QUANTITATIVE PROTEOMICS ANALYSIS OF LYMPH NODES FROM PIGS INFECTED BY PORCINE CIRCOVIRUS TYPE 2 (PCV2) BY 2-DE, $^{18}O/^{16}O$ LABELING AND LINEAR ION TRAP MASS SPECTROMETRY

Ramírez-Boo M.[1], Serrano H.[2], Núñez E.[2], Jorge I.[2], Navarro P.[2], Vázquez J.[2], Fernández L.[3], Segalés Q.[3], Garrido J. J.[1] y Moreno A.[1]

[1]Dpt. Genética, Universidad de Córdoba. CSIC. Córdoba;
[2]Laboratorio de Química de Proteínas y Proteómica, CBMSO (CSIC-UAM), Madrid;
[3]Universitat Autónoma de Barcelona (CReSA), Barcelona

PCV2 is the causal agent of postweaning multisystemic wasting syndrome in pigs, characterized by progressive weight loss, dyspnoea, enlargement of inguinal lymph nodes, depletion of lymphocytes and an altered pattern of cytokines. The mechanism whereby the virus causes the disease and the reason why only some animals become diseased remain unclear. To study the immune response associated with virus infection, ten piglets were divided into 2 groups: control (n = 4) and inoculated with PCV2 at 7 days of age (n = 6). Piglets were euthanized and necropsied on day 29 p.i and inguinal lymph nodes samples were collected. Lymph node protein extracts for each group were pooled, split into two equal aliquots and analyzed by two different proteomics strategies: a classical approach based on the differential 2-DE pattern and a stable isotope labeling approach combining SDS-PAGE protein fractionation, "in-gel" digestion, $^{18}O/^{16}O$ peptide labeling and peptide identification and quantification by LC-MS/MS. 2-DE analysis revealed 45 spots that were differentially expressed at a FDR of 5 %, corresponding to 31 unique proteins. In the second approach peptides were identified by using the pRatio method, and the quantitative results analyzed using QuiXoT. Among 1,493 identified proteins, 794 could be quantified, from which 65 proteins were found differentially expressed at a FDR of 5%. We used the Ingenuity Pathway Analysis package to analyze and compare the obtained results. Association of differentially regulated proteins with canonical pathways highlighted two major processes: acute phase response signalling and NRF-2-mediated oxidative stress response. Other canonical pathways associated with differentially expressed proteins were that of TGF-β, and the integrin and actin signalling pathways.