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Review

Animal models of papillomavirus pathogenesis

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Abstract

Tumorigenesis due to papillomavirus (PV) infection was first demonstrated in rabbits and cattle early last century. Despite the evidence obtained in animals, the role of viruses in human cancer was dismissed as irrelevant. It took a paradigm shift in the late 1970s for some viruses to be recognised as 'tumour viruses' in humans, and in 1995, more than 60 years after Rous's first demonstration of CRPV oncogenicity, WHO officially declared that 'HPV-16 and HPV-18 are carcinogenic to humans'. Experimental studies with animal PVs have been a determining factor in this decision. Animal PVs have been studied both as agents of disease in animals and as models of human PV infection. In addition to the study of PV infection in whole animals, in vitro studies with animal PV proteins have contributed greatly to the understanding of the mechanisms of cell transformation. Animal PVs cause distressing diseases in both farm and companion animals, such as teat papillomatosis in cattle, equine sarcoids and canine oral papillomatosis and there is an urgent need to understand the pathogenesis of these problematic infections. Persistent and florid teat papillomatosis in cows can lead to mastitis, prevent the suckling of calves and make milking impossible; heavily affected animals are culled and so occasionally are whole herds. Equine sarcoids are often recurrent and untreatable and lead to loss of valuable animals. Canine oral papillomatosis can be very extensive and persistent and lead to great distress. Thus the continuing research in the biology of animal PVs is amply justified. BPVs and CRPV have been for many years the model systems with which to study the biology of HPV. Induction of papillomas and their neoplastic progression has been experimentally demonstrated and reproduced in cattle and rabbits, and virus-cofactor interactions have been elucidated in these systems. With the advancements in molecular and cell culture techniques, the direct study of HPV has become less problematic and understandably research efforts have shifted in focus from animal to human PVs. However, there are still areas in which studies on animal PVs will continue to provide answers to questions pertaining to virus biology. One of these questions is the involvement of HPV in oesophageal and bladder cancer in humans as is the case for BPV in cattle. Another is the site of viral latency. Lymphocytes have been proposed as a site of latency for both BPV and HPV but only experiments performed in animals could clarify this point. Animal PVs have been instrumental in the development of vaccines as cattle, rabbit and more recently dog all provide the opportunity to study vaccination in the natural host. Several anti-HPV vaccines, both prophylactic and therapeutic, based on those developed in animals, are now in clinical trials with encouraging results. In vitro studies with two animal PV early proteins, the transcriptional regulator E2 and the oncoprotein E5, among others, have contributed to the elucidation of viral gene control and cell transformation. BPV E2 was the first viral product to be identified as a transcriptional regulator; more recently, its association with mitotic chromosomes has been suggested as a mechanism for the partition of viral genomes between daughter cells, and its L2-mediated localisation in the sub-nuclear compartments PODs is believed to favour viral DNA encapsidation. E5 is the major transforming protein of several BPVs. Many of the function of E5 proteins have been

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first established for BPV E5 and later validated for HPV E5. E5 interacts with 16k ductin/subunit c and this interaction is deemed responsible for the down-regulation of gap junction intercellular communication and the inhibition of acidification of endomembranes. E5 activates growth factor receptors and numerous kinases, including cdks, and down-regulates expression of MHC class I. Thus E5 would help the establishment of viral infection by promoting both cell proliferation and immune evasion. Despite the extensive studies on vaccination in animals, E5 has not been tried in animal models as a possible anti-papillomavirus vaccine. A recent study has reported that vaccination of mice with HPV-16 E5 in a recombinant adenovirus reduced the growth of tumours induced by E5-expressing cells. Perhaps this is an instance in which work on animal PVs should follow HPV and the potential for E5 vaccination should be validated in naturally occurring animal models.

Keywords: Animal papillomavirus models; BPV; CRPV; COPV; HPV; Disease; Cancer; Progression; Cell transformation; Vaccination

1. Introduction

Papillomaviruses are strictly species-specific and, even in experimental conditions, do not infect any other host than their natural one. The only known case of cross-species infection is the infection of horses and other equids by bovine papillomavirus (BPV) type1 and 2. Given the apparently insurmountable problem of species-specificity, no animal model of human papillomavirus (HPV) infection exists and papillomavirologists have had to rely for direct experimentation on animal papillomavirus systems.

Animal papillomaviruses have been invaluable in the investigation of virus biology, its relationship with the host, the host immune response to the virus and in the development of the first anti-papillomavirus vaccines. The direct link between papillomavirus infection and neoplasia, and the relationship between virus and environmental co-carcinogens was first established for animal papillomaviruses, particularly cottontail rabbit papillomavirus (CRPV), BPV, and canine oral papillomavirus (COPV).

The results obtained in these animal systems contributed in no small measure to the official recognition by WHO in 1995 of HPV-16 and HPV-18 as 'carcinogenic to humans', of HPV-31 and HPV-33 as 'probably carcinogenic to humans', and of HPV types other than 16, 18, 31 and 33 as 'possibly carcinogenic to humans' (IARC, 1995).

Given that the oncogenicity of HPVs has been recognised, that HPV-transgenic mice are available for in-depth carcinogenicity studies, and that new and more sophisticated in vitro systems have been developed which allow the completion of the full life cycle of HPV, is there still any need to investigate animal papillomaviruses? The answer to this question can only be a resounding 'yes', on at least three counts: animal papillomaviruses are agents of diseases in farm and companion animals, still provide in vivo models for HPV infection, carcinogenesis and vaccination, and experimental investigation of their molecular biology continues to unearth novel features that point the way to studies on HPVs.

These three points will be discussed in detail in the rest of this review.

2. Papillomaviruses cause distressing diseases in farm and companion animals

2.1. BPVs

In cattle BPV infection causes skin warts (BPV-1 and -2), papillomatosis and cancer of the upper gastrointestinal (GI) tract (BPV-4), papillomatosis of teats and udder (BPV-1, -5 and -6) and penis (BPV-1) and cancer of the urinary bladder (BPV-1 and -2) (Campo, 1998). Cancer of the upper GI tract and urinary bladder develop as a result of the interactions between the virus, chemical carcinogens and immunosuppressants present in bracken fern (Campo, 1997a). This will be discussed in greater detail below, but immunosuppression is instrumental in the development of widespread and persistent papillomatosis of the upper GI tract (Fig. 1). Animals that develop such extensive

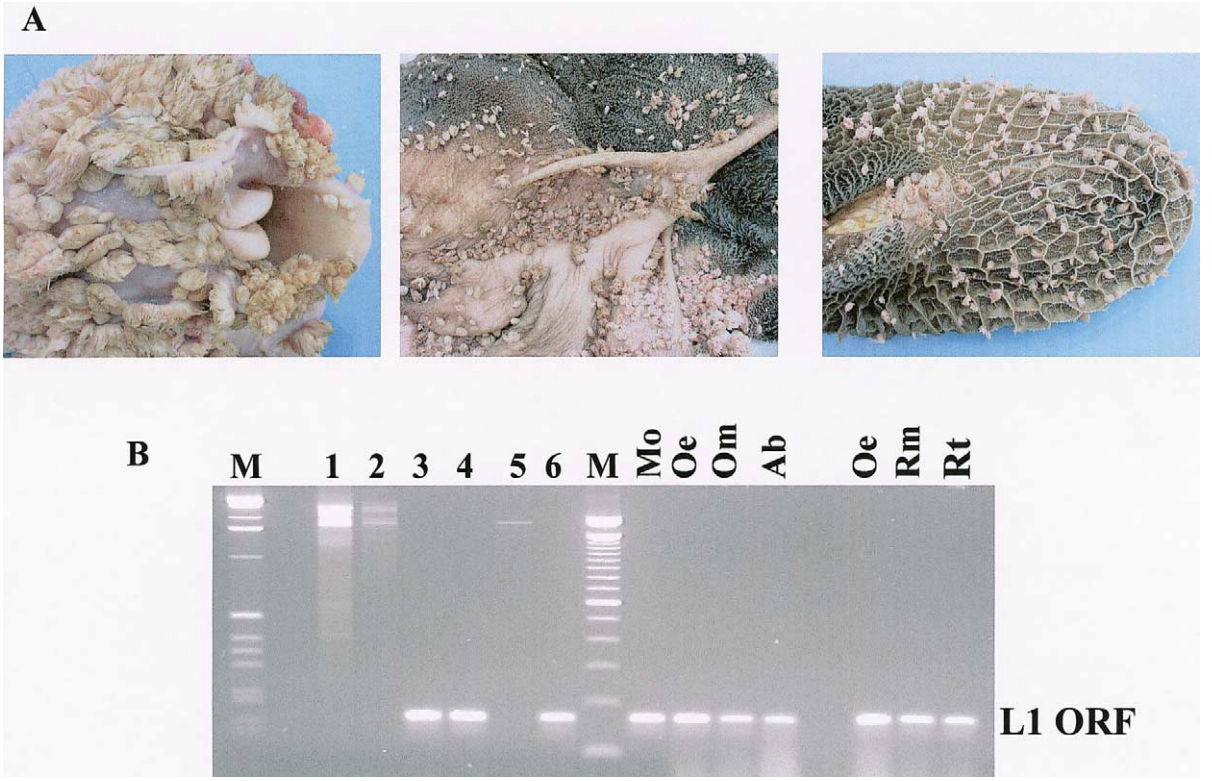

Fig. 1. (A) Florid papillomatosis of pharynx (left panel), rumen (middle panel) and reticulum (right panel) in a calf. (B) PCR amplification of viral L1 ORF from papilloma biopsies. M, 1 kb and 100 bp molecular weight markers; lanes 1–6, recombinant viral DNA from BPV-1-6, respectively (note the primers amplify only subgroup B BPVs); Mo, mouth; Oe, oesophagus; Om, omasus; Ab, abomasus; Rm, rumen; Rt, reticulum; unmarked lane, blank.

papillomatosis have difficulties in eating and breathing and have to be culled. If the papillomatosis is not too severe, the animals survive but are at great risk of developing squamous cell carcinomas (see below).

The teats and udders of cows are subjected to infection by three different types of BPV (Fig. 2) (Campo, 1998). This disease, especially if caused by BPV-6, is not only a health problem but has also economic consequences, as cows with teat papillomas cannot be milked, young calves cannot suckle, and often the peduncolated papillomas snap off, the sites become infected and mastitis may ensue with distortion of the milk canals. Occasionally, entire herds have to be culled if the papillomatosis does not regress.

Papillomatosis of the penis interferes with the normal function of bulls and the animals have to be sacrified.

BPV-1 and -2 are the only papillomaviruses that can infect a host of a different species. Horses, donkeys and mules develop sarcoids tumours as result of BPV infection (Fig. 3; Olson and Cook, 1951). The tumour is locally invasive, refractory to treatment and can lead to the animal being sacrified. The economic loss is important both for the racing industry and even more so for the farmers and peasants of the developing countries, for whom often the mule or donkey is the only livelihood. The tumour is not permissive for virus replication and infection is abortive; the BPV DNA is present in the tumour cells in multiple episomal copies and its early genes are transcribed, indicating an active role of the virus in sarcoid aetiology (Nasir and Reid, 1999). The late genes also can occasionally be transcribed but no virion has ever been detected in sarcoids. It is possible that virus is produced at a particular develop-

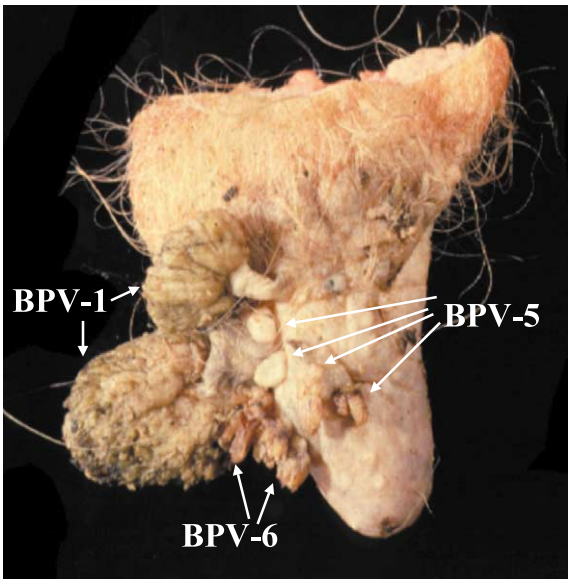

Fig. 2. Teat papillomas in a cow. Large frond fibropapillomas are caused by BPV-1; small oval fibropapillomas ('rice grain') by BPV-5, and small frond epithelial papillomas by BPV-6.

mental stage of the tumour and this point requires further investigation.

No mutation has been found in the p53 gene in sarcoids, but the p53 protein is likely to be involved in sarcoid pathogenesis as it is constitutively expressed, appears to be sequestered in the cytoplasm and has lost its transcription transactivation properties as judged by the absence of transcription of the p53-controlled mdm2 gene (Fig. 3; L. Nasir, personal communication).

More work needs to be done for a better understanding of the role of BPV in the induction and pathogenesis of equine sarcoids.

2.2. COPV

COPV induces papillomas in the oro-pharynx of dogs. As in the case of BPV-4, the papillomatosis can be very widespread and distressing (Fig. 4). The papillomas can spread to other areas of the body and the animal has to be euthanased. Again, like BPV-4 induced papillomas, COPV papillomas can progress to squamous cell carcinomas (Nicholls and Stanley, 1999).

3. Animal papillomaviruses as in vivo models for HPV infection and carcinogenesis

Animal papillomaviruses have been instrumental in the demonstration of the virus oncogenicity and of its synergy with co-factors, either environmental or genetic. Cancer of the upper GI tract and the urinary bladder in cattle and carcinoma of the skin in rabbits are the result of complex interactions between a papillomavirus, chemical carcinogens and immunosuppressants, and the host genetic make-up.

3.1. BPV-4 and cancer of the upper GI tract

In cattle feeding on bracken fern the BPV-4-induced papillomas of the upper GI tract are at a high risk of progressing to cancer (Fig. 5). Bracken fern contains immunosuppressants and chemical carcinogens and its presence in animal feed causes a variety of diseases (Campo, 1997a,b and references therein). The contribution of viral, immunological and chemical factors in neoplastic progression of BPV-4 papillomas was first established in the field (Jarrett et al., 1978) and then in experimental conditions (Campo et al., 1994b). Epidemiological and molecular studies have allowed the determination of the events that take place during carcinogenesis (Fig. 5C). BPV-4 infects the mucosa of the upper GI tract, expresses its transforming proteins and induces papillomas. These are benign hyperproliferative lesions which in healthy immunocompetent animals regress due a cell mediated immune response. In cattle eating bracken fern the immune system is chronically depressed by the immunosuppressants present in the plant and no effective response can be mounted against the virus or the infected cells. The papillomas spread widely (see Fig. 1) and present a vastly expanded target for the chemical carcinogens of the bracken fern. The rapidly dividing papilloma cells start invading the underlying derma and full transformation to squamous carcinoma ensues. During the carcinogenic process, the number of the cellular receptors for epidermal growth factor increases, the ras gene is activated and the p53 gene is mutated (Smith et al., 1987; Campo et al., 1990; Scobie, 1996). These

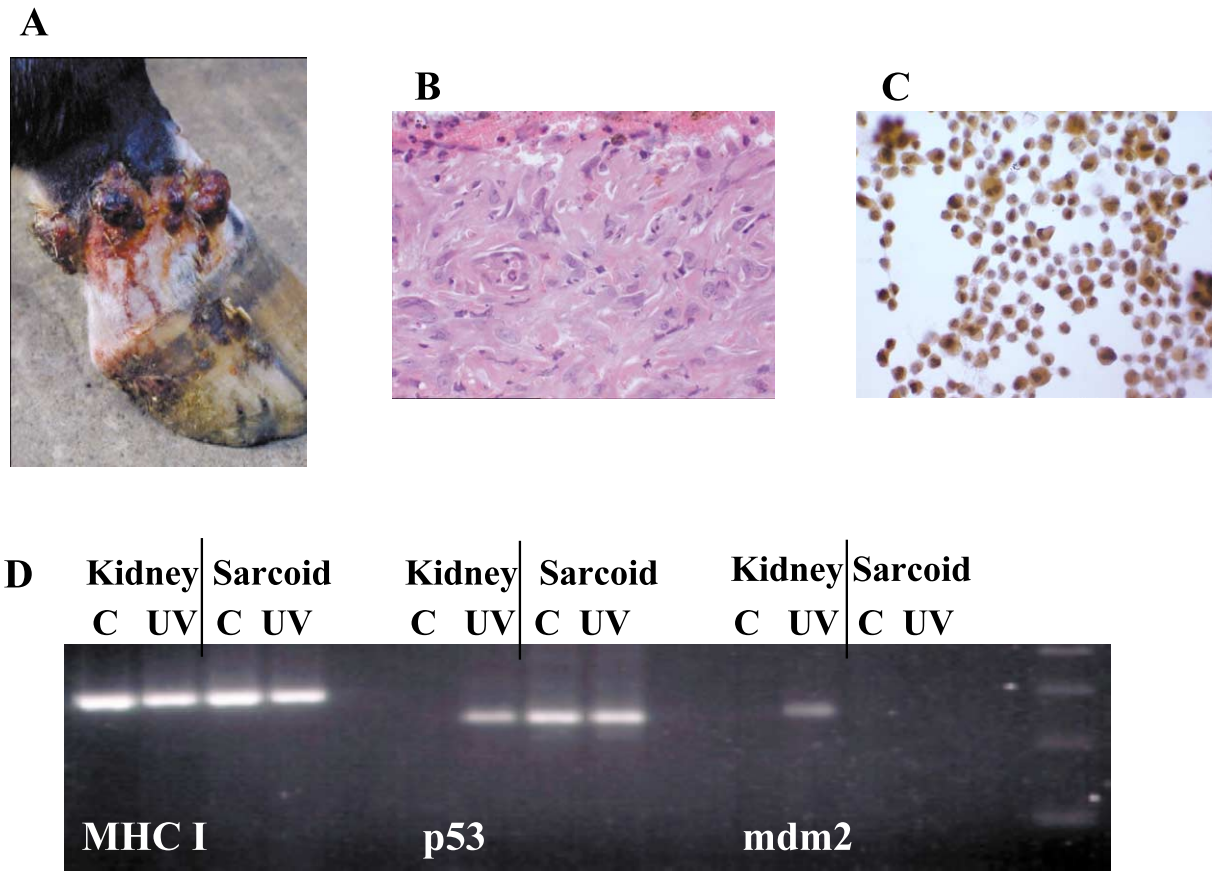

Fig. 3. Equine sarcoids. (A) sarcoid on the hoof of a horse; (B) histological section (H&E) of an equine sarcoid; (C) immunostaining of p53 in a sarcoid cell line (note the extensive cytoplasmic localisation of p53); (D) the transcriptional function of p53 is impaired in a sarcoid cell line; mRNA for MHC I heavy chain, p53 and mdm2 was amplified by RT-PCR in kidney control cells and in sarcoid cells in normal growth conditions and after exposure to UV light. As expected, there is no difference in MHC I heavy chain mRNA; p53 mRNA is induced in kidney cells after UV treatment but is constitutively present in sarcoid cells; mdm2 mRNA is induced after UV treatment in kidney cells but not in sarcoid cells. Images and data courtesy of L. Nasir.

transforming events are probably due to the bracken fern chemicals, but, although this has been proved in vitro, it remains to be established in vivo.

3.2. HPV and bracken fern in human GI cancers

In humans too, exposure to bracken fern, whether in the diet or as spores, has been epidemiologically linked to oesophageal cancer in several parts of the world (Alonso-Amelot et al., 1996; Alonso-Amelot and Avendano, 2001; Hirayama, 1979; Marliere et al., 1999; Villalobos-Salazar et al., 1995). All forms of culinary bracken have been shown to be carcinogenic in experimental animals (Santos et al., 1987, 1992) and DNA adducts have been found in the upper GI tissue of mice fed bracken extracts or bracken spores (Povey et al., 1996). HPV type 16 has been found in approximately 50% of cancers and precancers of the oesophagus (Chang et al., 1990; Togawa et al., 1994; Cooper et al., 1995; Suzuk et al., 1996; de Villiers et al., 1999), particularly in developing countries, and we ourselves have detected HPV-16 DNA in biopsies of oesophageal cancer from one area of Brasil (Fig. 6) (Campo et al., 1999), where bracken fern is a common component of human diet and the relative risk

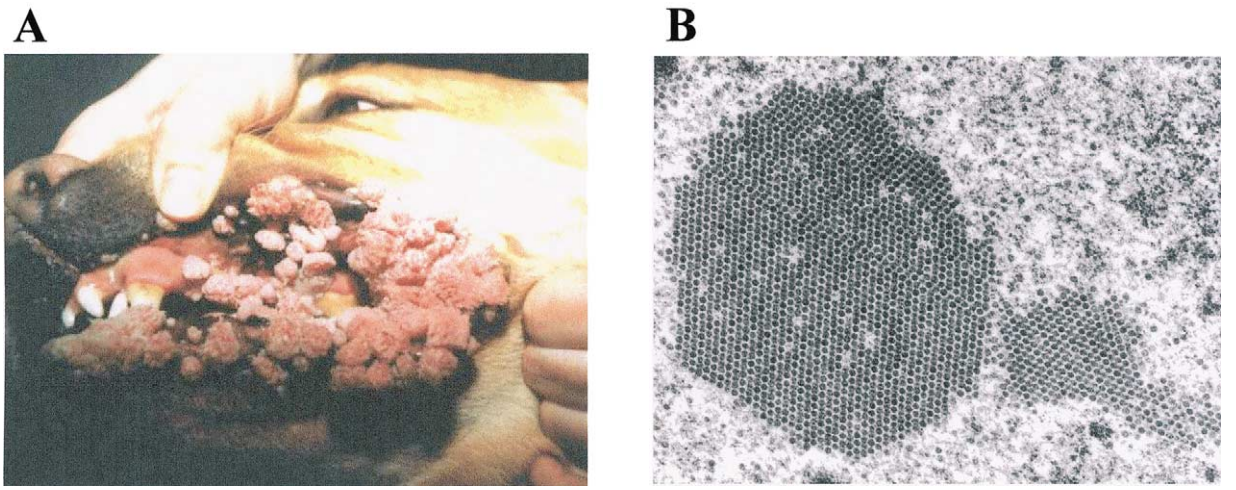

Fig. 4. (A) Extensive persistent oral papillomatosis induced by COPV in a dog; (B) paracrystalline arrays of COPV in an oral papilloma. Images courtesy of R. Moore.

for bracken fern exposure and oesophageal carcinoma has been estimated to be 5.47 (Marliere et al., 1999). We have also found that, as is the case for BPV-4 (Connolly et al., 1998), the transcriptional promoter of HPV-16 is trans-activated by quercetin, one of the mutagens contained in bracken fern (Fig. 6; Campo et al., 1999). The trans-activation of the papillomavirus promoter results in an increased expression of the viral transforming proteins and hence in increased cell transformation. These findings suggest that some cases of cancer of the upper GI tract in humans may have the same aetiology as in cattle, i.e. papillomavirus and bracken, and open up the possibility that the molecular mechanisms elucidated for cell transformation and cancer in cattle operate also in humans.

3.3. BPV-1/2 and cancer of the urinary bladder

Cattle feeding on bracken fern are also affected by cancer of the urinary bladder (Campo, 1997a, 1998). The involvement of bracken and BPV-1 or BPV-2 in bladder carcinogenesis has been recognised for a long time, and more recently this neoplastic progression has been reproduced experimentally (Campo et al., 1992). The several types of bladder cancer observed in field cases were also experimentally induced in cattle infected with BPV-2 and kept on a diet of bracken fern (Fig. 7). A carcinogenic process, similar in outline to the one established for BPV-4 (see Fig. 5C) has been recognised (Fig. 7C). BPV-1/2 infects the epithelium of the urinary bladder (perhaps as a secondary infection deriving from infection of the paragenital area) and establishes an abortive infection, with no production of virions. The bracken immunosuppressants allow the formation of pre-neoplastic lesions, which are made to become malignant by the chemical carcinogens of the plant. As in carcinomas of the upper GI tract, the ras gene is activated (Campo et al., 1990), and expression of the tumour suppressor fragile histidine tetrads (FHIT) locus is down-regulated (Borzacchiello et al., 2001). Interestingly fragile sites are often disrupted by integration of HPV DNA in cervical cancers (Popescu et al., 1990) and alterations of FHIT expression has been observed in many cervical carcinomas (Greenspan et al., 1997).

3.4. HPV and urinary tract tumours in humans

The involvement of HPV in cancer of the urinary bladder in humans is still an unresolved issue. HPV DNA has been found in a number of

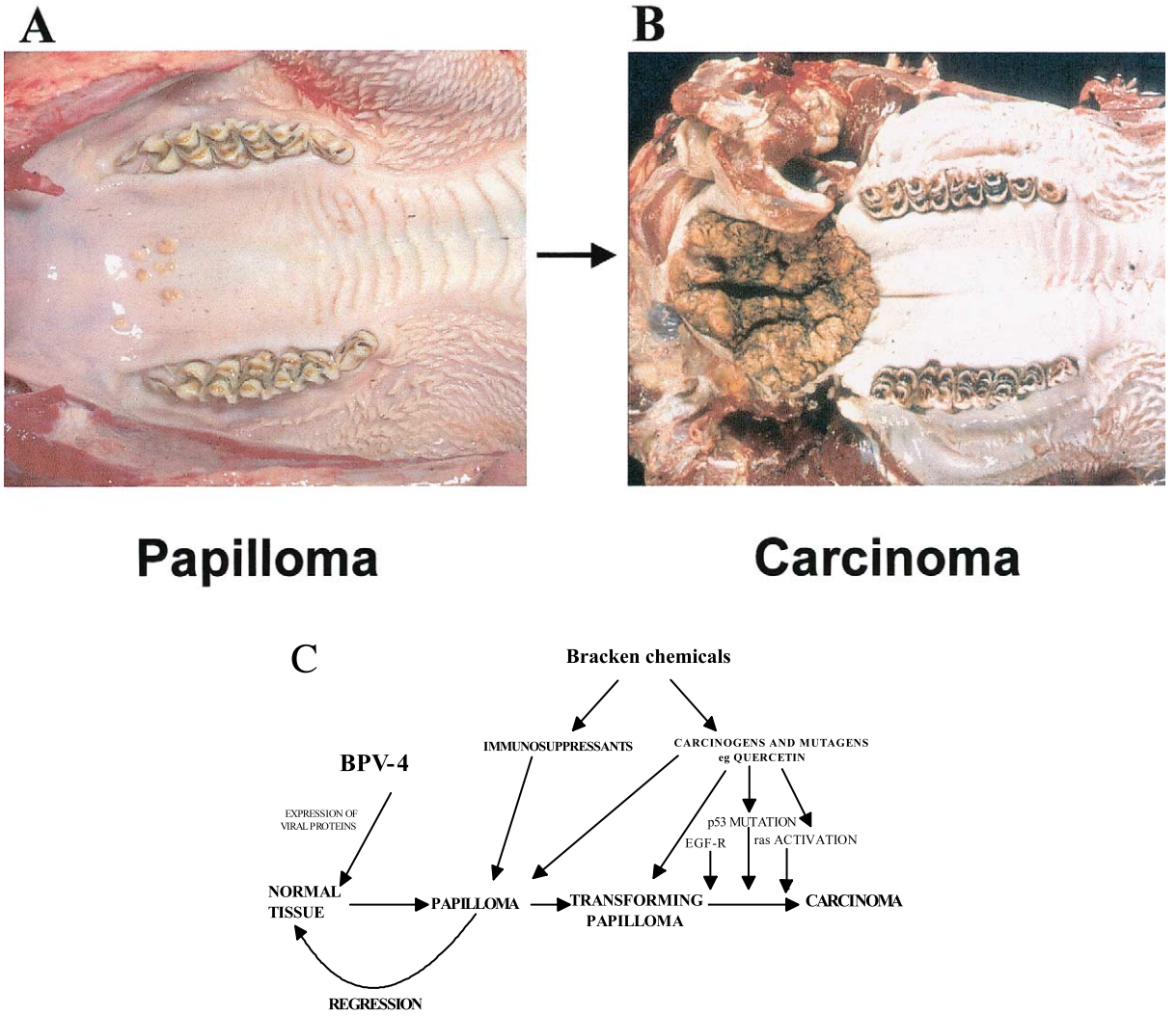

Fig. 5. Papilloma–carcinoma progression in cattle. (A) BPV-4 papillomas of the palate; (B) squamous cell carcinoma of the palate; (C) diagrammatic representation of the multi-step nature of neoplastic progression of upper GI tract papillomas.

cases of urinary tract cancer, sometime ascribed to secondary spread from penile or vulval condylomas. However, other reports dispute the presence and the involvement of HPV in this type of human cancer (Oliver et al., 1998). It is conceivable that the virus may be instrumental only in a proportion, perhaps a minority, of urinary tract cancer in selected populations (Khaled et al., 2001). If this were the case, it would explain the contradictory results.

3.5. *Papillomavirus latency*

Like many viruses, papillomavirus can establish a latent infection. The viral genome can be often found in normal epithelia with no clinical sign of disease, and normal epithelium is the accepted site of latent infection. However, viral genomes can also reside in lymphocytes, both in cattle and in humans (Campo, 1998; Campo et al., 1994a and references therein). Latent infection of lympho-

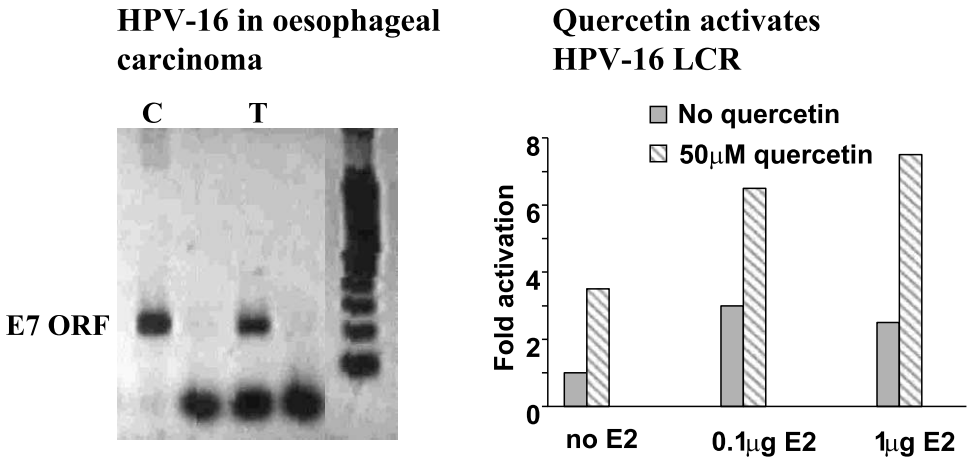

Fig. 6. Possible involvement of HPV and bracken in carcinogenesis of the upper GI tract in humans. Left panel, amplification of the E7 ORF by PCR in an oesophageal carcinoma from Minas Gerais, Brasil, where people include bracken fern in their diet; C, control E7 plasmid, T, oesophageal cancer. Right panel, the bracken fern mutagen quercetin increases the transcriptional activity of the LCR of HPV-16 both in the presence and absence of the viral trans-activator E2.

cytes has been established in experimental cattle (Stocco dos Santos et al., 1998). The genomes appear to be maintained as episomes but whether the E1 and E2 genes are transcribed it is not known (E1 and E2 proteins are necessary for episomal DNA replication and maintenance). More work needs to be done to resolve this point.

3.6. CRPV and skin carcinoma

The role of CRPV in skin carcinogenesis of domestic rabbits, and the interplay of the virus with chemical carcinogens, such as tar, was well established at the beginning of the 20th century. Given the relative ease with which CRPV DNA induces papillomas, the CRPV papilloma–carcinoma disease is a very powerful system in which to study which viral genes are necessary for papilloma induction and malignant progression and how the host genetic constitution contributes to neoplasia. Using mutated forms of infectious viral DNA it was established that the E7 gene is necessary for papilloma induction (Brandsma et al., 1991) while the E4 gene can be dispensed for the induction of papillomas but not for viral DNA amplification and expression of the late structural proteins (J. Doorbar, personal communication).

3.7. Virus polymorphism, host genetic constitution and tumour progression

The influence of the host genetic constitution on disease progression is made clear by the observation that the majority of papillomas persist in a benign state in cottontail rabbits, whereas in domestic rabbits the majority of papillomas progress to cancer (Kreider and Bartlett, 1981). Even among domestic rabbits, however, the rate of progression differs, with some animals appearing more prone to cancer development. Genetic and molecular studies have demonstrated that rabbits with a DRA-C/DQA-G MHC class II haplotype are more susceptible to tumour progression than rabbits with a DRA-B/DQA-E haplotype (Fig. 8; Han et al., 1992, 1994). MHC class II presents peptides to T-lymphocytes and association of a particular MHC class II haplotype with tumour progression suggests that antigen presentation by that haplotype may be sub-optimal. The complex interaction between virus and host is further highlighted by the fact that CRPV variants in the E6 gene present a higher risk for cancer progression in association with DRA-B/DQA-E haplotype (Fig. 8; Salmon et al., 2000). A similar association between MHC class II, HPV E6 variants and increased risk of cancer progression has been observed also in humans (Wank and

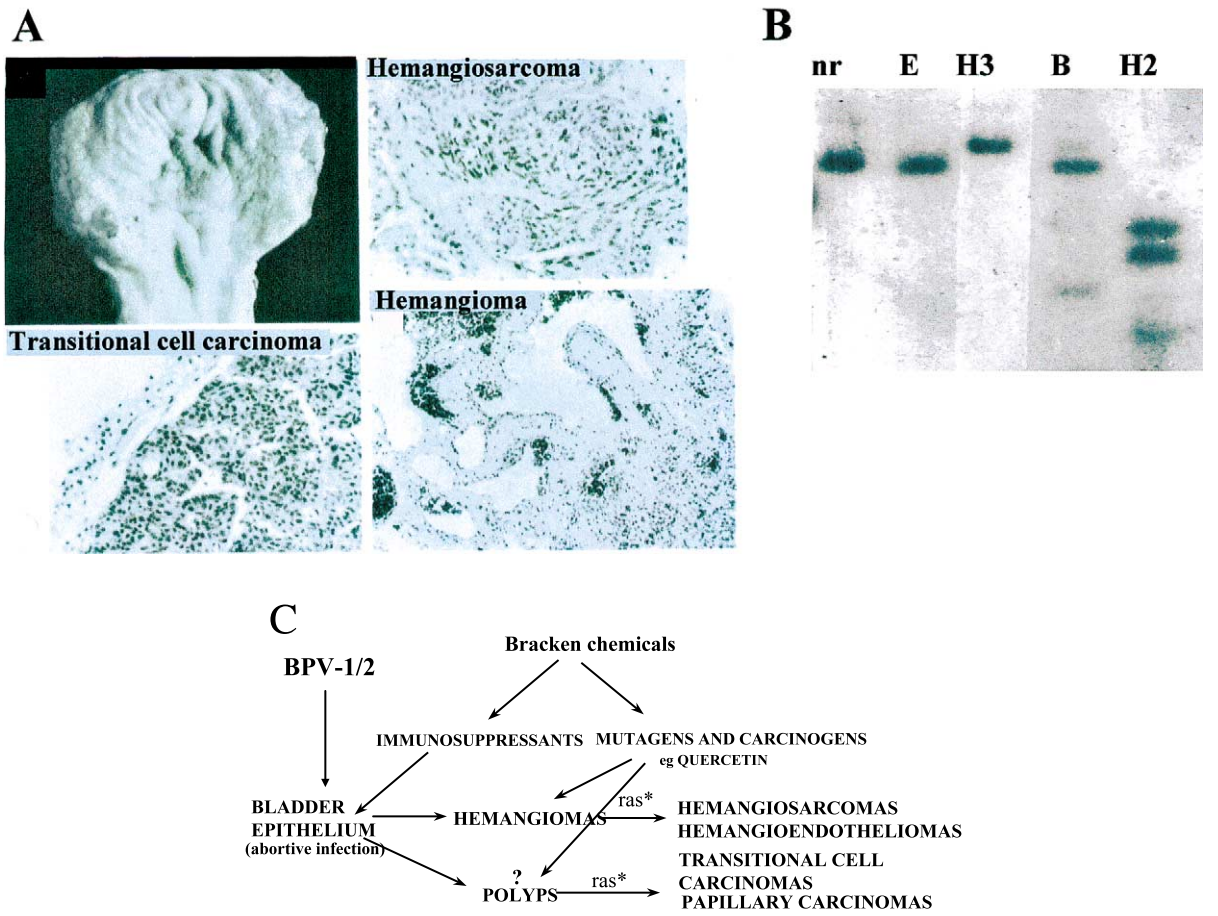

Fig. 7. BPV-2 is involved in urinary bladder cancers in cattle. (A) Urinary bladder with cancers and histological section (H&E) of three different types of bladder cancers; (B) southern blot of episomal BPV-2 DNA from a bladder carcinoma; (C) diagrammatic representation of carcinogenesis of the urinary bladder.

Thomssen, 1991; Zehbe et al., 2001). These findings stress the delicate balance between the pathogen and its host, and how otherwise innocuous genetic differences can tip this balance away from the norm and into an unfavourable condition for both the host, now harbouring cancer, and the virus, now incapable of replicating infectious progeny in the cancer.

4. Animal papillomaviruses as models for antiviral vaccines

The animal models of papillomavirus infection have proved extremely valuable in the search and development of anti-viral vaccines. From the 'first generation' vaccines based on inactivated virus, to the latest DNA vaccines, the animal systems have shown that vaccination against papillomavirus is feasible and effective both prophylactic and therapeutically. The instances of successful vaccines against animal papillomaviruses are too many to enumerate here and several reviews on vaccination in animals are available (Campo, 1997b; Schiller and Lowy, 2001). A point that deserves comment is the fact that vaccines based on the same viral gene/protein can be effective either prophylactically, providing protection from infection/disease or therapeutically, inducing regression of early lesions, thus challenging the old, and perhaps

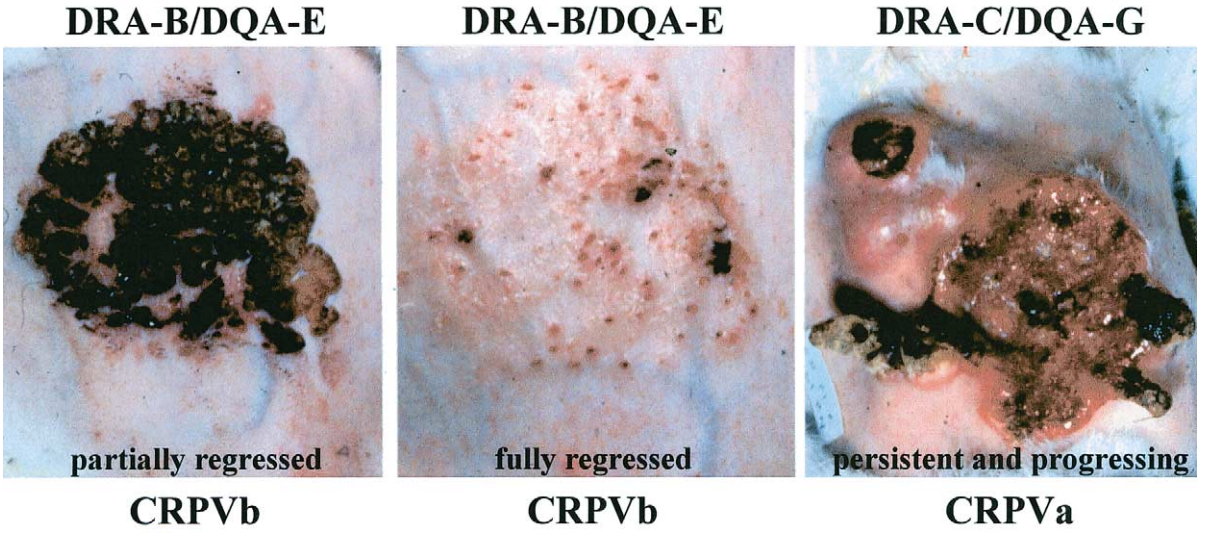

Fig. 8. CRPV papilloma regression or progression is determined by MHC class II haplotype and CRPV variants. Left and middle panels show a partially regressed and a fully regressed papilloma induced by CRPVb in a DRA-B/DQA rabbit; the right panel show a progressing papilloma induced by CRPVa in a DRA-C/DQA-G rabbit. Images courtesy of F. Breitburd. Modified with permission from Breitburd and Coursaget (1999).

naïve, belief that viral structural protein vaccines would provide protection from, and non-structural protein vaccines cure of, virus-induced lesions. It is worth emphasising that, following trials in animals, numerous clinical trials have started in humans with vaccines against HPV which are closely based on vaccines developed against animal papillomaviruses (Borysiewicz et al., 1996; Harro et al., 2001; Lehtinen et al., 2000).

5. Novel features of the molecular biology of animal papillomaviruses

The ability of BPV-1 or its genome to transform cultured cells opened up the investigation into the molecular aspects of viral gene transcription, viral DNA replication and cell transformation. These investigations were soon followed by similar studies on HPVs and research efforts understandably shifted in focus from BPV to HPV. Although BPVs have taken a back seat, many interesting aspects of viral biology have been recently uncovered in investigations of BPVs.

5.1. E2, mitotic chromosomes and PODs

BPV-1 E2 associates with mitotic chromosomes (Bastien and McBride, 2000; Ilves et al., 1999) via its amino terminal portion allowing its DNA binding domain to remain associated with viral genomes. Thus, E2 mediates the association of the viral genomes with the mitotic chromosomes resulting in efficient distribution of the BPV genomes into the daughter cells. It has still to be determined if HPV E2 is likewise involved in viral chromosome segregation, but it would be surprising if it did not.

A further role for E2 in the regulation of the viral life cycle has come from the recent observation that BPV-1 E2 can enhance the packaging of plasmid DNA into pseudovirions (Zhao et al., 2000) and appears to be directed to POD domains by the minor capsid protein L2 (Day et al., 1998; Heino et al., 2000). POD domains are nuclear structures associated with virus replication, and localisation of E2 to these structures is suggestive of its role in promotion of virion assembly. We have shown recently that also in the case of HPV-16, E2 is directed to PODs by L2 and that this localisation affects both the transcription and the

replication functions of E2 (Cordano et al., unpublished observations).

5.2. E5 and MHC I down-regulation

Major histocompatibility complex class I (MHC I) is responsible for the presentation of antigenic peptides to effector T-cells and therefore, plays a critical role in immune surveillance. β2-Microglobulin and chaperones associate with MHC I heavy chain in the endoplasmic reticulum where peptides are loaded onto the MHC I heavy chain in a pH-dependent process (Gromme et al., 1999; Reich et al., 1997). The complex is transported from the endoplasmic reticulum through the Golgi apparatus, where dissociation of MHC I from chaperons takes place (van Leeuwen and Kearse, 1996), to the plasma membrane for recognition by T-cells (Cresswell et al., 1999).

We have recently shown that BPV E5 prevents the transport of MHC I to the cell surface (Ashrafi et al., 2002) and sequesters MHC I in the Golgi cisternae (Marchetti et al., in press). Down-regulation of MHC I by E5 takes place at different levels, including reduced transcription of the MHC I heavy chain gene, lower levels of the MHC I heavy chain protein and impeded transport of the MHC I complex to the cell surface (Ashrafi et al., 2002). It is not yet known how E5 achieves down-regulation of MHC I, but recent results suggest that the retention of MHC I in the Golgi membranes is due to the E5-induced alkalinisation of the Golgi apparatus (Marchetti et al., in press). Lack of surface MHC I is observed also in cells transformed by HPV E5 proteins (O'Brien and Campo, 2002; Ashrafi et al., unpublished observations), indicating that this is a common function of E5 proteins.

By down-regulating MHC I, E5 would help to evade host immunosurveillance and thus the establishment of a successful infection.

6. Conclusions

Animal papillomavirus systems have been ground breaking in the recognition of the oncogenic nature of the virus, the elucidation of the relationship between virus and co-factors and the development of anti-viral vaccines. The latest insights in BPV E2 and E5 functions show that animal papillomaviruses can still lead the way and contribute significantly to our understanding of virus biology.

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