eP136
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ANALYSIS OF THE PROTEOME OF A MODIFIED VACCINIA VIRUS ANKARA-BASED VACCINE PROVIDES INSIGHT INTO THE EFFECTS OF TRANSGENIC MODIFICATION ON VIRUS PHYSIOLOGY

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Several Modified Vaccinia Virus Ankara (MVA)-based live virus vaccines are at advanced stages of clinical trials against infectious diseases, agents of bioterrorism and for cancer immunotherapeutics. Successful development and licensure of this class of vaccines is however confounded by issues of safety and immunogenicity. In this study a global proteome analyses was performed on a recombinant (r)MVA versus a non-recombinant (nr)MVA during infection of a permissive cell-line with the aim of revealing changes in the proteome of the virus due to the genetic modification of its genome. BHK-21 cells was infected with nrMVA or its recombinant variant, hanpMVA (MVA containing influenza virus haemagglutinin (ha) and nucleoprotein (np) genomic cDNA inserts), and a quantitative (protein expression) as well as a qualitative (protein post-translational modification) proteomic study employing both forward and reverse stable isotope labelling by amino acids in cell culture (SILAC) was conducted. BHK-21 cells labelled with the SILAC $^{13}C_6$- Lysine and Arginine were mock-infected or infected with nrMVA or hanpMVA and incubated in parallel for 6 hours in SILAC media. After incubation, infected and mock-infected cells were combined in 1:1 ratio before protein extraction. Following SDS-PAGE, protein-bands were trypsin-digested and analyzed by LC-MS/MS to determine relative peptide abundance and posttranslational modifications of virus encoded proteins. Several posttranslational modification sites on the virus proteins were identified. MASCOT searches of the NCBInr database identified a total of 52 different differentially expressed MVA proteins, 32 of which were identified in both forward and reverse SILAC. Further validation of protein expression was performed by Western Blotting on selected proteins. Temporal protein expression analysis using the predicted and experimental expression data as listed by the Poxvirus Bioinformatics Resource Centre (http://www.poxvirus.org/vaccinia_orthologs.asp) revealed that 29% of the total differentially expressed proteins are early class proteins; late proteins accounted for 48%; early/late proteins – 23% and intermediate proteins – 2%. Of the 52 differentially expressed proteins, 39 were up-regulated while 10 were down-regulated. Early proteins were more represented in the down-regulated group of proteins. This result helps to understand the effects of transgenic modification on MVA physiology, which have implications for safer and more effective MVA-vectored vaccine.