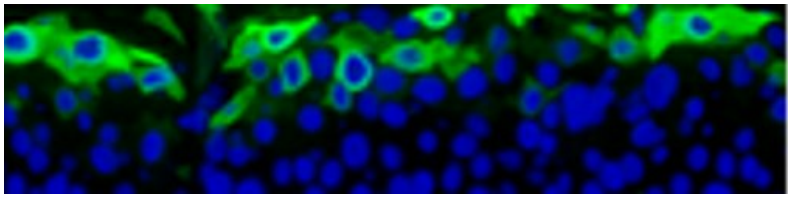
Human Papillomavirus Molecular Pathogenesis



Group leader: **Sally Roberts (/staff/profiles/cancer/roberts-sally.aspx)**

Overview

The research of the our group is focussed on human papillomavirus (HPV), a small DNA tumour virus which is now recognized as the cause of cervical cancer and other anogenital cancers, and more recently as contributing to the aetiology and pathogenesis of a subset of head and neck cancers. The principle focus is to improve understanding of the molecular interactions between HPV and its human host, with a view to identifying new therapeutic targets for the treatment of HPV-associated diseases.

Our research group

Central to our research direction is the use of cell models based on the host cell of the virus – the keratinocyte. The introduction of HPV genomes into primary keratinocytes enables establishment of cell lines whose stratification in organotypic raft culture permits the recapitulation of the complete virus life cycle. Moreover, the manipulation of these cell models reflects virus changes observed in the development of cancer and thus also allows the investigation of virus-host interactions that contribute to the development of malignancy.

Stemming from our work on HPV, the group also has an interest in keratinocyte biology, with specific interest in understanding the function of cellular proteins that contain protein interaction domains known as “PSD95/DLG1/ZO1 or PDZ domains”; a subset of these have tumour suppressor properties and are the substrates of the HPV E6 oncoprotein.

The group has strong local (**Parish (/staff/profiles/cancer/parish-jo.aspx)** , **Woodman (/staff/profiles/cancer/woodman-ciaran.aspx)**) and international collaborative links with groups interested in virus persistence, virus-induced epigenetics programming and in virus oncology. We have also developed close ties with our clinical colleagues (Mehanna) to develop the translational aspects of our research portfolio.

Current projects

Open all sections

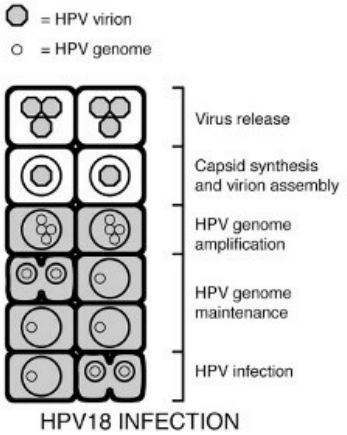
The life cycle of this group of viruses is dependent on keratinocyte differentiation. However, infection begins in the undifferentiated basal keratinocytes, where the viral genome is replicated as a low-copy number (~50-100 copies/cell) episome, a process that requires HPV E1 and E2 functions. HPV proteins E6 and E7 act to expand the population of infected keratinocytes once they migrate from the basal layer by stimulating cell cycle entry and cell survival, while E5 functions contribute to retention of the proliferative state. The virus utilizes the host cell’s replication machinery that has been reactivated in these cells to amplify the HPV DNA to many thousands of copies per cell. Finally, the capsid proteins L1 and L2 are produced and new progeny assembled in the highly differentiated squames. One of the most abundant proteins expressed during the HPV life cycle is the viral protein E4, a small protein that is heavily modified by various posttranslational modifications.

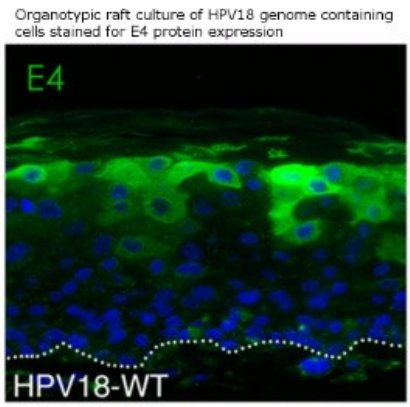
Whilst some 120 HPV genotypes have been identified only thirteen of these are associated with human cancers that arise in the anogenital tract and the oropharynx. The most prevalent types found in these cancers are HPV16 and HPV18. The two major HPV oncoproteins are E6 and E7 and they dysregulate important cellular pathways controlling cell growth and survival.

Regulation of human papillomavirus life cycle by E4 functions (supported by *Cancer Research UK*)

We have a longstanding interest in the HPV E4 protein. Our work has been instrumental in understanding E4 function; we have identified functionally important sequence motifs within the protein and have shown that proteolytic processing of E4 significantly contributes to the functional diversity of the viral protein. Studies from our group have also shown E4 to mediate number of diverse biological actions, including the inhibition of cellular DNA synthesis; a mode of action that might enable the virus to access the host replication machinery without competition from the host. We have also shown that E4 interacts with important host cell factors such as the cellular serine arginine kinase SRPK1. This virus-host association may regulate the activity of the kinase to promote virus gene expression in differentiating keratinocytes.

Our more recent investigations are focussed on gaining a comprehensive understanding of E4 function by using a cell-based model of HPV18 replication that uses primary human keratinocytes - the natural host cell of the virus - transfected with HPV18 genomes.



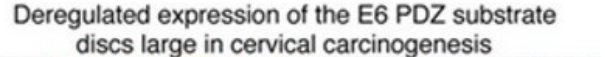


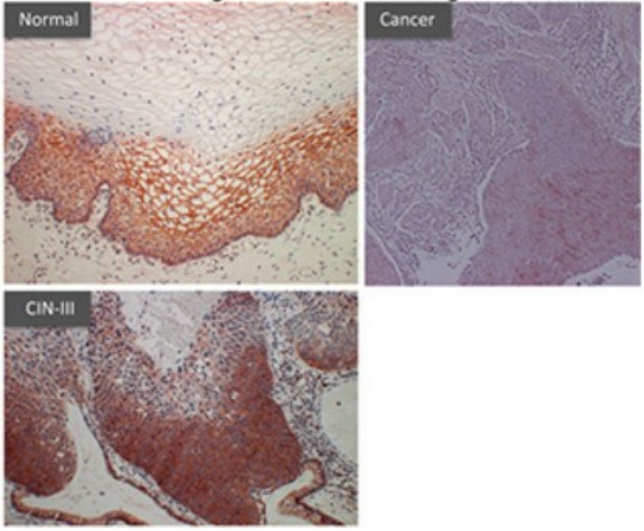
This model has been proven to be a robust system of HPV18 propagation to produce infectious virus following stratification by growth in organotypic raft culture. Attenuation of E4 expression in this HPV18-cell model has shown a requirement for E4 functions for efficient productive replication of the virus. Genetic analysis of the *E4* gene now focuses on targeting specific sequence elements of known function and the assessment of the effects of the mutations on the complete profile of virus functions, including the ability of the mutant genomes to support production of infectious virus is ongoing.

Role of oncogenic human papillomavirus E6-PDZ interactions in the virus life cycle and in human papillomavirus-induced malignancy (supported by *The Wellcome Trust* and the *Medical Research Council*)

The E6 and E7 protein functions deregulate cellular pathways controlling cell proliferation and survival. These functions are necessary for virus productivity, but in persistent infection with high-risk HPV types, they can render infected cells genetically unstable, which can lead to immortalization and in rare instances malignancy. These oncogenic characteristics have been attributed to the ability of E6 and E7 to target and disrupt the p53 and retinoblastoma tumour suppressor pathways respectively. However, other actions of these proteins are involved; including the capacity of E6 to target a select group of PSD95/DLG1/ZO1 (PDZ) domain-containing proteins. Many of these E6 substrates have roles in the regulation of cell growth and polarity and some are also potential tumour suppressor proteins, e.g discs large (DLG1). Moreover, we have shown dysregulation of DLG1 in cervical carcinogenesis.

Targeting of PDZ substrates is mediated by a C-terminal class I PDZ-binding motif (PBM), a unique motif of E6 proteins of high-risk types. There is also evidence that the interaction between E6 and PDZ proteins can be negatively regulated via phosphorylation of the E6 PBM by protein kinase A. Importantly, we have shown that the activity of the E6 PBM is linked to the morphological transformation of human keratinocytes.





By using our cell-based model of HPV18 replication combined with genomes containing mutations that disrupt PDZ targeting or the regulation of this virus–host interaction we aim to understand how these interactions contribute to virus replication and to understand how these interactions can be controlled by changes in phosphorylation. These studies will also provide insight into how the E6-PDZ interactions contribute to HPV malignancy. This project is in collaboration with Lawrence Banks at the International Centre for Genetic Engineering and Biotechnology in Trieste, Italy.

Using cell-based models to investigate the role of human papillomavirus in oropharyngeal cancer (supported by *Cancer Research UK* and *Coventry and Warwickshire NHS Trust*)

Squamous cell carcinoma of the head and neck (HNSCC) is the sixth most common cancer worldwide with diagnosis of 600,000 cases each year. The most important risk factors for the development of these cancers is high consumption of alcohol and high tobacco use. However, a subset of these cancers, most often arising in the tonsil and base of tongue (oropharyngeal site) is caused by infection with high-risk HPV types that also cause cancers of the anogenital tract. Whilst the overall incidence of HNSCC is decreasing the incidence of HPV-associated HNSCC is on the rise in some parts of the world.

Individuals presenting with HPV associated oropharyngeal cancer (OPC) respond better to treatment with DNA inducing therapies than individuals with HPV-negative tumours. Currently there is no difference in the management of HPV-positive or HPV negative carcinomas. However, since HPV positive OPC is now recognized as a distinct disease entity changes in treatment between the two groups needs to be considered. An option for less radical treatment for HPV-positive tumours eliminating unwanted side-effects of more aggressive treatment being some of the driving forces. The management of this disease will however rely on a better understanding of the mechanisms of high risk HPV infection in the tonsillar crypts and knowledge of the virus-host interactions responsible for malignant progression of these infections, as well as identification of virus-host interactions that determine a better response of these tumours to DNA damage-inducing therapies.

To underpin such studies we are developing relevant cell-based models that recapitulate the full spectrum of stages of orpharyngeal carcinogenesis from infection with oncogenic HPV to the development of carcinoma (both HPV negative and HPV-positive). These models will then be used to understand HPV-host interactions important for malignant progression, response to treatment and as a valuable collection to screen for compounds with antineoplastic activities. This project is in collaboration with Hisham Mehanna in the School of Cancer Sciences, University of Birmingham.

Selected publications

- **Roberts, S**., Delury, C., Marsh, E. (2012) The PDZ protein discs large (DLG): the “Jekyll and Hyde” of the epithelial polarity proteins. *FEBS J*, 279, 3549-58 doi: 10.1111/j.1742-4658.2012.08729
- Leonard, S., Wei, W., Collins, S. I., Pereira, M., Diyaf, A., Constandinou-Williams, C., Young, L. S. Young, **Roberts, S**., Woodman, C. B. (2012) Oncogenic human papillomaviruses impose an instructive pattern of DNA methylation changes which parallel the natural history of cervical HPV infection in young women. *Carcinogenesis,* 33, 1286-93.
- Mehanna, H., Beech, T., Nicholson, T., El-Hariry, I., McConkey, C., Paleri, V., **Roberts, S**. (2012) Prevalence of human papillomavirus in oropharyngeal and non-oropharyngeal head and neck cancer – systematic review and meta-analysis of trends by time and region. *Head and Neck* DOI 10.1002/hed.22015.
- Knight, G. L., Pugh, A. G., Yates, E., Bell, I., Wilson, R., Moody, C. A., Laimins, L. A., **Roberts, S**. (2011) A cyclin-binding motif in human papillomavirus type 18 (HPV18) E1^E4 is necessary for association with CDK-cyclin complexes and G2/M cell cycle arrest of keratinocytes, but is not required for differentiation-dependent viral genome amplification or L1 capsid protein expression. *Virology*, 412, 196-210
- **Roberts, S**., Kingsbury, S. R., Stoeber, K., Knight, G. L., Gallimore, P. H., Williams, G. H. (2008) Identification of an arginine-rich motif in human papillomavirus type 1 E1^E4 protein necessary for E4 mediated inhibition of cellular DNA synthesis *in vitro* and in cells. *J. Virol*. 82, 9056-9064.

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