Bioinformatics tools for inferring immune-related functions from proteomic data

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The development of functional genomics technologies has lead to the generation of large amounts of data that needs to be structured for easier interpretation and full exploitation of the knowledge hidden in huge databases. This is only possible when we apply efficient analysis tools in order to discover new techniques for data storage, querying, extracting and mining.

In the present study, we use an experimental model of response to infection, based in the exposure of neutrophils to LPS from *Salmonella typhimurium*. Neutrophil activation by LPS involves the production of reactive oxygen intermediates, release of lipid mediators and cytokines, adhesion, and phagocytosis. However, recent studies indicate that a robust transcriptional response, mainly of cytokines, occurs in neutrophils after LPS stimulation, suggesting that this inflammatory cells do much more than just releasing mediators and bactericidal agents [1, 2].

The aim of this study was 2-fold. Firstly, it was intended to identify novel proteins involved in the swine neutrophils response to LPS by using a 2-dimensional gel electrophoresis (2-DE) approach. Despite of the rise of new technologies in quantitative differential proteomics, 2-DE and matrix-assisted laser-desorption ionization time of flight mass spectrometry (MALDI-TOF/MS) are still the most widespread methods for proteomics studies [3]. The differences in protein expression after LPS treatment may lead to the elucidation of protein functions or pathways of inflammatory processes, which could be involved in immune response against bacterial pathogens. And secondly, it used bioinformatics tools such as Ingenuity Pathway Analysis (IPA, www.ingenuity.com) and Cytoscape (www.cytoscape.org), to analyze how these altered proteins interact in a cellular context to perform certain biological functions.

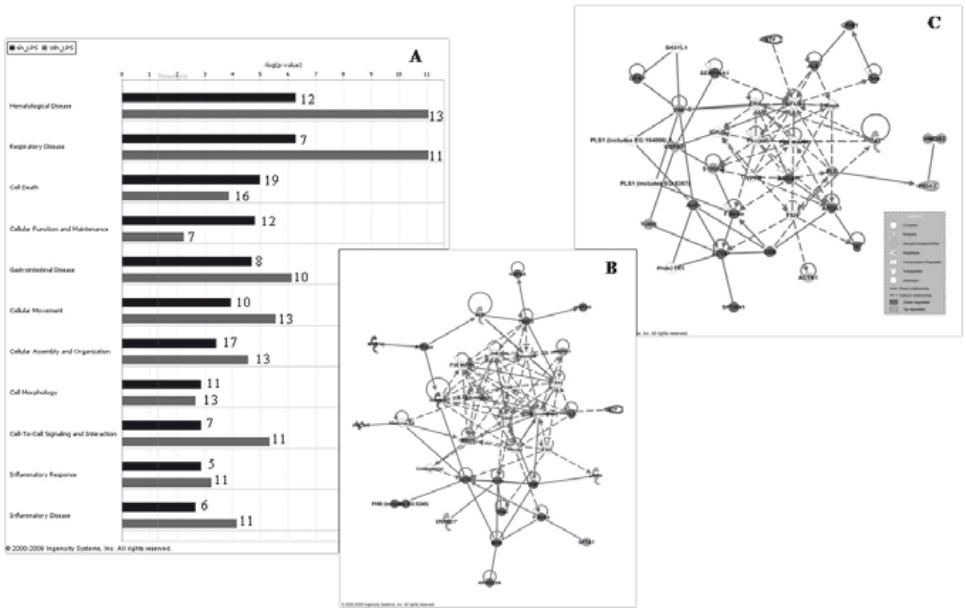

Figure 1. *Biofunctions and diseases significantly altered through the time course. Differentially expressed proteins were subjected to statistical analysis with a Student´s T-test and those categories with $p < 0.05$ were listed. The number of proteins in each category is shown at the right side of the bars.*

Blood samples were collected at the slaughterhouse from five Iberian healthy pigs and neutrophils were isolated with Dextran sedimentation and centrifugation through Ficoll-Paque. For LPS stimulation, the neutrophils were incubated for 6, 9 and 18 hours in presence or absence of 100 ng/ml LPS. Proteins were solubilized and the extracts were pooled and six replicate 2-DE gels for condition (untreated cells and treated with LPS) were analysed by 2-DE. The LPS-induced changes in proteins was subjected to statistical analysis with a Student's t test after checking normality by the Wilks-Shapiro test and those spots with $p< 0.05$ were analyzed by MALDI TOF-TOF (MS/MS).

The number of differentially expressed proteins in neutrophils after LPS treatment varied through the time-course. Up today, 44 and 31 proteins for 6 and 18 hours were identified respectively. Data analysis with IPA revealed that several immune response-related functions such as inflammatory and respiratory disease, cellular movement and organization or cell death were altered during the time-course (Figure 1).

Differentially expressed proteins in each timepoint were subjected to BiNGO plugin of Cytoscape [4] in order to elucidate the relationship among the altered GO functions through the time course in a unified conceptual framework. The results of this analysis are shown in Figure 2. Briefly, immune related functions such as killing and apoptosis are conserved both at 6 and 18 hours. Similarly, cellular metabolism was also altered through the time course.

In conclusion, LPS stimulation alters the patterns of protein expression in neutrophils, and the present results represent the first comprehensive study to better understanding a complex biological event such as the swine innate immune response. Bioinformatics provides many tools for analyzing protein data sets by visually exploring biological networks, including well-known examples such as IPA and Cytoscape. Since these tools can integrate graph drawing, information visualization, network analysis and biology, we can use them to interpret our results from a proteomic approach in an easy way.

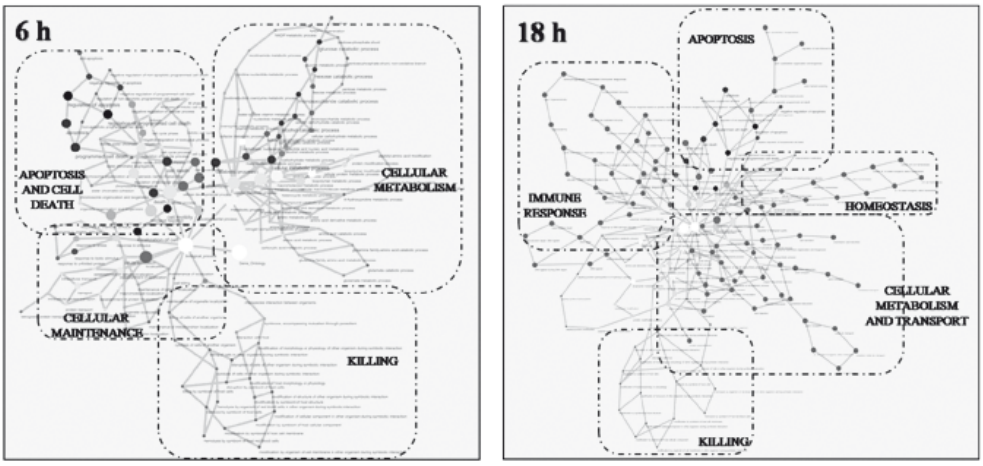

Figure 2. *BiNGO analysis for 6 and 18 hours. Nodes represent biological functions categories and edges represent the interaction among them. Statistical significance of each category is shown as grey scale, where black is the most significant function. Node weight indicates the number of interactions.*

References

[1] Malcolm KC, Arndt PG, Manos EJ, Jones DA, y Worthen GS. Microarray analysis of lipopolysaccharide-treated human neutrophils. Am J Physiol Lung Cell Mol Physiol 2003; 284: L663-L670.

[2] Fessler MB, Malcolm KC, Duncan MW y Worthen GS. Lipopolysaccharide Stimulation of the Human Neutrophil: An Analysis of Changes in Gene Transcription and Protein Expression by Oligonucleotide Microarrays and Proteomics. CHEST 2002; 121: 75S-76S.

[3] Monteoliva L y Albar JP. Differential proteomics: An overview of gel and non-gel based approaches. Briefings in Func Genomics and Proteomics 2004; 3: 220-239.

[4] Maere S, Heymans K and Kuiper M. BiNGO: a Cytoscape plugin to assess overrepresentation of Gene Ontology categories in Biological Networks. Bioinf Applic Note 2005; 16: 3448-3449.