Structural studies of innate immune signalling proteins and viral oncogenes

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Introduction.

Virus infections are associated with chronic pathologies and are the causative agents of various cancers. For example 78% of biopsies from patients with cervical cancer are positive for human papillomavirus (HPV) and new cancer-associated viruses are being discovered including the Merkel cell carcinoma polyomavirus (MCV). Our ability to control virus infection is often inadequate due to the limited availability of drugs that target many pathogenic viruses and an incomplete understanding of the host response to virus infection. As such there is a pressing need to increase our understanding of the host response to virus infection. To be effective it requires rapid and appropriate activation of inflammatory cytokine messengers to eliminate the spread of infection as quickly as possible. However, as this response often evolves at the cost of tissue damage, failure of resolution can result in inflammatory disorders and the further development of disease. Due to this inherent danger of an inappropriate response, the crucial steps are regulated by a growing number of cellular proteins. There remains a poor understanding of the molecular mechanisms utilised by these proteins. Additionally, it is clear that these signalling pathways are target for viral subversion and often deregulated by virus-encoded proteins to affect the anti-viral or inflammatory response. Thus, a clearer understanding of this area will arm us with better strategies to fight viral infection through the development of novel therapeutics.

Biophysical characterisation of viral oncogenes.

The human papillomaviruses are causative agents of a range of ano-genital and head and neck cancers. The E5 onco-protein transforms primary keratinocytes and causes cancerous lesions in the cervix of transgenic mice. The mechanism of E5 mediated transformation is not well understood. As E5 is theorised to function in the early stages of HPV persistence, we focussed on characterising the biochemical nature of E5 in an attempt to expose a drugable function. E5 is a highly hydrophobic membrane bound protein. We designed the first successful bacterial purification system for E5. Using a multifaceted approach consisting of biophysical characterisation, cell based assays and *in silico* modelling we demonstrate that E5 exists as a hexamer (Figure 1). Furthermore, we are the first to describe E5 as a novel viroporin or virally encoded ion channel. E5 is the first oncoprotein to be described as a viroporin. Collaborating with Prof. Colin Fishwick we are adopting a rational approach to E5 inhibitor design incorporating virtual screening of compounds tailored against our molecular model. This approach successfully produced the first small molecule inhibitor against E5 and represents a breakthrough in rational drug design against an *in silico* target.

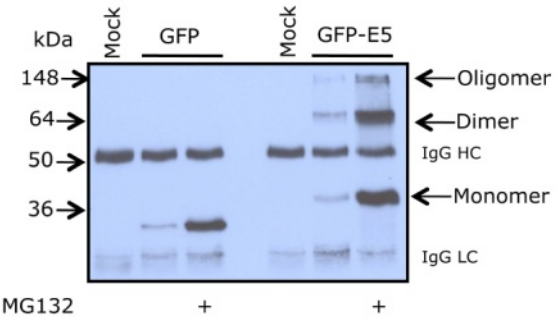

Figure 1: HPV16 E5 oligomerises in cells. SDS-PAGE and immunoblotted for anti-FLAG demonstrating E5 monomer, dimer and oligomer and levels of protein were enhanced in the presence of the proteasome inhibitor MG132. Non-specific IgG heavy and light chain are indicated.

Studies on regulators of the innate immune response

Studies from our laboratory have identified the protein optineurin as a negative regulator of the anti-viral response. The mechanism by which optineurin regulates the anti-viral response is currently unknown, although it may require an interaction with the protein kinase TBK1 and upstream signalling proteins that are ubiquitylated. Preliminary experiments demonstrated that optineurin forms a higher molecular weight oligomeric structure in response to viral nucleic acids (Figure 2). Our Royal Society funded studies are analysing the structural and molecular biology of optineurin in greater detail, with the eventual aim of solving the three dimensional structure of this critical mediator of signalling.

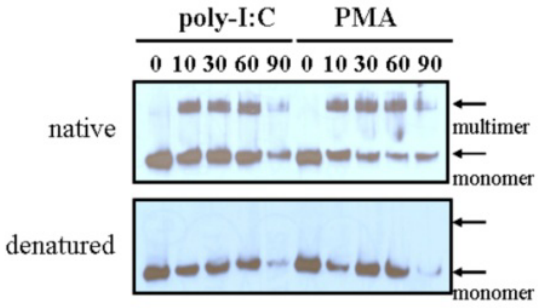

Figure 2: Formation of higher order structures by optineurin. Cells expressing optineurin were stimulated with the viral dsRNA mimic poly-I:C or treated with the mitogen PMA and lysed at the indicated time-point. Lysates were analysed either by non-denaturing native electrophoresis (top) or by denaturing.

Publications

Poole, E., Groves, I., Macdonald, **A.,** Pang, Y., Alcami, A. and Sinclair, J. (2009) Identification of TRIM23 as a co-factor involved in the regulation of NFκB by the Human cytomegalovirus gene product UL144. *J Virol* **83**:3581-90.

Short, J. and Macdonald, A. (2009). Viral evasion of interferon-stimulated genes. *Bio-Science Horizons,* **2:** 212-224.

Wittmann, M., Macdonald, A. and Renne, J. (2009) IL-18 and inflammation. *Autoimmun Rev*,**9**:45-48.

Gamlen, T., Richards, K., Mankouri, J., Hudson, L., McCauley, J., Harris, M. and Macdonald, A. (2010) Cytopathogenic BVDV NS3/4A induces apoptosis but is not required for inhibition of IFNβ induction. *J Gen Virol*, **91**:133-44.

Mankouri, J., Fragkoudis, R., Richards, K., Wetherill, L., Harris, M., Kohl, A., Elliott, R. and Macdonald, A. (2010) The nemo-related protein optineurin is a regulator of innate antiviral signalling. *PLoS Pathogens,* **19**:e1000778.

Funding

This work is funded by EPSRC, Yorkshire Cancer Research, MRC, BBSRC, The Royal Society and the University of Leeds.