sis of interacting partners of different proteins that are presented in TERMs [2]. This was accomplished by "pull-down" techniques and high-throughput protein identification of the captured ligands by mass spectrometry. For this end, synthetic biotinylated peptides spanning the C-terminal cytoplasmic end of these proteins were incubated with extracts from lymphoblast cell models, and then captured using Streptavidin-sepharose microbeads. Proteins interacting with the peptide baits were subjected to digestion and the resulting peptides systematically analyzed by HPLC-linear ion trap MS/MS mass spectrometry. Proteins from more than 50 pull-down analysis classified in 9 different experiments were automatically identified from the MS/MS spectra in a human database. We identified more than 2500 human proteins filtering with pRatio software developed by our group with an error rate of protein identification never superior to 5% [3-4].

We developed a robust statistical criterion based on the number of peptides of each protein identified in all the partners in the same pull down experiment ($p<0.01$). We also confirmed the interactions between the identified proteins and the baits considering the reproducibility in all the experiments. More than 200 specific interactions were differentially clustered revealing strong relationships among groups of peptide baits.

The validated proteins have been analyzed using System Biology tools (Ingenuity Pathways Analysis or IPA) (Figure 1). Interacting proteins from different baits were found to share biological functionality, and the groups of interactions showed a strong functional and biological relationship among them. This information suggests that proteins which link the citosolic domains of tetraspanins form a coherent network of interactions with their functional role between T lymphocytes and vascular endothelium in the processes of diapedesis.

References

[1] Hemler ME. Tetraspanin functions and associated microdomains. Nat Rev Mol Cell Biol. 2005;6:801-11.

[2] Barreiro O, Yáñez-Mó M, Sala-Valdés M, Sánchez-Madrid F. Endothelial tetraspanin microdomains regulate leukocyte firm adhesion during extravasation. Blood. 2005; 105:2852-61.

[3] Martínez de Bartolomé S., Navarro P., Martín-Maroto F., Vazquez J., López-Ferrer D., Ramos-Fernández A.,et al. Properties of Average Score Distributions of SEQUEST The Probability Ratio Method. Molecular & Cellular Proteomics 2008; 7:1135-1145.

[4] Navarro P, Vázquez J. A refined method to calculate false discovery rates for peptide identification using decoy databases. J Proteome Res. 2009;8:1792-6.

Use of ProteoMiner in Veterinary Research

Anna Marco, Gemma Rovira, Anna Bassols

Departament de Bioquímica i Biologia Molecular. Facultat de Veterinària. Universitat Autònoma de Barcelona. 08193 Cerdanyola del Vallès

One of the main applications of serum proteomics is the identification of new biomarkers for animal disease or animal production. However, potential obstacles to these studies are the poor performance of affinity serum depletion methods based on human antigens when using animal samples. In the present study, we have analyzed the efficiency and reproducibility of the ProteoMiner® beads with bovine and porcine serum samples.

When looking for biomarkers, serum is potentially the most valuable biological sample, because it contains thousands of different proteins and peptides and it is the most easily accessible, noninvasive, and widely collected sample [1]. Unfortunately, its content is dominated by a handful of high-abundant proteins, with their estimated concentration exceeding the low-abundant proteins highly by 10 orders of magnitude [2]. This large dynamic range exceeds

the analytical capabilities of traditional proteomic methods, making the detection of lower abundance serum proteins extremely challenging. In particular, most potential biomarkers are among those low-abundance proteins, secreted into the bloodstream by tissues or cells because of the disease process.

To detect these low-abundance proteins using currently available technologies, it is advisable to remove the most abundant proteins first. Many strategies have been developed for the removal of albumin and other high-abundance proteins; however they have a high specificity for human or laboratory animal proteins [3]. Recently, a new method for capturing low-abundance proteins has been commercially available, which is known under the trade name of ProteoMiner® and it is based on the use of a combinatorial peptide binding library, which affinity-captures and amplifies the low-abundance proteome [4].

Proteomics constitutes an interesting approach to veterinary medicine and animal production. In this sense, porcine and bovine livestock are the most interesting species due to their economical interest. Surprisingly, there are very few applications of serum proteomics to veterinary science. The ProteoMiner technology, since it is not based on an immunological approach, should be species independent, and thus of potential application to samples of veterinary interest.

The objective of the present work was to analyze the efficiency and reproducibility of the ProteoMiner system with bovine and porcine serum samples.

Blood was collected from the coccygeal vein in Holstein cows and from the cava vein in Duroc x (Landrace x Large White) pigs in a vacutainer tube with no added chemicals. Serum samples were treated with the ProteoMiner beads (Bio-Rad, Hercules, CA, USA); a relationship 1:10 (mg protein:µl resin) was used to optimize the yield of the procedure. First dimension was performed on pH 3-10, 24 cm Immobiline DryStrips (GE Healthcare, Uppsala, Sweden) under reducing and denaturing conditions. Then, strips were reduced and alkilated with equilibration buffer (50mM Tris-HCl pH 8.8, 6M urea, 30% (v/v) glycerol, 2% (w/v) SDS and bromophenol blue) and 1% (w/v) DTT or 2.5% (w/v) IAA, respectively. The second dimension was performed with home-made 12% polyacrylamide gels. Protein staining was performed with silver, following the Berkelman and Stendstedt protocol [5]. Gels were scanned with the Image Scanner III (GE Healthcare).

After treatment with the ProteoMiner system, a substantial enrichment of the low-abundance proteins was achieved and new spots were detected, whereas albumin and the light and heavy chains of immunoglobulins were less represented (Figure 1). These reagents are based on the affinity binding of all kind of proteins and thus they intend to capture and amplify the low-abundance proteome.

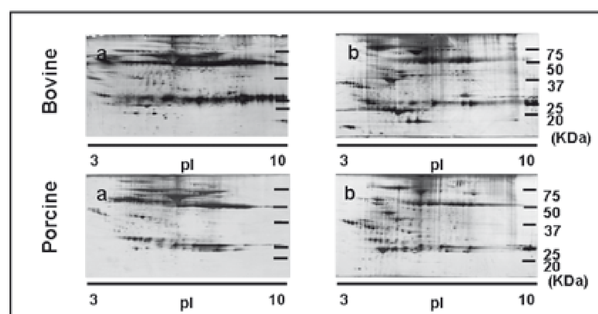

Figure 1. *Two-dimensional electrophoresis of bovine and porcine serum after treatment with the ProteoMiner beads: a) control, non-depleted serum; b) bound fraction ("enriched" serum).*

Reproducibility is necessary for accurate downstream analysis. To assess this characteristic in the ProteoMiner system, five technical serum replicates from cow and pig were prepared from aliquots of the same sample and run on a one dimensional 12% polyacrylamide gel, using 50 µg protein per lane. Gels were stained with silver and scanned as described above. Densitometry was performed with the Multi Gauge software (Fujifilm). As seen in Figure 2, the ProteoMiner system presents good reproducibility.

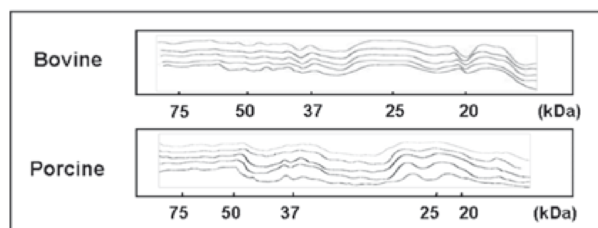

Figure 2. *Reproducibility analysis of the enriched low-abundance proteome achieved with the ProteoMiner beads on bovine and porcine samples. Densitometry of the bound fraction ("enriched" serum) obtained from one-dimensional SDS-PAGE gels is shown.*

These results clearly demonstrate that the ProteoMiner system is useful for bovine and porcine serum samples. While this is not a depletion method

to remove high abundance proteins, like others used for human biomarker identification, the ProteoMiner treatment will decrease the amount of these high-abundance proteins. This kind of strategies should widen the number of applications in serum animal proteomics.

This work was funded by grant AGL2006-02364/GAN from the Spanish "Ministerio de Educación y Ciencia". Part of the funding was financed by the FEDER program from the European Union. We thank Ms. Anna Vilalta for her excellent technical assistance.

References

[1] Adkins JN, Varnum SM, Auberry KJ, Moore RJ, Angell NH, Smith RD et al. Toward a human blood serum proteome: analysis by multidimensional separation coupled with mass spectrometry. Mol. Cell. Proteomics 2002; 1, 947-955.

[2] Anderson NL and Anderson NG. The human plasma proteome: history, character, and diagnostic prospects. Mol. Cell. Proteomics 2002; 1, 845-867.

[3] Echan LA, Tang HY, Ali-Khan N, Lee K and Speicher DW. Depletion of multiple high-abundance proteins improves protein profiling capacities of human serum and plasma. Proteomics 2005; 5, 3292-3303.

[4] Boschetti E and Righetti PG. The ProteoMiner in the proteomic arena: A non-depleting tool for discovering low-abundance species. J. Proteomics 2008; 71, 255-264.

[5] Berkelman T and Stenstedt T. 2-D Electrophoresis. Principles and methods. Amersham Biosciences, Uppsala, Sweden. 1998.

La Proteómica como vía para determinar el origen animal de los productos cárnicos

Miguel Ángel Sentandreu[1], Paul D. Fraser[2], Enrique Sentandreu[1], Leticia Mora[1], Peter M. Bramley[2]

[1] Instituto de Agroquímica y Tecnología de Alimentos (CSIC), P.O. Box 73, 46100 Burjassot, Valencia. España. [2] Center for Systems and Synthetic Biology, School of Biological Sciences, Royal Holloway, University of London, Egham TW20 0EX. Reino Unido

Resumen

En el presente trabajo se describe el desarrollo de un método para detectar la presencia de carne de pollo en mezclas de carne haciendo uso de la tecnología proteómica. Es un método simple y robusto que incluye una etapa de extracción de proteínas musculares, enriquecimiento de la proteína diana mediante isoelectroenfoque, digestión con tripsina de la misma y análisis de un péptido biomarcador específicos de la especie aviar mediante LC-ESI-MS/MS. Esta metodología ha permitido detectar la presencia de un 0,5 % de carne de pollo contaminando carne de cerdo. Además de su sencillez, el método presenta la ventaja de poder aplicarse indistintamente al análisis de carne fresca y cocinada, representando una alternativa interesante a los métodos que se están empleando actualmente para el control del origen animal de los productos cárnicos.

Texto principal

Los principales casos de adulteración en productos cárnicos se relacionan con la sustitución de carne de elevada calidad por carne de un menor coste con objeto de poder obtener un beneficio económico adicional. Para poder controlar el fraude que supone esta forma de proceder es necesario disponer de métodos de análisis robustos, sensibles y precisos. De entre las tecnologías más empleadas hasta el momento para este propósito hay que destacar los inmunoensayos y los análisis de ADN. A pesar del