# Studies of small DNA tumour viruses that cause disease in humans

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## Introduction

Members of the *Papovaviridae*, which includes the Papillomaviruses and Polyomaviruses, are the causative agents of a number of severe diseases in humans. Notable examples include cervical cancer, which is exclusively associated with infection with human papillomaviruses, and polyomavirus-associated nephropathy (PVAN) and progressive multifocal leukoencephalopathy (PML) caused by the BK and JC polyomaviruses, respectively. Current therapeutic strategies to treat these virus-associated maladies are lacking. We undertake a broad ranging analysis of these viruses in an effort to identify new targets for therapeutic intervention. These studies have revealed novel information about these viruses.

## Results

**Human papillomavirus:** We use an integrated approach to understand the roles of the least understood virus encoded proteins in the HPV life cycle and in cancer development. In particular we have focussed on the E5 oncoprotein. This highly hydrophobic membrane protein is expressed by all cancer-associated HPV isolates but its role in transformation and the life cycle is not clear. In 2012 we developed the first system to study recombinant E5 and subsequently demonstrated that it was a novel member of the viroporin family of virus encoded ion channels. This has opened up the possibility of developing small molecule inhibitors targeting the channel function of E5. In addition, we study the host pathways essential both for transformation and the HPV life cycle. Using three-dimensional

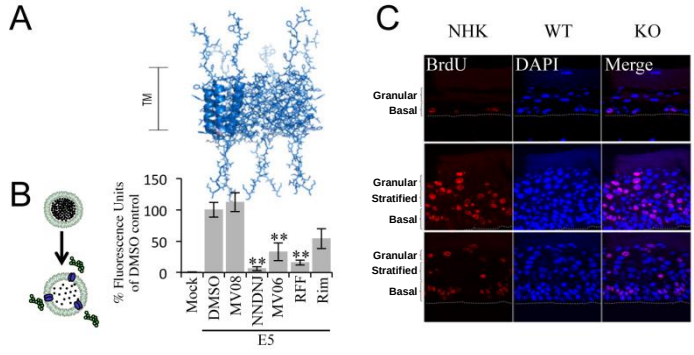


**Figure 1: (a)** *In silico* modelling of the HPV16 E5 proteins. Energy minimisation models depicting a hexameric channel structure. **(b)** Screen for novel inhibitors of E5 viroporin activity. Panel of known viroporin inhibitors and bespoke compounds generated from the *in silico* model were tested for the ability to prevent E5-mediated dye release. **(c)** Genetic studies of E5 function using E5-knockout viruses in primary 3D organotypic raft culture models. BrdU incorporation measures the levels of unscheduled DNA replication in the suprabasal layers of the epithelium (red stain) in normal human keratinocytes (NHK), cells infected with wild-type HPV18 (WT) and cells infected with an HPV18 genome lacking E5 expression (KO).

organotypic raft culture models – we grow skin in a dish – we have shown that E5 manipulates growth factor pathways to enhance keratinocyte proliferation and to delay terminal differentiation, both of which are essential for the HPV life cycle. Current projects include studying novel E5 binding partners and understanding how these contribute to HPV infection and pathogenesis. In addition we have embarked on a novel study to ascertain the global changes to the host proteome upon HPV infection.

**Human polyomaviruses:** Members of this family, including BK, JC and Merkel cell polyomavirus, are associated with disease in humans. Despite their clear association with disease there is a paucity of understanding of their basic biology and as such we are using a wide ranging series of experiments to understand their life cycles and to identify novel targets

for antiviral therapeutics. We have identified a novel immune evasion strategy for the Merkel cell polyomavirus and are uncovering the roles of the enigmatic agnoprotein in the polyomavirus life cycle. We are also using the latest advances in structural biology to gain an unprecedented understanding of the fundamental make-up of polyomavirus particles. This information may herald crucial advances in anti-viral drug design.

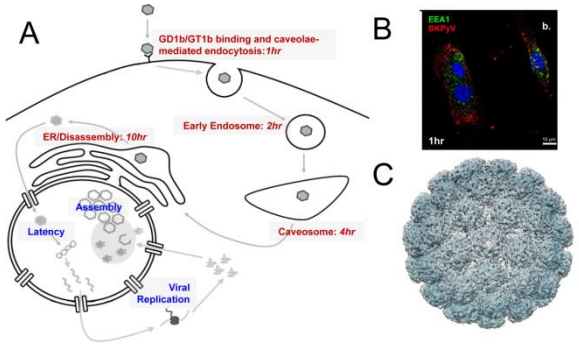


**Figure 2: (a)** Schematic of the current understanding of BK polyomavirus (BKPyV) life cycle. **(b)** Immunofluorescence with Alexafluor954 labelled BKPyV infected human renal proximal tubular epithelial (RPTE) cells. **(c)** Cryo-electron microscopy structure determination of the native BKPyV virion.

## Publications


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