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 have established a multi-disciplinary research group to 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 have focussed our analysis on the least understood of the three transforming proteins encoded by this virus. The E5 protein is a small membrane protein expressed by all carcinogenic papillomaviruses. Little is understood of the role of E5 in the virus life cycle or its mechanisms of pathogenesis. We discovered that E5 functions as a virus-encoded ion channel or "viroporin" (Wetherill *et al*., 2012). We utilised *de novo* models of an E5 channel complex (Figure 1A) to identify small molecule inhibitors of E5 channel function and are currently using these models to reveal the functional determinants of E5 channel function *in vitro*. In addition we have established organotypic raft culture systems that mimic the natural three-dimensional nature of human skin (Figure 1B). These model systems allow us to grow HPV in the laboratory and provide an ideal opportunity to test our *in vitro* findings in a physiologically relevant system.

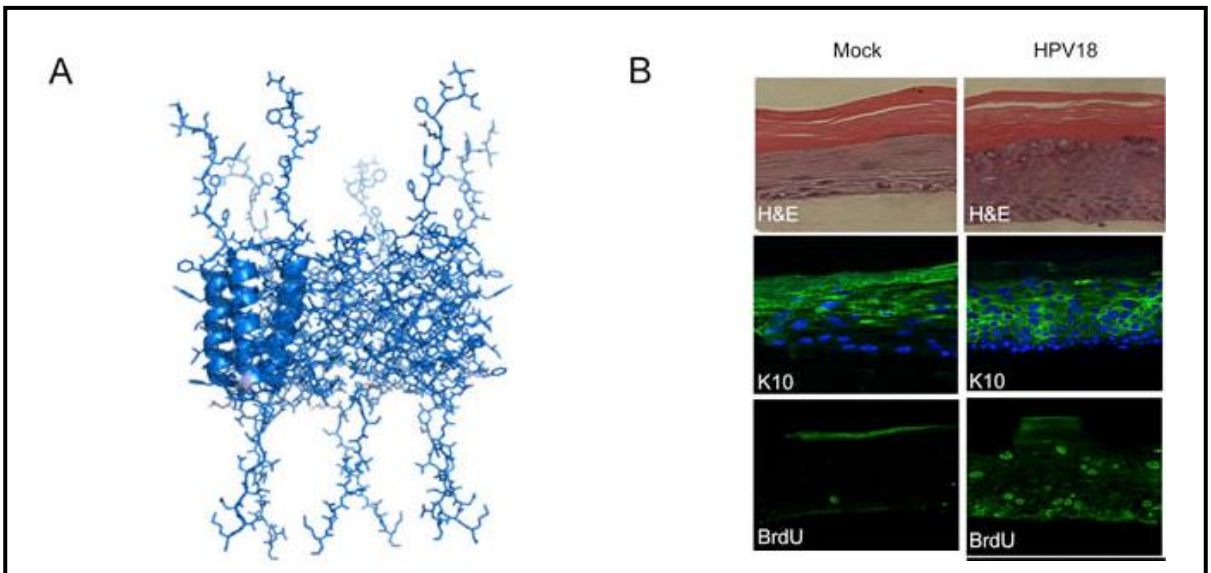

Figure 1: (a) *in silico* molecular modelling of an E5 channel complex (Wetherill et al., 2012). Molecular predictions, based on energy minimisation, suggest that the E5 channel is composed of a hexamer, with a hydrophilic region protruding from the membrane and a luminal pore lined with residues that mediate ion flow. **(b)** Immunohistochemistry of an organotypic raft culture section from control and HPV containing primary keratinocytes. Cells are stained with haematoxylin and eosin to show general morphology and keratin 10 as a marker of keratinocyte differentiation. BrdU staining highlights the increased host cell DNA replication occurring in cells containing HPV. Probing for a HPV late protein, E4 highlights a productive HPV life cycle in the raft culture model.

Human polyomaviruses: Our analysis currently covers three major polyomaviruses associated with disease in humans. These are the JC, BK and Merkel polyomaviruses. In collaboration with Prof. Adrian Whitehouse (UoL), we are dissecting the role of the Small T antigen of Merkel polyomavirus in transformation. Our studies have shown that this virus protein is an efficient and powerful inhibitor of the host innate immune response and is capable of preventing an inflammatory response. This may have profound implications for the persistent nature of virus infection and allow Merkel to persist in the host despite the presence of an immune response. In parallel studies we are beginning to understand more about the enigmatic agnoproteins that are encoded by BK and JC viruses. Our preliminary biochemical characterisation of these proteins suggests that they are ideal targets for antiviral therapeutics and work in 2013 will continue to target these proteins for study.

Publications

Wetherill, L., Holmes, K., Verow, M., Mueller, M., Howell, G., Harris, M., Fishwick, C., Stonehouse, N., Foster, R., Blair, G., Griffin, S. & Macdonald, A. (2012) High-risk human papillomavirus e5 oncoprotein displays channel-forming activity sensitive to small-molecule inhibitors. *J. Virol.* **86:** 5341-5351.

Wu, W., Macdonald, A., Hiscox, J. & Barr, J. (2012) Different NF-kappa B activation characteristics of human respiratory syncytial virus subgroups A and B. *Microb. Pathog.* **52:** 184-191.

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Collaborators

External: S. Roberts (Birmingham), N. Coleman (Cambridge), S. Graham (Glasgow) and M. Imperiale (University of Michigan, USA).
Leeds: B. Turnbull (School of Chemistry), M. Webb (School of Chemistry), D. Tomlinson (Faculty of Biological Sciences) and M. McPherson (Faculty of Biological Sciences).