Bovine papillomavirus infections in animals

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Bovine papillomaviruses (BPV) are DNA oncogenic viruses inducing hyperplastic benign lesions of both cutaneous and mucosal epithelia in cattle. Six (BPV 1-6) different viral genotypes have been characterized so far; they are all strictly specie-specific even if BPV 1/2 may also infect horses inducing fibroblastic tumors. The benign lesions may regress or develop to cancer once the virus synergizes with environmental carcinogenic co-factors. Among these the bracken fern is the most extensively studied. The BPV associated tumors have veterinary and agriculture relevance in its own right but they have also been studied as relevant model of human papillomavirus (HPV). Recent insights into BPV biology open new fields of speculation about the role of these viruses in inducing neoplastic transformation of cells other than epithelial.

Keywords: Bracken fern; cancer; papillomavirus; ptaquiloside;

1. Introduction

Bovine papillomaviruses (BPV) belong to the Papillomaviruses (PV) family. These are small DNA viruses infecting humans as well as many domestic and wild species of animals and birds causing benign hyperproliferative lesions of both mucosal and cutaneous epithelia [1-3]. BPV cause both benign and malignant epithelial and mesenchymal tumors in cows and equids. They are strictly specie-specific and, even in experimental conditions, do not infect any other host than the natural one. The only known case of cross-species infection is the infection of horses and other equids by BPV type 1 (BPV-1) or BPV type-2 (BPV-2) [4]. Papillomavirus infections usually regress, but occasionally they develop to cancer.

2. The virus

Six BPV types (BPV 1-6) have been characterised associated with different histopathological lesions. The different six genotypes have been classified into three groups [5]:

Xi-papillomaviruses encompassing the pure epitheliotropic BPV-3;BPV-4 and BPV-6; Delta-papillomaviruses encompassing BPV-1 and BPV-2 associated to fibropapillomas (i.e. benign tumors of both epithelium and underlying derma) and Epsilon-papillomavirus comprising the BPV-5 whose genome seems to share similarities with the formers two BPV groups.

The BPV virion is a non-enveloped structure of 55-60 nm diameter containing a double-stranded covalentely closed circular DNA of approssimatively 8000 nucleotides. Three different regions compose the genome: the long control region (LCR) and two regions encoding for early and late genes.

The LCR is the genome region containing signals for both viral DNA replication and transcription. E2 regulates BPV transcription at LCR level. The E2 sites may be also bound by different cellular transcription factors and the E2 can also bind to mitotic chromosomes resulting in efficient distribution of the BPV genome into daughter cells.

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3. BPV gene products

3.1 E5

The papillomavirus E5 proteins are short hydrophobic polypeptides (from 83 amino-acid residues in human papillomavirus type 16 (HPV-16) to 42 residues in BPV-4), many of which have transforming activity. BPV-1 E5 oncogene encodes for a 44-amino acid protein that is the major BPV transforming oncoprotein. It is a type II transmembrane protein which is expressed in the deep layers of the infected epithelia [6-8] and is largely localized to the membranes of the endoplasmic reticulum (ER) and Golgi apparatus (GA) of the host cells [9-10]. BPV E5 is expressed in the cytoplasm of both basal and suprabasal transformed epithelial cells with a typical juxtanuclear pattern due to its localization in the GA. It may be also expressed in neoplastic cells of mesenchymal origin such those of endothelial origin. BPV E5 has no intrinsic enzymatic activity and its transformation activity is related to the activation of several kinases, from growth factor receptor to cdk cyclins. E5 interacts with the 16-K subunit c protein, a component of the vacuolar H^+ -ATPase pump [11]. This proton pump acidify the lumen of intracellular compartments, (endosomes, lysosomes, and GA) that process growth factors so that E5 binding may result in alteration of this processing. Another consequence of E5 mediated impaired acidification is the down-regulation (both *in vivo* and *in vitro*) of the Major Histocompatibility Complex class I (MHC-I) expression, representing one of the mechanisms by which the BPV evade the immunoresponse by the host [12].

The mechanism by which BPV-1 E5 induces cell transformation lies in its binding to and activation of the cellular β receptor for the platelet-derived growth factor (PDGFβ) [13]. The activation of endogenous PDGFβ receptors is characterized by the formation of stable E5-receptor complexes, persistent tyrosine phosphorilation of the receptor, its dimerization and cellular transformation. This interaction takes place also in naturally occurring urinary bladder cancer [14].

3.2 E6

The BPV-1 E6 gene of Xi BPV encodes an oncoprotein of 137 amino acids. It binds to paxillin blocking its interaction with vinculin and the focal adhesion kinase [15]. It also binds to several others cellular proteins being able to transform cells alone by itself.

3.3 E7

The BPV E7 gene encodes a 127 amino-acids zinc binding protein which cooperates with E5 and E6 in inducing cell transformation. Once E7 is co-expressed with E5 and E6, its transformation capacity increases many folds [16], and such co-expression may also occur in tumors of mesenchymal origin [17]. BPV-1 E7 transformation function correlates with its binding to a cellular target p-600 [18].

3.4 L1 and L2

The BPV late proteins L1 and L2 are expressed into the more differentiated epithelial cells [7]. The former mediates virus interaction with cellular receptors, the latter induces virion assembly by binding to viral DNA.

4. Natural history of BPV infection

Infection by Delta-PVs leads to transformation of subepithelial fibroblasts followed by epithelial acanthosis and then papillomatosis, while infection by Xi-PVs induces transformation only of the epithelial component. Virus replication can take place only in keratinocytes undergoing terminal

differentiation to squamous epithelium, so it is seen only in the epithelial component of the tumors and only at certain stages of its development. Virus replication has never found in fibroblasts where the BPV genome is present in a nonintegrated episomal form [19], although BPV viral gene expression has been recently found in tumors of mesenchymal origin such those arising from blood vessels (haemangioma and haemangiosarcoma) [17].

Papillomas and fibropapillomas may occur in different organs in cattle and different BPV genotypes are found.

BPV-1, BPV-5 and BPV-6 are associated to papillomas of the teats and udders in cows [20]. This can become a great economic problem once the papillomas spread around the primary tumors and the cows cannot be milked, veals are unable to suckle properly and the site may become infected.

Epithelia of both prepuce and penis may be infected by BPV-1 resulting in fibropapillomas. The tumors can spread along the perineum and even up toward the back; they can become necrotic and cause loss of reproductive functions.

5. BPV-4 and gastrointestinal tumors

In cattle, BPV-4 infects the mucosa of the upper gastrointestinal (GI) tract leading to the formation of papillomas and/or fibropapillomas [21]. BPV-4 infection and associated tumors of the upper GI tract have been found in Brazil, Nasampolai Valley of Kenya, the Western Highlands of Scotland and in the South of Italy [22,23].

Healthy cattle normally recover from papillomatosis in approximately one year time through a cell-mediated immune response [24], but some animals may even die due to widespread papillomatosis if they are not able to reject the infection. Chronic exposure to immunosuppressants leads to the persistence and spreading of the papillomas. Commonly, immunosuppression in cattle results from exposure to bracken fern [25], but may even due to some other factors such as infection with bovine viral diarrhoea virus [26].

The fern induces immunosuppression and the fibropapillomas spread; the animals with extensive papillomatosis are at high risk to develop cancer such as squamous cell carcinoma. The latent BPV is activated and full malignant transformation depends on others mutagens such as quercetin and ptaquiloside that act synergistically with the virus in the carcinogenic process, triggering BPV gene expression and leading to the development of cancer.

The BPV-4 E7 oncoprotein cooperates with quercetin for neoplastic transformation, in so doing the ras oncogene is activated, the p53 is mutated and the number of the cellular receptors for epidermal growth factors is increased [20]. These transforming events are probably due to bracken fern mutagens, but still remain to be established *in vivo*.

It is wothnoting that some human GI cancer may have the same etiology: papillomavirus and bracken suggesting that similar molecular mechanisms underlying bovine cancer may even occur in humans.

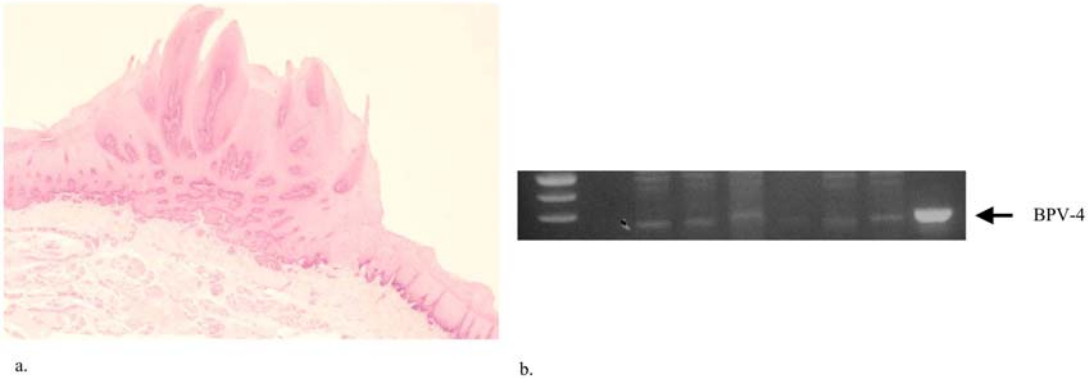

Fig.1. a. Histological appearance of oesophageal papilloma. b. PCR amplification of bovine oesophageal papilloma. The arrow on the right indicates the position of the BPV-4 amplification product.

6. BPV-1/2 and urinary bladder tumors

In cattle, tumours of the urinary bladder are commonly associated with a syndrome known as Chronic Enzootic Haematuria due to prolonged ingestion of bracken fern with a prevalence as high as 90% in adult animals [27]. Field cases of urinary bladder cancer in cattle occur wherever the bracken-fern is spread. The disease is known to occur in continental Europe, Azores Islands, in some regions of Kenya, Brasil, New Zealand, India and in China. Human exposure to bracken fern directly or indirectly through milk from bracken eating cattle has been linked to human GI cancer [28].

The involvement of the BPV-1/2 in bladder carcinogenesis has been recognised for a long time [29] and the relationship among the virus and fern has been experimentally reproduced [30].The cancer are of both epithelial and mesenchymal origin (mostly haemangioma and its malignant counterpart) with different epithelial histological variants identified [31-33]. The epithelial tumors express the urothelium tumor marker Uroplakin III [34,35].and do not metastatise frequently probably due to the content of sialic acid and gangliosides [36].

The BPV-2 is involved in both epithelial and mesenchymal tumors, testifying that the virus is not a pure epitheliotropic agent in its natural host [17]. The virus infects the urinary bladder mucosa inducing an abortive and latent infection with no production of virions. The exposure to immunosuppressants, mutagenic and carcinogenic principles from bracken triggers viral gene expression leading to cell transformation. In both epithelial and mesenchymal cancers the BPV-2 E5 oncoprotein is expressed [37,17] and is in complex with the activated form of the PDGFβ receptor [14]. Additionally, in urothelial cancers the telomerase activity is upregulated [37], the expression of ras oncogene [38] and cyclooxygenase-2 (COX-2) [39] is increased and, as already observed in HPV associated cervical cancer, the fragile sites are disrupted and the expression of the tumor suppressor fragile histidine tetrads (FHIT) is down-regulated [40].

Lymphocytes from bracken feeding cows harbour BPV DNA [41], which has been found also into the blood stream [42,43] and chromosomal abnormalities have been demonstrated [44].

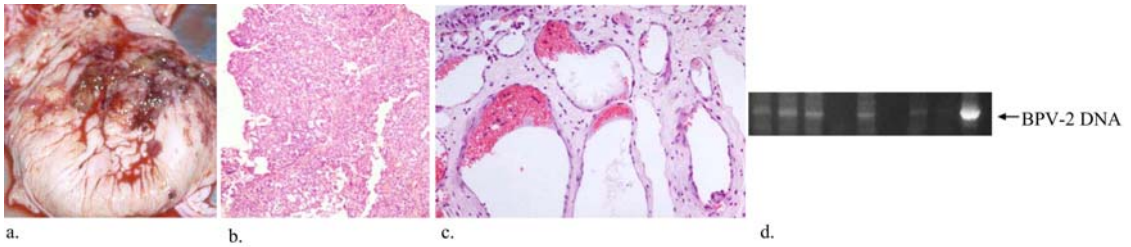

Fig. 2. a. Macroscopic appearance of bovine urinary bladder tumor; b. Histological appearance of urothelial carcinoma grade II; c. Histological appearance of cavernous haemangioma of the urinary bladder; d. PCR amplification of bovine urinary bladder cancer. The arrow on the right indicates the position of the BPV-2 amplification product.

7. BPV-1/2 and equine sarcoids

The sarcoids are benign tumors of fibroblastic skin origin affecting horses, mules and donkeys. They are locally invasive often occurring at sites of previous injury or scarring.

Clinically, five different types of sarcoids can be distinguished: Occult sarcoid: is an hairless circular area of the skin; Verrucous: tumors with wart-like appearance; Fibroblastic sarcoids present as a fleshy mass; Nodular sarcoids consist of firm masses lying under the skin and mixed sarcoid show a combination of features of verrucous, fibroblastic and nodular types [45].

It is the most common dermatological neoplasm reported in horses. The most common sites of appearance is the skin of the head, ventral abdomen, legs and the paragenital region [46].

Despite the failure to isolate any papillomavirus from the sarcoids, a large body of evidence strongly support the hypothesis that BPV is the etiological agent of this tumour.

Both BPV-1 and BPV-2 have been detected in sarcoid tumours with the BPV-1 being the predominant type [47-49].

The BPV exists as episomally [50] and its major oncoprotein E5 is expressed, thus suggesting the viral genes are expressed [51,52].

Equine sarcoids is a biologically attractive tumor since it is the only known case of natural cross-species PV infection. Moreover, while BPV infection in cattle produce benign lesions that may regress, the sarcoids are non-permissive for virus production, locally aggressive and non regressing [46].

Cell cycle regulatory proteins are involved in the pathogenesis of equine sarcoids: p53 is stabilised in sarcoid cells being expressed in the nuclei as well as in perinuclear region, however its transactivation function is abrogated [53,54]. Low levels of cell proliferation are characteristic of sarcoids with no overexpression neither of cyclin A, $p27^{kip1}$ nor of CDK-2 [54].

The loss of p53 function and the low levels of cell proliferation indicate that sarcoid cellular and molecular pathology may not be associated with abnormal cell cycle control mechanisms.

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