Cell PRESS

The clinical importance of understanding the evolution of papillomaviruses

Ignacio G. Bravo[1,2], Silvia de Sanjosé[2,3] and Marc Gottschling[4]

[1] Genomics and Health, Center for Public Health Research (CSISP), Avda, Cataluña 21, 46020 Valencia, Spain
[2] CIBER Epidemiology and Public Health (CIBERESP), Barcelona, Spain
[3] Infections and Cancer, Catalan Institute of Oncology, Barcelona, Spain
[4] Department of Biology I, Systematic Botany and Mycology, GeoBio-Center, Ludwig-Maximilians-Universität München, Munich, Germany

A significant fraction of human cancers is associated with infections by different papillomaviruses (PVs). In other vertebrates, the presence of specific PVs is also associated with different neoplasias. The popular view of PVs conceives them to be largely static and relies on generalized assumptions that have rarely been rigorously tested such as: virus–host codivergence, strict tissue tropism and host-specificity, their very low mutation rate and the absence of recombination. Here, we want to stress the need and the medical importance of understanding the evolutionary history and present-day dynamics of PVs. Understanding the way that PV genomes have evolved will clarify the link between a given genotype and the phenotypic and clinical outcome of the corresponding viral infection.

Papillomavirus infection and the link with cancer

Papillomaviridae are small non-encapsulated viruses with a double-stranded DNA circular genome of approximately 8 kbp. Papillomavirus (PV) DNA has been recovered from the skin and lesions of many mammalian species. Moreover, PVs or their genetic material have also been found in birds and turtles. Many PVs seem to cause asymptomatic infections and have been recovered from the healthy skin of many mammals [1]. Negative results in certain mammals could reflect inadequacies in experimental techniques or extinction/sorting events in certain host species. Other PVs induce conspicuous infections of the epithelia and give rise to hyperkeratotic lesions, such as plantar and hand warts in humans caused by HPV1, oral warts in dogs caused by CPV1 and horny warts in the cottontail rabbit caused by SfPV1. Infections by particular human PVs, such as HPV6 and HPV11, cause genital warts and are among the most common sexually transmitted infections.

Infectious agents can account for 20% of the global cancer burden [2]. Several PVs are recognized by the World Health Organization as human carcinogens [3], as the link between cancer of the cervix and infection by so-called 'high-risk' human PVs (e.g. HPV16 or HPV18) is well established [4]. World estimations in 2004 attribute

Glossary

Amniota (amniotes): are a monophyletic group of tetrapods with eggs adapted to the terrestrial environment.
Apomorphy (evolutionary novelty): is any feature novel to an evolutionary lineage and its descendants. The concept of synapomorphies and 'the search for the sister group' are suitable only to infer close relationships. Sister groups comprise two monophyletic entities that are closer related to each other than to any other taxon. Monophyletic groups are characterized by autapomorphies (i.e. unique to a particular taxon), whereas sister groups share synapomorphies.
Biological classification: is a conceptual operation to partition a given collection of individuals with regard to resemblance and common ancestry. The current basis of PV classification [66] is the mutual comparison of molecular sequence data and the subsequently inferred phylogenetic trees, refined by phenotypic clinical manifestations of infections.
Convergent evolution: is the acquisition of (sometimes highly) similar biological traits in only distantly related lineages. Such similarity refers to analogous structures, in contrast to homologous structures, and can appear as independent adaptations to similar environments and habitats with comparable ecologies.
Divergence: is an evolutionary process by which new biological species (and subsequently clades) arise. This (probably most important) process includes a separation event (by means of geographical, cultural or behavioral barriers), a divergence phase and a final isolation event. For sexually active organisms this is also known as speciation.
Homology: refers to a historical continuity, in which morphological features in related taxa are similar in pattern or form because they evolved from a corresponding structure in a common ancestor. Homology criteria are 'position', 'structure' and 'specific quality'.
Monophyletic group: is a taxon that forms a clade, thus consisting of an ancestor and all its descendants. Each PV taxon at the species and genus level has been identified monophyletic as inferred from molecular phylogenetic trees [15,17,65,66]. Non-monophyletic groups (paraphyletic and polyphyletic) are of little use for analyses of evolutionary processes.
Paraphyletic group: is a taxon consisting of an ancestor and some, but not all, of its descendants. Human Alpha-PVs are paraphyletic, as they share a common ancestor but do not include other Alpha-PVs, such as chimpanzee and macaque PVs that do not infect humans.
Plesiomorphy: is an ancestral state of a character, present in the ancestor of a group of taxa. Taxa that are held together by plesiomorphies are paraphyletic, as they usually exclude one or more taxa that show autapomorphies. Evolutionary polarity of characters is determined by outgroup comparison.
Polyphyletic group: is a taxon that does not share a common ancestor. Human PVs, consisting of at least six only distantly related lineages, exemplify such a polyphyletic group.

Corresponding author: Bravo, I.G. (bravo_ign@gva.es).

0966-842X/$ – see front matter doi:10.1016/j.tim.2010.07.008 Trends in Microbiology, October 2010, Vol. 18, No. 10

Systematics: is the philosophy of organizing nature.
Taxon: is a group of one or more organisms that a taxonomist adjudges to be a unit. It is not a requirement to provide a taxon with a name and a rank, although it is frequently done. The usage of scientific names is governed by one of the six Nomenclature Codes (including the International Code of Virus Classification and Nomenclature), which sets out rules to determine the scientific name that is correct for a particular taxon. The regulations of the particular codes differ considerably.
Taxonomy: the usage of sets of organic (including molecular) data guided by systematic principles to sort organisms into different taxa. It includes identification and naming of organisms.

more than 270,000 deaths to cervical cancer, 85% of them in developing countries (http://www.who.int/hpvcentre/en/). Globally, PVs account for more than 30% of all infection-associated cancers in humans, as they are also putatively involved in cancers of the penis, vagina, vulva, anus, perianal region and head and neck [2]. The 2008 Nobel Prize in Physiology and Medicine awarded to Harald zur Hausen "for his discovery of human papilloma viruses causing cervical cancer" acknowledges the importance of this connection (http://nobelprize.org/nobel_prizes/medicine/laureates/2008/). Two vaccines using capsid proteins as immunogens from the most clinically relevant human PVs that cause cervical cancer have been recently licensed [5,6] and seem to offer at least mid-lasting protection (4–6 years) [7,8]. Certain PV-related malignancies could thus become preventable diseases, but projections for 2030 still foresee more than 470,000 deaths and almost 4 million years of life lost due to cervical cancer in the absence of a widespread application of human PV vaccines (http://www.who.int/healthinfo/global_burden_disease/projections/en/index.html).

The importance of knowing the diversity, taxonomy (see Glossary) and evolution of PVs for diagnostic and therapeutic approaches has been increasingly acknowledged over the past few years [9–11]. PV research relies on evidence-based experiments, primarily investigating the present-day effects of gene expression or suppression, virus–host interactions at a molecular level and epidemiology. The persuasive power of an evolutionary virology approach lies in the assessment of the phylogenetic history of a group. Genealogical relationships allow for the reconstruction of evolutionary mechanisms as causes for biological diversity and a battery of high-quality tools are currently available to infer phylogenetic trees. However, our understanding of the origin and evolution of these viruses lacks a solid scientific background in many cases, despite the enormous advances in basic and medical biology of PVs. Thus, we summarize in this opinion article the importance of understanding the evolutionary history of PVs for an improved evaluation of the clinical manifestations induced by viral infections and the variety of evolutionary mechanisms driving PV diversification.

Virus–host codivergence alone does not account for PV diversity and their occurrence on specific hosts

On the basis that PV phylogeny resembles to some extent the phylogeny of their hosts [12,13], it is commonplace in PV literature to read that these viruses have codiverged with their hosts. If codivergence alone was the driving force for present-day PV diversity, then (i) all PVs infecting the same host should be monophyletic and (ii) the topologies of the phylogenetic trees of hosts and of PVs should be entirely congruent. However, this hypothesis is frequently rejected, as many only distantly related PVs infect the same host species. Moreover, phylogenetic trees of the viruses and their hosts are largely incongruent. PVs infecting humans are the most striking example: they are not monophyletic but are polyphyletic and have different close relatives present on different, non-human host species. The same is true for PVs infecting chimpanzees, gorillas, macaques, multimammate rats, dogs, cats and cattle (Figure 1) [14–16]. Multigene phylogenetic trees of PVs indicate four large, diverse and well-supported clades at high taxonomic level that accommodate most of the known PV diversity [15,17,18]. Distantly related placental host species are represented in each of the supertaxa and the initial radiation of PVs might have predated the divergence of large placental groups, which occurred sometime in the Mesozoic era [19]. The origin of mammalian skin might have provided new habitats for the viruses, presumably in the form of new cell types or new histological microenvironments and new ecological niches have subsequently been established. Later on, diversification of mammalian hosts could have further triggered diversification of PVs.

PV evolutionary history

We believe that the phylogenetic relationships among PVs are compatible with an evolutionary scenario that considers multiple driving forces for viral diversification. The last common ancestor of the amniotes was already infected by at least one ancestral PV and mammals are one of the major lineages of the amniotes. An ancestral, probably cutaneotropic PV, might have diverged into a small number of different PVs, possibly linked to the differentiation of mammalian skin [20]. The last common ancestor of mammals might thus have already been infected by several different PVs.

One early PV lineage, probably with a (derived) mucosal tropism gave rise to the supertaxon comprising Alpha- and Omicron-PVs and all their close relatives. This supertaxon includes viruses infecting primates, carnivores, swine and cetaceans (Figure 1, red clade). The medically important group of Alpha-PVs belongs to this supertaxon, with viruses associated with cervical cancer in humans [4]. A viral lineage within Alpha-PVs secondarily shifted tropism from mucosal tissue towards skin, leading to the extant species 2, 3, 4, 14 and 15, usually associated with common skin warts. However, Alpha-PVs infecting humans are not monophyletic, as several non-human viruses are nested within the (paraphyletic) human viruses in a highly polyphyletic pattern. The hypothesis of codivergence in a generalized form is thus rejected here. Therefore, it needs to be determined whether additional evolutionary mechanisms such as an adaptive radiation into different ecological habitats in the ancestor of humans, apes and monkeys could better explain such conflicting tree topologies.

A second early PV lineage gave rise to a large clade of primarily cutaneotropic PVs including the Beta- and Xi-PVs and all their close relatives (Figure 1, green clade).

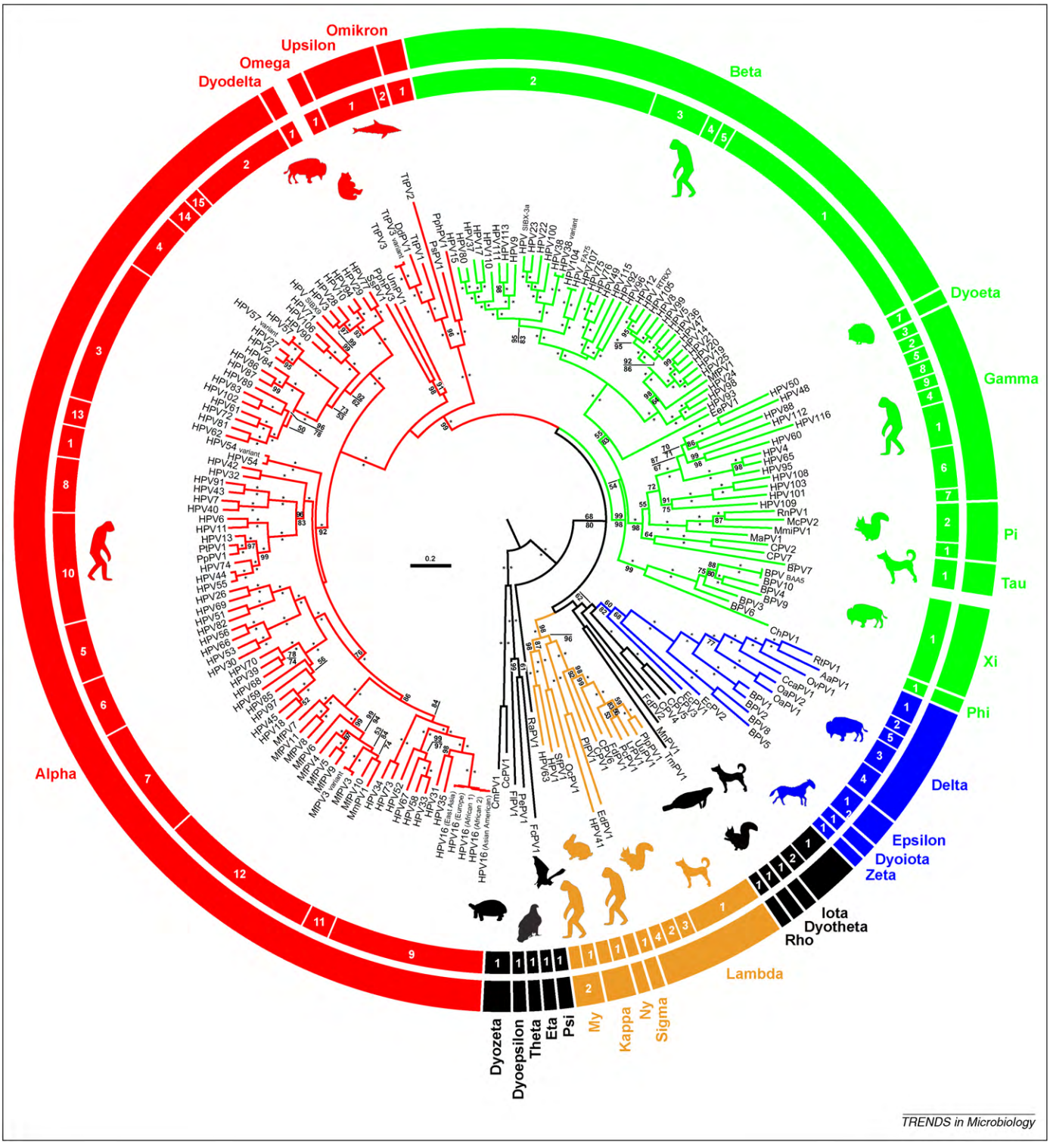

Figure 1. the best-known maximum likelihood phylogenetic tree for PVs. This tree was constructed using the concatenated E1–E2–L1 genes, aligned at the amino acid level with MUSCLE (http://www.drive5.com/muscle/), back-translated into nucleotides, filtered with GBLOCKS (http://molevol.cmima.csic.es/castresana/Gblocks.html) and calculated with RAxML (http://wwwkramer.in.tum.de/exelixis/software.html), both at the amino acid level (using generalized time-reversible, four gamma categories and three partitions, one per gene) and at the nucleotide level (using generalized time-reversible, four gamma categories and nine partitions, one per codon position per gene). Support after 1000 bootstrap replicates for either reconstruction is given above and below the nodes, respectively (asterisks indicate maximal support). Color codes highlight the four PV supertaxa. PVs that cannot be assigned yet with confidence to a supertaxon are labeled in black. Silhouettes represent the hosts infected by the corresponding viruses.

Hosts of this supertaxon are heterogeneous and comprise primates, rodents, carnivores, ruminants, hedgehogs and even marsupials. Clear congruence between phylogenetic trees of viruses and their hosts is rare and human PVs are not monophyletic within this supertaxon either. Many viruses of this supertaxon have been found in healthy skin and are apparently not associated with lesions [16,21]. Others cause malignant transformation depending on the genetic susceptibility of the host or on the coexposure to carcinogens. For example, infections of dogs by CPV3 [22] or of humans by HPV5 or HPV8 [23] are associated with the development of squamous cell carcinoma in

individuals suffering from the hereditary disease epidermodysplasia verruciformis. Some Beta- and Gamma-PVs have secondarily developed mucosal tropism in humans [24,25]. In cattle, BPV4 together with the carcinogen ptaquiloside from bracken fern can cause carcinoma of the gastrointestinal tract [26], whereas treatment of the oral mucosa with a chemical carcinogen and physical wounding results in malignant transformation associated with MaPV1 in hamster [27].

A third PV lineage gave rise to the supertaxon of Delta- and Zeta-PVs and all their close relatives, infecting perissodactylans and ruminants (Figure 1, blue clade). Delta-PVs are often quoted as an example of virus–host codivergence, but the absence of congruence between viruses and hosts phylogenies is paradigmatic here: (i) PVs infecting cattle (BPV1, BPV2, BPV5 and BPV8) are a paraphyletic assemblage and not monophyletic like their hosts; (ii) sheep PVs are closely related to (monophyletic) cervid PVs but not to the other bovid PVs; and (iii) PVs infecting horses (EcPV1 and EcPV2) are polyphyletic. Pathology associated with some of these viruses is unique, as infection involves fibroblasts and gives rise to fibropapillomas in cattle (BPV1 and BPV2) or roe deer (CcaPv1) [26,28]. In cattle exposed to bracken fern, BPV1 and BPV2 also have the potential to cause cancer of the urinary bladder [29].

A fourth PV lineage gave rise to the supertaxon including Kappa- and Lambda-PVs and all their close relatives, with primates, rodents, lagomorphs and carnivores as hosts (Figure 1, ochre clade). Evolution of Lambda-PVs is usually taken as an example of virus–host codivergence [12] because the knowledge about PV diversity on different hosts is higher than for most other PV genera. However, Lambda-PVs infecting canids are not monophyletic and neither is the relationships among the felid hosts [19], nor have the relationships among the corresponding viruses been sufficiently resolved to reliably conclude this hypothesis. Thus, a solid case of cophylogenetic relationships between PVs and their mammalian hosts supported by identical tree topologies, even on a local scale, is still required. Viruses in this supertaxon are heterogeneous in the pathological outcome of the infection [30], with some causing oral lesions (CPV1 in dogs and SfPV1 in cottontail rabbits), cutaneotropic viral plaques (FPV1 in cats) and plantar warts (HPV1 in humans).

Tissue tropism, host specificity and recombination

Virus–host codivergence might play an important role in the evolution of PVs, but a single driving force cannot fully account for PV diversity and for the phylogenetic relationships among the viruses [15]. Therefore, additional evolutionary mechanisms, such as recombination, need to be considered. To date, no recombinant PV strain has been experimentally shown and the potential for PV recombination is thus considered low. However, depending on the region of the genome analyzed, phylogenetic inconsistencies within Alpha-PVs [10,31] and of Upsilon- and Omicron-PVs [32], as well as *in silico* indications for ancient recombination events in Alpha-PVs [33,34] and the multiple events of gene gains and losses, help build a case for the plausibility of recombination in PVs. Moreover, two defective viruses can trans-complement each other in a rabbit model and cause a productive infection [35]. The virus isolated from the marsupial *Perameles bougainville*, with genomic features from both PVs and polyomaviruses [36], also suggests a broad potential for recombination and/or a chimeric origin and/or modular evolution of PVs.

Strict host specificity of PVs might act as a barrier that prevents close physical contact between different viruses, but a series of PVs infect a variety of phylogenetically distant hosts ('heterologous PV infections'). For example, BPV1 and BPV2 infect cattle but also infect close relatives of cattle such as water buffalo and giraffe [37]. Moreover, these viruses naturally infect more distantly related species as well, including horse, donkey, tapir and zebra [38,39] and cause fibrosarcomas when inoculated into rodents [40]. Such a host switch lowers the evolutionary pressures on the PVs present in the original host and might allow for changes in infection timing and virulence [41]. Even if the initial replication steps are not efficient, evolution upon host switch could ultimately lead to the colonization of a new host [42]. This seems to have been the case with BPV1 and BPV2 in horses, where genetic material from these viruses is found on the skin of more than two-thirds of healthy, stabled horses [43]. The genetic changes in viral sequences retrieved from horses further suggest an adaptation process to the environment provided by the new host [44]. More examples for heterologous PV infections include the presence of feline sarcoma PV on bovine skin [45], the presence of HPV9 in lesions of a cat [46], the possible transient infections of zookeepers with animal PVs [16] and interspecies infection between macaques [47].

The mechanisms for heterologous PV infections are relatively poorly understood at present. Domestication of animals, as well as humans, might have played a major role [16]. Horizontal skin-to-skin contact seems to be enough for PVs to spread among humans [48], even if the infection is transient and non-productive in many cases. Humans might acquire asymptomatic infections during birth, in the first weeks of life and throughout their lifetime [49–51]. The high prevalence of antibodies against PV proteins in the general population also indicates that humans and other animals are exposed to PVs from the earliest stages of life [52,53]. As in many other viral groups, arthropods could act as vectors for PV dispersal, as suggested by the presence of BPV1 and BPV2 DNA in the face fly feeding on horse sarcoids [54]. At present, we lack further information about this virus–vector–host system. The zoonotic potential and the medical implications for the corresponding transmission route need to be explored.

PVs are considered primarily epitheliotropic, but corresponding viral genetic material has been retrieved from a large variety of tissues. In humans, DNA and mRNA from different PVs have been detected in the blood of patients suffering from cervical cancer [55,56] as well as in healthy blood donors [57,58]. Viral DNA has been isolated from peripheral blood mononuclear cells, milk, urine, seminal fluid and sperm cells of animals infected with BPV1, BPV2 and BPV4 [39,59,60]. Genomic material of MnPV1 and McPV2 was found in anogenital lesions of multimammate rats but also in their livers, spleens,

kidneys and gastrointestinal tracts [61]. In horses, BPV1 can also be found in peripheral blood mononuclear cells and these cells might serve as stepping stones during the infection process [62]. Presence of DNA, however, does not necessarily imply an effective infection and more research is necessary to interpret such results.

The implications of the plausibility of recombination, the presence of a single PV in a variety of tissues within the same host and the presence of the same PV in a variety of hosts might be profound. Medically, the possibility of PV transmission via blood transfusion [57,58] or via an arthropod vector [54] cannot be excluded, although transmission might be less efficient than the well-established mucosal–mucosal pathway. From an evolutionary perspective, the provision of new habitats (i.e. either new cell types or new host species) and their colonization facilitates an adaptation process that results in generalist rather than in specialist viruses [63]. Generalist viruses are usually capable of successfully exploiting a variety of habitats, whereas specialist viruses tend to have lower efficiency when changing environment [41]. Different PVs show differences in their host diversity: some appear to be highly host-specific (although it should be said that most PVs have been isolated only once), whereas others display a wider host range. In this regard, BPV1 and BPV2, which are capable of infecting different cell types and different hosts, are paradigmatic. However, they might not be the only PVs with such a phenotype.

PV taxonomy and nomenclature

Taxonomy and systematics are human creations that help guide research by providing a framework and defining limits in a world of an uncountable richness in biological diversity. An unambiguous naming system is a necessary prerequisite for any further reproducible and experimental approach, particularly in times of an exponential increase in our knowledge of viral diversity. The two pillars that sustain biological classification are genealogy and similarity [64]. The Study Group of Papillomaviruses within the International Committee on Taxonomy of Viruses has pioneered the introduction of both concepts into PV classification. The guidelines issued in 2004 defined clear-cut nucleotide similarity values to delimitate variants, types and genera [65]. The recently updated taxonomy [66] has further improved the classification by including common ancestry as a criterion and refraining from strict limits for the demarcation of PV types and genera. This acknowledges that alignment-based sequence similarity values differ between PV clades [17], as is also the case for cellular organisms [67].

Despite the quality of the current PV classification system, some issues should nevertheless be reconsidered in future. First, nomenclature based on the Greek alphabet for naming taxonomic entities will not be able to cope with, and remain informative for, the enormous diversity of PVs likely to be discovered [68]. Second, PV nomenclature is currently linked to the scientific names of the hosts, which is problematic in several respects. Names such as Human PV, Bovine PV and Canine PV might imply that viruses infecting the same host, or with a similar tropism, are evolutionarily related, but this is frequently not the case. Moreover, the current virus taxonomy rules implicitly assume that the first detection of a PV occurred in the original host. Thus, BPV1 would have been termed EcPV1, if it had been originally detected in a horse. Creative solutions for such problems as well as the revision of possible alternative naming approaches should be the goal for future PV classification updates. Third, genus is still the highest-level category within Papillomaviridae and all genera comprise assemblages of equal rank by definition, without any more detailed systematic structure. Equalizing Alpha- and Beta-PVs at the same taxonomic rank impedes information about their degree of relationship and they are only distantly related: Alpha-PVs are more closely related to TtPV2, causing genital condylomata in the bottlenose dolphin, than to any other PV infecting primates. The same is true for Beta-PVs, which are more closely related to EePV1, detected in a hedgehog, than to any other PV infecting primates.

Comparative biology of distantly related PVs infecting the same host species can be highly informative, as it might suggest events of convergent evolution while being medically informative [69]. However, convergent evolution should not be confused with similarities that arise from common ancestry (i.e. homologies). Highlighting the criterion of common ancestry will thus pinpoint medically interesting questions. An example is the possible chimeric nature of HPV54 that shows a differential relationship with high-risk and low-risk PVs (so-called because of their association with cervical cancer) according to the genome region analyzed. However, the current anthropocentric view of PVs ignores common ancestry between certain human and non-human PVs. This could result in a loss of opportunity for medical research, for example choice of (in)appropriate animal models [70].

Finally, there is strong evidence for the existence of monophyletic clusters of generic PV clades or 'supertaxa' [14,15,17,18] that should be considered for future PV classification. An exhaustive classification of encaptic biological hierarchy, as visualized in a phylogenetic tree, would require as many categories as nodes, but the number of systematic categories available is principally limited. A phylogeny-based PV classification that acknowledges the existence of monophyletic assemblages of PVs at different taxonomic levels would allow for fine-scale information about relationships and might also simplify nomenclature. Recognizing the existence of supertaxa and subsequent categorizing of PV genera within them would be an example of such changes and is exemplified by colors in Figure 1.

Concluding remarks

The popular view on PVs assumes that these viruses are evolutionary static and diverge so slowly that it is not necessary to integrate viral evolution into biological, medical and epidemiological models. The focus of attention of the large PV community should be on these widespread but unidimensional assumptions, as well as on the risks and misconceptions associated with them. If we can explain and understand how PVs have evolved and make inferences on ancestral properties, then we might be able to elucidate the path that leads from viral genotype to phenotype (Box 1). Identification of differences and

Box 1. Open research questions

- What are the evolutionary relationships between PVs, polyomaviruses and the recently discovered viruses that display characteristics of both of them? Are they the result of an ancestral recombination event?
- What were the histological, cellular, molecular and/or environmental changes that drove the basal diversification in PVs and led to the extant supertaxa?
- For particular PVs, phylogenetic reconstructions render different topologies when based on the early genes E1–E2 and on the late genes L1–L2. Do these inconsistencies reflect ancestral recombination events or rather convergent evolution during adaptation to similar host environments?
- Certain Delta-PVs can infect distantly related hosts and other examples of putative horizontal transmission have also been reported for different PVs. Some arthropod species could even act as vectors for PV infection. Should we rethink our paradigm of PVs being strictly species-specific? What would be the impact of broad host tropism in PV evolution? What is the potential for zoonosis in PV?
- PV genetic material and mRNA have been found in different tissues other than epithelia. Should we rethink our paradigm of PVs being strict epitheliotropic? What would be the impact of broad cellular tropism in PV evolution?
- Within the largely mucosal Alpha-PVs some viruses are cutaneotropic (e.g. HPV2 and HPV3), whereas within the largely cutaneotropic Beta- and Gamma-PVs some viruses are mucosotropic (e.g. HPV98 or HPV104). Can we find molecular correlates of this tropism shift? Can we find a molecular signature for PVs infecting mucosa, skin or both?
- Many only distantly related PVs infect humans. The same is true for multimammate rats, dogs, cats and cattle. There is thus evidence to consider every amniote as host of many different PVs. Can we find molecular correlates of convergent evolution in distant viruses that infect the same host? Can we find a molecular signature for PVs infecting humans, for viruses infecting cattle and so on?
- Within Alpha-PVs, high-risk HPVs are monophyletic (if we include with them some macaque PVs), whereas low-risk HPVs, associated with benign lesions, are paraphyletic. The potential to cause cancer might thus be a derived state, an autapomorphy of high-risk HPVs. Can we find a molecular signature for this potential?
- The prevalence of high-risk HPVs is not evenly distributed in the cases of cervical cancer. Can we find a molecular correlate for this exacerbated association between certain high-risk HPVs and cervical cancer? Can we find evidence for convergent evolution in HPV16 and HPV18 that makes them different from the rest of Alpha-9 and Alpha-7 PVs, respectively? Or are they exploiting different mechanisms for malignization?

similarities between distantly related viruses that infect the same host species also has explanatory potential for medical research. Only such an integrative perception might eventually shed light onto what makes particular PVs trigger cancer, whereas others simply give rise to benign transformations.

Acknowledgements

I.G.B. is funded by the Spanish Ministerio de Ciencia e Innovación (MICINN) Ramón y Cajal program and CGL2009-08350.

References

1 Antonsson, A. *et al.* (2003) Prevalence and type spectrum of human papillomaviruses in healthy skin samples collected in three continents. *J. Gen. Virol.* 84, 1881–1886
2 Zur Hausen, H. (2009) The search for infectious causes of human cancers: where and why. *Virology* 392, 1–10
3 IARC (2007) *IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 90: Human Papillomaviruses*. International Agency for Research on Cancer
4 Muñoz, N. *et al.* (2003) Epidemiologic classification of human papillomavirus types associated with cervical cancer. *N. Engl. J. Med.* 348, 518–527
5 Villa, L.L. *et al.* (2006) Immunologic responses following administration of a vaccine targeting human papillomavirus Types 6, 11, 16, and 18. *Vaccine* 24, 5571–5583
6 Harper, D.M. *et al.* (2004) Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet* 364, 1757–1765
7 Villa, L.L. *et al.* (2006) High sustained efficacy of a prophylactic quadrivalent human papillomavirus types 6/11/16/18 L1 virus-like particle vaccine through 5 years of follow-up. *Br. J. Cancer* 95, 1459–1466
8 Harper, D.M. *et al.* (2006) Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet* 367, 1247–1255
9 Bernard, H-U. *et al.* (2006) Genome variation of human papillomavirus types: phylogenetic and medical implications. *Int. J. Cancer* 118, 1071–1076
10 Bravo, I.G. and Alonso, Á. (2004) Mucosal human papillomaviruses encode four different E5 proteins whose chemistry and phylogeny correlate with malignant or benign growth. *J. Virol.* 78, 13613–13626
11 Schiffman, M. *et al.* (2009) Classification of weakly carcinogenic human papillomavirus types: addressing the limits of epidemiology at the borderline. *Infect. Agent Cancer* 4, 8
12 Rector, A. *et al.* (2007) Ancient papillomavirus-host co-speciation in Felidae. *Genome Biol.* 8, R57
13 Bernard, H.U. (2005) The clinical importance of the nomenclature, evolution and taxonomy of human papillomaviruses. *J. Clin. Virol.* 32S, S1–6
14 Lange, C.E. *et al.* (2009) Three novel canine papillomaviruses support taxonomic clade formation. *J. Gen. Virol.* 90, 2615–2621
15 Gottschling, M. *et al.* (2007) Multiple evolutionary mechanisms drive papillomavirus diversification. *Mol. Biol. Evol.* 24, 1242–1258
16 Antonsson, A. and Hansson, B.G. (2002) Healthy skin of many animal species harbors papillomaviruses which are closely related to their human counterparts. *J. Virol.* 76, 12537–12542
17 Bravo, I.G. and Alonso, Á. (2007) Phylogeny and evolution of papillomaviruses based on the E1 and E2 proteins. *Virus Genes* 34, 249–262
18 Chan, S-Y. *et al.* (1995) Analysis of genomic sequences of 95 papillomavirus types: uniting typing, phylogeny, and taxonomy. *J. Virol.* 69, 3074–3083
19 Bininda-Emonds, O.R. *et al.* (2007) The delayed rise of present-day mammals. *Nature* 446, 507–512
20 Garcia-Vallve, S. *et al.* (2005) Papillomaviruses: different genes have different histories. *Trends Microbiol.* 13, 514–521
21 Forslund, O. (2007) Genetic diversity of cutaneous human papillomaviruses. *J. Gen. Virol.* 88, 2662–2669
22 Tobler, K. *et al.* (2006) Detection of the prototype of a potential novel genus in the family Papillomaviridae in association with canine epidermodysplasia verruciformis. *J. Gen. Virol.* 87, 3551–3557
23 Feltkamp, M.C. *et al.* (2008) Betapapillomaviruses: innocent bystanders or causes of skin cancer. *J. Clin. Virol.* 43, 353–360
24 de Villiers, E.M. and Gunst, K. (2009) Characterization of seven novel human papillomavirus types isolated from cutaneous tissue, but also present in mucosal lesions. *J. Gen. Virol.* 90, 1999–2004
25 Ekstrom, J. *et al.* (2010) Three novel papillomaviruses (HPV109, HPV112 and HPV114) and their presence in cutaneous and mucosal samples. *Virology* 397, 331–336
26 Campo, M.S. (2006) Bovine papillomavirus: old system, new lessons? In *Papillomavirus Biology: From Natural History to Vaccine and Beyond* (Campo, M.S., ed.), Caister Academic Press
27 Iwasaki, T. *et al.* (1997) Presence of a novel hamster oral papillomavirus in dysplastic lesions of hamster lingual mucosa induced by application of dimethylbenzanthracene and excisional wounding: molecular cloning and complete nucleotide sequence. *J. Gen. Virol.* 78, 1087–1093

28 Erdelyi, K. *et al.* (2008) Characterisation of the first complete genome sequence of the roe deer (*Capreolus capreolus*) papillomavirus. *Virus Res.* 135, 307–311
29 Campo, M.S. (2002) Animal models of papillomavirus pathogenesis. *Virus Res.* 89, 249–261
30 Munday, J.S. and Kiupel, M. (2010) Papillomavirus-associated cutaneous neoplasia in mammals. *Vet. Pathol.* 47, 254–264
31 Narechania, A. *et al.* (2005) Phylogenetic incongruence among oncogenic genital alpha human papillomaviruses. *J. Virol.* 79, 15503–15510
32 Rector, A. *et al.* (2008) Genomic characterization of novel dolphin papillomaviruses provides indications for recombination within the Papillomaviridae. *Virology* 378, 151–161
33 Angulo, M. and Carvajal-Rodriguez, A. (2007) Evidence of recombination within human alpha-papillomavirus. *Virol. J.* 4, 33
34 Varsani, A. *et al.* (2006) Evidence of ancient papillomavirus recombination. *J. Gen. Virol.* 87, 2527–2531
35 Hu, J. *et al.* (2009) Papillomavirus DNA complementation in vivo. *Virus Res.* 144, 117–122
36 Woolford, L. *et al.* (2007) A novel virus detected in papillomas and carcinomas of the endangered western barred bandicoot (*Perameles bougainville*) exhibits genomic features of both the Papillomaviridae and Polyomaviridae. *J. Virol.* 81, 13280–13290
37 Silvestre, O. *et al.* (2009) Bovine papillomavirus type 1 DNA and e5 oncoprotein expression in water buffalo fibropapillomas. *Vet. Pathol.* 46, 636–641
38 Bogaert, L. *et al.* (2005) Detection of bovine papillomavirus DNA on the normal skin and in the habitual surroundings of horses with and without equine sarcoids. *Res. Vet. Sci.* 79, 253–258
39 van Dyk, E. *et al.* (2009) Detection of bovine papillomavirus DNA in sarcoid-affected and healthy free-roaming zebra (*Equus zebra*) populations in South Africa. *J. Virol. Methods* 158, 141–151
40 Robl, M.G. and Olson, C. (1968) Oncogenic action of bovine papilloma virus in hamsters. *Cancer Res.* 28, 1596–1604
41 Turner, P.E. and Elena, S.F. (2000) Cost of host radiation in an RNA virus. *Genetics* 156, 1465–1470
42 Antia, R. *et al.* (2003) The role of evolution in the emergence of infectious diseases. *Nature* 426, 658–661
43 Bogaert, L. *et al.* (2008) High prevalence of bovine papillomaviral DNA in the normal skin of equine sarcoid-affected and healthy horses. *Vet. Microbiol.* 129, 58–68
44 Nasir, L. and Campo, M.S. (2008) Bovine papillomaviruses: their role in the aetiology of cutaneous tumours of bovids and equids. *Vet. Dermatol.* 19, 243–254
45 Munday, J.S. and Knight, C.G. (2010) Amplification of feline sarcoid-associated papillomavirus DNA sequences from bovine skin. *Vet. Dermatol.* 21, 341–344
46 Munday, J.S. *et al.* (2007) Feline cutaneous viral papilloma associated with human papillomavirus type 9. *Vet. Pathol.* 44, 924–927
47 Chen, Z. *et al.* (2009) Genomic diversity and interspecies host infection of alpha12 *Macaca fascicularis* papillomaviruses (MfPVs). *Virology* 393, 304–310
48 Hernandez, B.Y. *et al.* (2008) Transmission of human papillomavirus in heterosexual couples. *Emerg. Infect. Dis.* 14, 888–894
49 Antonsson, A. *et al.* (2003) General acquisition of human papillomavirus infections of skin occurs in early infancy. *J. Clin. Microbiol.* 41, 2509–2514
50 Gottschling, M. *et al.* (2009) Cutaneotropic human beta-/gamma-papillomaviruses are rarely shared between family members. *J. Invest. Dermatol.* 129, 2427–2434
51 Plasmeijer, E.I. *et al.* (2010) Transmission of betapapillomaviruses between domestic partners in an Australian community. *J. Clin. Virol.* 47, 216–218
52 Michael, K.M. *et al.* (2008) Seroprevalence of 34 human papillomavirus types in the German general population. *PLoS Pathog.* 4, e1000091
53 Lange, C.E. *et al.* (2009) Detection of antibodies against epidermodysplasia verruciformis-associated canine papillomavirus 3 in sera of dogs from Europe and Africa by enzyme-linked immunosorbent assay. *Clin. Vaccine Immunol.* 16, 66–72
54 Finlay, M. *et al.* (2009) The detection of Bovine Papillomavirus type 1 DNA in flies. *Virus Res.* 144, 315–317
55 Tseng, C.J. *et al.* (1999) Detection of human papillomavirus types 16 and 18 mRNA in peripheral blood of advanced cervical cancer patients and its association with prognosis. *J. Clin. Oncol.* 17, 1391–1396
56 Kay, P. *et al.* (2005) Detection of HPV 16 and HPV 18 DNA in the blood of patients with cervical cancer. *J. Med. Virol.* 75, 435–439
57 Bodaghi, S. *et al.* (2005) Could human papillomaviruses be spread through blood? *J. Clin. Microbiol.* 43, 5428–5434
58 Chen, A.C. *et al.* (2009) Human papillomavirus DNA detected in peripheral blood samples from healthy Australian male blood donors. *J. Med. Virol.* 81, 1792–1796
59 Roperto, S. *et al.* (2008) Detection of bovine papillomavirus type 2 in the peripheral blood of cattle with urinary bladder tumours: possible biological role. *J. Gen. Virol.* 89, 3027–3033
60 Lindsey, C.L. *et al.* (2009) Bovine papillomavirus DNA in milk, blood, urine, semen, and spermatozoa of bovine papillomavirus-infected animals. *Genet. Mol. Res.* 8, 310–318
61 Nafz, J. *et al.* (2008) A novel rodent papillomavirus isolated from anogenital lesions in its natural host. *Virology* 374, 186–197
62 Brandt, S. *et al.* (2008) Peripheral blood mononuclear cells represent a reservoir of bovine papillomavirus DNA in sarcoid-affected equines. *J. Gen. Virol.* 89, 1390–1395
63 Cooper, L.A. and Scott, T.W. (2001) Differential evolution of eastern equine encephalitis virus populations in response to host cell type. *Genetics* 157, 1403–1412
64 Mayr, E. (1994) Ordering systems. *Science* 266, 715–716
65 de Villiers, E-M. *et al.* (2004) Classification of papillomaviruses. *Virology* 324, 17–27
66 Bernard, H.U. *et al.* (2010) Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments. *Virology* 401, 70–79
67 Castresana, J. (2001) Cytochrome *b* phylogeny and the taxonomy of great apes and mammals. *Mol. Biol. Evol.* 18, 465–471
68 Bernard, H-U. (2005) The clinical importance of the nomenclature, evolution and taxonomy of human papillomaviruses. *J. Clin. Virol.* 32, 1–6
69 Garcia-Vallve, S. *et al.* (2006) Different papillomaviruses have different repertoires of transcription factor binding sites: convergence and divergence in the upstream regulatory region. *BMC Evol. Biol.* 6, 20
70 Borzacchiello, G. *et al.* (2009) Human papillomavirus research: do we still need animal models? *Int. J. Cancer* 125, 739–740

Have your say

Would you like to respond to any of the issues raised in this month's *TiM?* Please contact the Editor (etj.tim@elsevier.com) with a summary outlining what will be discussed in your letter and why the suggested topic would be timely. You can find author guidelines at our new website:

http://www.cell.com/trends/microbiology