marchetti et al., 2009). The down-regulation of MHC class I expression by BPV-1 E5 has also been demonstrated in equine sarcoids in vitro (Marchetti et al., 2009).

3. Papillomavirus entry and life cycle

PVs are described to have a stringent tropism for cutaneous and/or mucosal epithelia (Chow and Broker, 2006). The viruses cannot actively penetrate the skin of their host. Therefore, abrasion is one of the prerequisites for PV infection. Basal epidermal cells provide the appropriate primary surface and secondary receptor molecules for virion attachment and uptake. There is evidence for surface heparan sulphate proteoglycans (HSPG) representing initial PV attachment sites (Joyce et al., 1999; Giroglou et al., 2001). Subsequent PV endocytosis possibly involves clathrin- and caveolin-mediated mechanisms (Day et al., 2003; Smith et al., 2007), and/or may necessitate the presence of tetraspanin-enriched microdomains (TEMs) (Spoden et al., 2008). It is suggested that PVs bind to receptors expressed by a wide