Studies of small DNA tumour viruses that cause disease in humans

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Introduction

Members of the Papillomavirus and Polyomavirus families 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 Merkel cell carcinoma (MCC) caused by the BK and MCV 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. Our studies have revealed novel information about these viruses.

Results

Human papillomavirus: Infection with HPV causes ~6% of human cancers, including nearly all cervical carcinomas, as well as many other anogenital tract tumours. The past decade has also witnessed an epidemic of head and neck squamous carcinomas (HNSCC), primarily in white, middle aged men, which are also associated with HPV. Despite FDA-approved prophylactic vaccines, the burden of HPV-associated malignancy will remain high for decades due to their limited availability in low income countries, poor coverage where access is possible, and the long latency period separating infection from carcinogenesis. Although HPV-specific antivirals are needed, their development is hindered by an incomplete understanding of the virus life cycle and essential interactions with host factors within the infected keratinocyte.

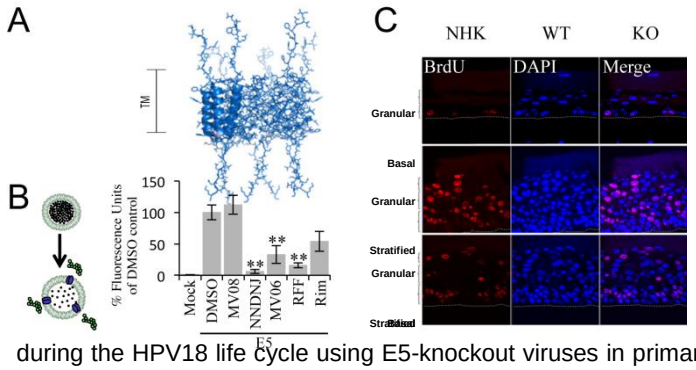

Figure 1: (A) *In silico* modelling of the HPV16 E5 proteins. Energy minimisation models depicting a hexameric channel structure. (B) Screen for novel inhibitors of E5 viroporin activity. Panel of known viroporin inhibitors and bespoke compounds generated from the *in silico* model were tested for the ability to prevent E5-mediated dye release. (C) Genetic studies of E5 function during the HPV18 life cycle using E5-knockout viruses in primary 3D organo-typic 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). Loss of E5 results in a significant reduction in DNA replication.

Using an approach incorporating molecular and cellular biology with cutting edge cell culture and cancer models we explore the roles of the HPV encoded E5, E6 and E7 oncoproteins in the virus life cycle and in transformation. Our studies have revealed new information on these proteins, in particular how they interact with host factors that are essential for virus replication. For example, we identified the E5 protein as an ion channel or viroporin. This has opened up the possibility of developing small molecule inhibitors targeting the channel function of E5. In addition, we have shown that E5 hijacks the epidermal growth factor receptor (EGFR) to drive keratinocyte proliferation, which is necessary for both virus replication and transformation (2).

Human polyomaviruses: Members of this family, including BK and Merkel cell polyomavirus (MCV), 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

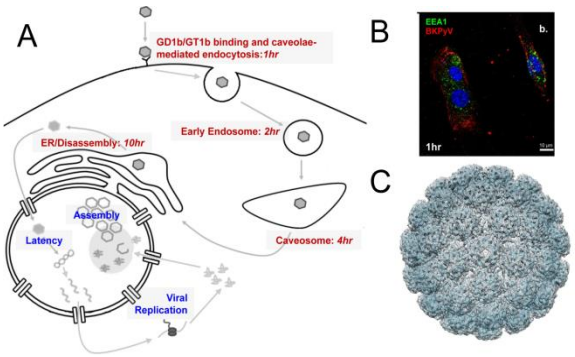

Figure 2: (A) Schematic of the current understanding of BK polyomavirus (BKPyV) life cycle. (B) Immunofluorescence with Alexafluor954 labelled BKPyV infected human renal proximal tubular epithelial (RPTE) cells. (C) Cryo-electron microscopy structure determination of the native BKPyV virion.

antiviral therapeutics. We have described roles for MCV proteins in immune evasion (1) and in collaboration with Prof. Adrian Whitehouse identified mechanisms of MCV-mediated cancer metastasis (3). With Prof. Neil Ranson we are using the latest advances in cryo-electron microscopy to gain an unprecedented understanding of the fundamental make-up of polyomavirus particles and how they mediate their interactions with host receptor molecules. This information may herald crucial advances in antiviral drug design. In more recent studies we have focussed on BK virus entry and egress mechanisms and identified a vital role for a host ion channel in polyomavirus entry to primary kidney cells. Importantly, a commonly available drug targeting this channel prevents BK infection. Further work will validate this channel as a novel therapeutic strategy to target BK-associated disease and mechanistic studies will shed light on why it is needed for virus infection.

Publications

Abdul-Sada H., Muller M., Mehta R., Toth R., Arthur J.S.C, Whitehouse W. & Macdonald A. (2017) The PP4R1 sub-unit of protein phosphatase PP4 is essential for inhibition of NF-κB by Merkel polyomavirus small T antigen. *Oncotarget* **8**:25418-25432.

Wasson C.W., Morgan E.L., Müller M., Ross R.L., Boxall S., Hartley M, Roberts S. & Macdonald A. (2017) Human papillomavirus type 18 E5 oncogene supports cell cycle progression and delays epithelial differentiation by modulating growth factor receptor signalling. *Oncotarget.* **8**:103581-103600.

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Collaborators

University of Leeds: Neil Ranson and Adrian Whitehouse (FBS), Stephen Griffin (LICAP), Richard Foster and Andrew Wilson (School of Chemistry).

External: Sally Roberts (Birmingham), Nick Coleman (Cambridge), Sheila Graham (Glasgow) and Simon Arthur (Dundee).