Minireview

1st International Workshop on Papillomavirus E5 Oncogene—A report

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A B S T R A C T

The 1st International Workshop on Papillomavirus E5 Oncogene was held in Capri, Italy, 27–28 May 2010. Here we present a brief report of the various lectures which addressed the multiple facets of the E5 protein.

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The 1st International Workshop on Papillomavirus E5 Oncogene was held at Villa Orlandi on the stunningly beautiful island of Capri, Italy, organised by Giuseppe Borzacchiello and Franco Roperto, University of Naples, Saveria Campo, University of Glasgow, and Aldo Venuti, Regina Elena Institute for Cancer Research, Rome. The workshop was held under the auspices of the International Papillomavirus Society, the Italian Society of Virology, the Italian Association for Veterinary Pathology and the European Society of Veterinary Pathology. The purpose of the workshop was to bring together the relatively small community of virologists stubborn enough to work on the papillomavirus E5 oncoprotein in order to stimulate in-depth discussion and promote understanding of the role of E5, primarily HPV E5 and BPV E5, in cell transformation and the virus life cycle. The workshop was a success. Most of the E5 virologists from all over the world were able to attend, the presentations were highly interesting and the discussion lively and energetic. (We will talk about the food and wines later!).

First, a few words of introduction for the papillomavirus E5 protein for the non-aﬁcionados. E5 is a small hydrophobic protein of 83 amino acid residues in HPV-16, 44 in BPV-1 and 42 in BPV-4, the most studied E5 proteins. E5 is located in the cell endomembrane compartments, primarily the Golgi apparatus in the case of BPV E5 and the ER in the case of HPV E5. BPV E5 has a single pass transmembrane domain with short non-membrane associated N- and C-termini on opposite sites of the membrane, N-terminus cytoplasmic and C-terminus lumenal; HPV-16 E5 has three transmembrane pass domains and again the N- and C-terminus domains are on opposite membrane sides, but in this case the N-terminus is luminal and the C-terminus cytoplasmic. The BPV and HPV proteins share functions but, generally speaking, BPV E5 is the more “potent” protein, at least in terms of cell transformation (but see below the presentation of Paul Lambert!). In this report, presentations on HPV E5 will be dealt with first, then those on BPV E5 and finally those on the possibility of using E5 as a target for anti-papillomavirus therapy.

Given the location of E5 in the cell endomembranes, the workshop was opened appropriately enough with a Plenary Lecture on the functions of the Golgi apparatus delivered by Antonella De Matteis (Naples), who emphasised that the Golgi is not a mere site of passage of various biomolecules, but it has also regulatory functions in cell cycle (the Golgi fragments at mitosis and blocking Golgi fragmentation stops mitosis) and signal transduction (many kinases and G proteins are activated at the Golgi). It follows that either positive or negative interference by E5 with any of these processes would have a profound and cascade effect on many cell functions, such as signal transduction, apoptosis, cell proliferation and stalling of protein trafﬁc. Indeed the effect of E5 on these cellular processes was discussed in the presentations that followed.

Dick Schlegel (Georgetown) presented data showing that E5 enhances signalling by EGF-R not so much by inhibition of endosome acidification, but by altering vesicle fusion events and therefore EGF
endocytic traffic and processing. These data would also explain the observed E5 interference with the traffic of MHC molecules, cholesterol, gangliosides and lipid raft proteins. A very interesting part of this presentation was the detection of E5 protein by mass spectrometry. The presence of E5 protein, especially HPV E5, is often inferred by the presence of “E5 mRNA” but detection of the protein has proved almost insurmountably difficult given the physical and biological characteristics of the protein, such as extreme hydrophobicity, very low amounts and membrane localisation. Thus the actual proof that HPV-16 E5 does exist in SiHa and CaSkI cells, and presumably in other cells in which E5 has been studied almost as an act of faith, is very welcome indeed!

The interference of E5 with the cell membranes was confirmed by an additional presentation. Brian Ceresa (Oklahoma) reported the formation of binucleated cells upon expression of E5. This seems to be a property of E5 from high risk HPV only as it is not observed with E5 from HPV not associated with cancer, nor in cells expressing only E6 and E7. Binucleated cells are a result of cell–cell fusion rather than endonuclear division. Although E5 binucleated cells fail to propagate, the addition of E6/E7 suppresses the cell cycle checkpoint and at least some cells escape growth arrest and become transformed, a further indication of synergy between E5 and the other HPV oncoproteins. How does E5 promote cell–cell fusion? Does (at least some) E5 reside on the plasma membrane mediating fusion directly? Or is it an indirect effect exerted from the endomembranes? These points still need resolution.

E5 increases cell motility and decreases cell adhesion to the substrate. Eeva Auvinen (Helsinki) showed that several cellular microRNAs are altered in cells expressing HPV-16 E5. Three microRNAs significantly altered by E5 are miR-203, miR-324–5p, both down-regulated, and miR-146a, which is up-regulated. Interestingly and gratifyingly, some of the targets of these microRNAs are the same genes the expression of which has been found altered in previous work by the same author, namely genes involved in cell motility, adhesion and proliferation. How E5 affects microRNA expression remains to be established.

E5-promoted activities were also studied in trophoblast and cervical cells by Véronique Fontaine (Brussels). Because of their invasive properties, trophoblast cells are a good model for studying mobility and invasion. Although E5 on its own is toxic and impairs viability, in the presence of E6 and E7 it reduces cell adhesiveness and increases migration and invasiveness. These events are accompanied and presumably partly caused by downregulation of E-cadherin and upregulation of NF-κB and AP-1.

The impact of E5 on signal transduction was addressed also by Francesca Belleudi (Rome). In particular E5 seems to interfere with KGF-induced KGF-R transport to the degradative pathway. Immuno-fluorescence showed that the localization of endocytic dots corresponding to internalized KGF-R is different in E5 expressing cells compared to control cells, which display a more peripheral distribution. These data suggest that E5 might be involved in the shifting of KGF-R trafficking from degradation to recycling, thus potentiating the signal.

The role of HPV-16 E5 in skin and cervical cancer was addressed in a Plenary Lecture, sponsored by the Association for International Cancer Research, by Paul Lambert (Madison). Mice transgenic for E5, expressed in the basal layer of epithelium under the control of the K14 promoter, develop hyperplasia and skin tumours, the severity of which correlates with the expression level of E5. E5-induced hyperplasia is dependent on EGF-R, in agreement with previous in vitro studies in a number of laboratories. One interesting facet of neoplastic progression in the K14E5 mice is that the exophytic papillomas convert to endophytic ones before the onset of cancer. The biological significance of the inversion remains to be elucidated. The K14E5 transgenic mice were also investigated in the context of cervical cancer. When treated with oestrogen for 6 months, the mice develop CIN I and CIN II but not frank carcinomas. In these conditions, when combined with either HPV-16 E6 or E7, E5 induces more severe neoplasia than in mice expressing only one oncogene, showing that E5 contributes to the neoplastic phenotype. Even more interestingly however, when oestrogen treatment continues for 9 months, E5 transgenic mice develop cervical carcinomas, thus uncovering the ability of E5 to induce cancer on its own, independently of other oncogenes. Does a similar process happen in humans? This is a difficult question to answer, but, given that many observations made in transgenic mice have been confirmed in humans, it would seem reasonable to assume that E5 has a role in human cervical cancer. This assumption goes somewhat against the widely accepted argument that the E5 region is lost in cancers as a result of integration of the viral genome in the E2 region. It is however to be kept in mind that approximately 40% of cervical cancers maintain extrachromosomal copies of the HPV genome capable of expressing E5. It is also possible that E5 contributes to the early stages of carcinogenesis, and E6 and E7 take over later on. The recent detection of E5 protein in cells (see above) should clarify this point.

Lou Laimins (Chicago) described the novel association of HPV-16 and HPV-31 E5 with Bap31 and A4. Bap31 is a chaperone involved in quality control of, for instance, MHC molecules; A4 is a putative ion channel protein of the endoplasmic reticulum; Bap31 and A4 physically interact with each other. E5 and Bap31 physically interact and colocalise in perinuclear structures. Deletion of the C-terminus of E5 does not affect colocalisation of the two proteins but prevents their interaction. The biological significance of this interaction is demonstrated by the decrease in colony formation and impaired proliferative capacity of E5 expressing cells upon the inhibition of Bap31. E5 also binds and colocalises with A4 independently of Bap31, in fact the addition of Bap31 destabilises the E5–A4 complex. How the interaction between E5 and A4 contributes to cell transformation remains to be established.

Bap31 featured also in the Plenary Lecture given by Saveria Campo (Glasgow) who bridged the HPV and BPV E5 sessions by reporting the effect of E5 proteins on the processing and surface expression of MHC class I. Both BPV and HPV E5 retain MHC class I in the endomembrane compartments, thus decreasing the number of MHC I molecules on the cell surface. BPV E5 and HPV E5 both interact directly with the heavy chain component of the MHC I complex, but they use different domains: BPV-1 and BPV-4 E5 bind heavy chain via their C-terminus, whereas HPV-16 E5 binds heavy chain via its first transmembrane domain, and precisely the leucine pairs present in this region. Intriguingly, this E5 domain shows homology with the third transmembrane domain of Bap31. Bap31 chaperones MHC I in its transit to the cell surface. Putting together the data presented by Laimins and Campo, a possible picture of the relationship between E5, MHC I and Bap31 emerges, whereby membrane-bound E5 would displace Bap31 from MHC I, maybe taking advantage of its own interaction with Bap31, and thus retain MHC I in the ER/Golgi. As MHC I is the main presenter of antigenic peptides to CD8+ cytotoxic T lymphocytes (CTLs), its absence from the cell surface would have profound effects on the recognition and elimination of infected cells. Indeed, using mouse cells expressing human MHC I (HLA-A2) and E5 and loaded with an E6 peptide, Campo showed that E6 peptide–specific CTLs are not capable of recognising these cells to the extent they do control cells. It is therefore likely that E5 downregulates the host immune response also during a natural infection, promoting the persistence of transformed cells and thus neoplastic progression.

And so to BPV E5. In his Plenary Lecture Dan DiMaio (Yale) discussed how BPV-1 E5 can be used as a scaffold for the generation of small membrane-bound proteins that can modulate, say, receptors. The primary mechanism by which BPV-1 E5 transforms cells is via the binding and activation of the PDGF β receptor in absence of ligand. Large libraries of small E5-like proteins randomised in their transmembrane domain were tested for their activities by focus forming
assays. All proteins that can induce foci activate the PDGF β receptor even if they show no significant homology with wild type E5. One protein, selected for its ability to confer erythropoietin-independence on growth factor depended cells, fails to activate the PDGF β receptor but activates the human erythropoietin receptor (EPO-R) instead, inducing erythroid differentiation and proliferation of human hemo-poietic progenitor cells, with obvious pharmacological applications. Other proteins were selected that inhibit cell surface expression of a G protein-coupled receptor (GPCR). Because GPCRs have many physiological activities, including acting as HIV receptors, this result raises the exciting possibility of a reagent against HIV infection. Clearly there is more to BPV-1 E5 than the induction of warts!

Maria Peleteiro (San Miguel, Azores) reported the results of a survey of various molecular markers in bovine urinary bladder tumours associated with BPV infection. Uroplakin III, a specific urothelial marker, is lost in aggressive bladder tumours, suggesting that it would be a good marker for the diagnosis of high grade cancers. Conversely cyclin D1 and p53 are overexpressed in high grade cancers. E5 is expressed in tumours confirming its involvement in bovine bladder carcinogenesis.

Nunzia Corteggio (Naples) from Borzacchiello’s lab discussed the signal transduction pathways induced by the activation of the PDGFR-β-R when bound to E5 in both equine sarcoids and bovine urinary bladder cancer (see later for BPV and equine sarcoids). PDGFR-β-R and PI3K physically interact as do PDGFR-β-R and the Grb2-Sos complex. PI3K-Akt and Grb2-Sos-Mek-Erk signals are all potentiated in cancer, but, as in vitro models, the levels of Erk and Mek proteins are not significantly overexpressed. E5 therefore impacts on signal transduction without altering the level of the proteins involved.

Sante Roperto (Naples) confirmed the presence of E5 protein in peripheral blood mononuclear cells of cattle suffering from urinary bladder tumors expressing E5. Roperto defined the subset of blood cells that express E5 as primarily CD4 and CD8 T lymphocytes although also B lymphocytes and monocytes appear to be involved. E5 is detected in lymphocytes when the neoplastic lesions of the urinary bladder are in the initial stages suggesting its early involvement in the pathology. What could be the effect of E5 expression in lymphocytes? E5 could inhibit both MHC class I and class II thus profoundly disabling the immunological role of blood cells. By inducing at the same time the proliferation of blood cells it could also contribute to the spread of infection via the blood stream. These tantalising possibilities remain to be proven and further evidence of the action of E5 (and other BPV proteins) in lymphocytes is eagerly awaited.

Although papillomaviruses are normally strictly species-specific, BPV-1 can jump species and infect equids, causing a fibroblastic tumour called sarcoid. Both Lubna Nasir (Glasgow) and Sabine Brandt (Vienna) discussed the role of BPV-1 in general and E5 in particular in the pathology of sarcoids. Nasir showed that sarcoid cells are invasive and degrade collagen. This aspect of equine cell transformation is at least in part due to E5. E5 increases expression of matrix metalloproteinase 1 (MMP-1, collagenase), with increased degradation of collagen and matrix invasion. How E5 increases MMP-1 and its activity is not yet clear. In the MMP-1 promoter there are AP-1 binding sites which are essential for MMP-1 transcription. However, although E5 has been reported to activate AP-1, in this case it does not appear to act on the MMP-1 promoter. An intriguing alternative is that E5 stabilises MMP-1 mRNA via an AU-rich stretch at the 3’ end of the RNA, but this possibility has not been tested.

Brandt showed conclusive evidence that BPV-1 infection in horses does not involve only the derma, as so far believed, but also the epidermis. Viral DNA and E5 RNA are found in the epidermis, although in lesser amounts than in the dermis. The sequences of E5 in the dermis and epidermis are the same, but different from wild type “bovine” E5, as previously reported by the Nasir group. Another interesting point presented by Brandt is the occasional presence of BPV capsomeres in the epidermis of sarcoids, raising the exciting possibility that the sarcoid may be productive for infectious virus, contrary to what was hitherto believed.

Moving toward the clinical significance of E5 research, Aldo Venuti (Rome) showed that E5 interferes with apoptosis induced by taxanes. In particular the paclitaxel-induced up-regulation of caspases 3 and 8 is dramatically inhibited by E5. Data from a limited number of patients suggest that E5 expression may lead to a worse response to treatment with taxanes.

Rosella Francconi (Rome) reported the production of recombinant DNA vaccine expressing the BPV E5 protein fused to the coat protein of PVX plant virus. This fusion enhanced the “visibility” of the tumour antigen rendering more efficacious the immunological response, at least in a mouse model. Starting clinical trials in cattle suffering of BPV induced pathologies (i.e. enzootic haematuria/bladder carcinoma) will ascertain the effectiveness of anti-E5 immunotherapy in modifying the natural history of BPV-induced tumours.

Finally Lies Bogaert (Gent/Los Angeles) presented data on a tumour mouse model expressing the E5-E6 proteins of BPV and on the immunogenicity of 4 overlapping E5 peptides. All peptides were able to bind to H-2Kb and H-2Db molecules and were thus suitable candidates for vaccine. In particular the 19–36 E5 peptide was able to stimulate the strongest immunological response in vaccinated mice inducing higher number of IFN-γ secreting splenocytes.

Two conclusions can be drawn from this last session. The first is that E5 can interfere negatively with some therapeutic treatments and the second is that it can be used in immunotherapy. The neutralization of E5 therefore, whether immunological or chemical, could be advantageous in a variety of cancer treatments.

Thus, the science was great. Now, the promised description of food and drinks. Not only the lunch was delightful, with pizza, mozzarella, provolone, fresh tomato, pasta and other Mediterranean delicacies; the Gala Dinner was absolutely amazing, with wave upon wave of sea food dishes, from prawn to mussels, to tuna, to sword fish and any other creature that ever swam in the sea. And the chilled, dry, white wine kept coming too…… The reader will not be surprised to hear that the potent combination of powerful science and great food left the participants determined to have a 2nd, a 3rd, a 4th, etc. E5 workshop!