EXPERT REVIEWS

From Proteomics Research To Clinical Practice

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Third International Conference of the Hellenic Proteomics Society: From Proteomics Research to Clinical Practice

Nafplio, Greece, 30 March–1 April 2009

The third International Conference of the Hellenic Proteomics Society, *From Proteomics Research to Clinical Practice*, took place in Nafplio (Greece), from 30 March to 1 April 2009. This year the conference was dedicated to the application of proteomics in clinical practice. Many scientists from different European counties participated in the conference, which made this event unique in the field of proteomics for the southeastern region of Europe. Extensive presentations and discussions covered nearly every aspect of the modern point of view regarding the application of proteomics in various diseases, the quantitative peptidomics and proteomics approaches, and the advances of methodologies for biomarker discovery and validation.

The Hellenic Proteomics Society (HPS) was founded in 2004 by a multidisciplinary group of scientists led by Michael Foundoulakis (Academy of Athens, Athens, Greece), who was elected first President of the society. In order to establish proteomics in Greece, as well as in the broader area of southeast Europe, the HPS is a founded member of the European Proteomics Association (EuPA) and has organized many theoretical and laboratory workshops. In 2005, after theses activities, the HPS announced the organization of an International Conference on Proteomics in Greece, every second year. The First International Conference of the Hellenic Proteomics Society took place at the Biomedical Research Foundation of the Academy of Athens, from 22–25 May 2005. The conference was attended by scientists from 20 countries and the proceedings included presentations of topics related to proteomics, functional gemonics and bioinformatics [1,2]. The first HPS conference also included oral presentations from distinguished invited scientists, as well as scientists selected from the submitted abstracts.

In 2007, 2 years following the first conference, the HPS organized the Second International Conference at Kolimbari (near Chania) on the Greek island of Crete, from 23–25 May. The main purpose of the conference was to inform the attendees of the forthcoming extensive and multidisciplinary applications of proteomics; the conference was entitled '*From Discovery to Applications*'. Similar to the first conference, that event was attended by scientists worldwide, including biologists, medical doctors, bioinformatitians and mass spectrometry (MS) experts. Many issues were discussed but the highlight of the conference was the extensive discussions of the comparison of different gel-staining methods, including fluorescent dyes, silver staining, Coomasie blue and the possibility to apply MS for protein identification [3].

Third International Conference

The Third International Conference on Proteomics was organized by the HPS on 30 March–1 April 2009. The conference was entitled *From Proteomics Research to Clinical Practice* and took place in Nafplion (Greece) [4]. The old Nafplion city at the east of Peloponnesus is one of the most beautiful towns in Greece and was the first capital of the state. With two mountains crowned by medieval fortresses overlooking the town and a small fortress island in the entrance of its harbor, the city is full of beautiful old buildings, while important ancient sites, such as Myceneae, Argos and Epidavros surround the city.

The key-note lectures were delivered by Thierry Rabilloud (CEA, Grenoble, France), Pier Giorgio Righetti (Polytechnic of Milano, Milan, Italy) and Bruno Domon (ETH Zurich, Zurich, Switzerland). The first speaker, Rabilloud, under the title 'What Room for Electrophoretic Separations in Modern Proteomics?' presented

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1D and 2D electrophoresis (2DE)-based proteomics strategies, in terms of quantitative proteomics and sample complexity. He presented the different protein separation and identification methods, as well as methods for the protein quantitative and post-translation modification analysis. At the level of biomarker discovery and validation, Rabilloud mentioned that, although the whole methodology of proteomics is developed at the level of peptide identification, the "proteins do the job, not the peptides…".

The second speaker, Righetti, presented methods for sample preparation and low-abundance protein identification. He tried to address why 2% of the proteome of a biological sample remains a mystery through considering biomarkers as "gold nuggets in the proteomic arena". Righetti's presentation focused on the newly developed method for the low-abundance protein purification through sample treatment with hexapeptides bound to organic polymers. By this approach, all the globin isoforms, even the embryonic variety, became 'visible' in red blood cell proteome, while analysis of the urine proteome resulted in the identification of 471 proteins instead of the 96 proteins previously identified without the hexapaptides treatment.

The search for protein biomarkers includes issues related to protein function, post-translational modifications, splice variants and single-nucleotide polymorphisms, as well as clinical data (e.g., early diagnosis, disease progression and drug response). These challenges in proteomic biomarker discovery were the theme of the last presentation of the section by Domon. Amongst others, he mentioned that, for consistent biomarker identification, the reduction of sample complexity and analytical systems with high sensitivity and dynamic range are necessary. Thus, the biomarker discovery, qualification and validation are in need of simple, robust, quantitative and high-throughput analysis. In accordance with the aforementioned statements, Domon proposed the selected reaction monitoring (SRM) technology as effective for biomarker discovery and validation. SRM is performed on a triple quatropole-type instrument for MS on the basis of the targeted proteomic strategy. This MS-based technique enables the quantitative detection with high selectivity and sensitivity of a pretermined set of peptides derived of a selected protein mixture. Finally, part of Domon's presentation was dedicated to the glycosylation-pattern profile and to the glycoproteome as a possible source of biomarkers.

Given together, these presentations form the impression that biomarker identification and validation needs high-quality, high-throughput and very sensitive methods. The combination of techniques, including sample preparation, high-performance MS platforms and bioinformatics ensure the in-depth analysis of the sample and the effective biomarker discovery.

The first section of the following day was entitled 'Bringing -omics to the Basic Science and Clinical Service'. This section was divided in two parts: the first included presentations from invited speakers and the second consisted of scientific presentations.

The majority of the presentations were dedicated to technology development for the analysis of clinical samples. Marc Baumann (University of Helsinki, Helsinki, Finland) presented 'The Use of Mass Spectrometry for Tissue Imaging' (specifically MALDI imaging and MS-imaging MS [IMS]). The label-free method offers the advantage to detect all kinds of molecules (e.g., proteins, lipids, metabolites and drugs) present in a tissue slide. Furthermore, it was made clear that the method could peak a single mass from the spectra and, by software-driven analysis, it could determine the distribution of the mass in the tissue slide. Bauman mentioned that MS-IMS in the future will be closely related to clinical applications, such as electron microscopy and immunocytochemistry. In accordance to this, application of the specific method to Alzheimer's disease indicated that different kinds of α-amyloid peptides appeared in different parts of the brain.

Hans Voshol (Novartis Institutes for Biomedical Research, Basal, Switzerland), presented details of reverse protein arrays (RPAs), an analytical tool readily scaled to provide massive readouts from the same sample. RPAs were based on the spotting of the total protein content on a sample and the incubation of each spot by different antibody or the spotting of the total protein content of different samples and the incubation of the spots with the same antibody. Capturing the dynamic of cellular signaling pathways, which require extensive sampling and simultaneous quantitation of multiple analytes, RPA seems to be the ideal analytical tool for the analysis of such pathways. Although the method is totally dependent on the quality and availability of the antibodies, it has been applied in pharmaceutical research for the identification of new potential intervention points in disease stages related to the modulation of signaling pathways.

Vilhem Guryca (F. Hoffmann–La Roche Ltd, Switzerland), presented the 'deeper exploitation of the proteome with a combination of multidimensional peptide separations with accurate mass and time tags proteomic approach'. This approach allows peptide/protein identification through information extracted exclusively from peptide-accurate masses and chromatographic retention times, entirely by-passing the time-consuming MS-MS events during data acquisition. The analysis of complex proteomes is highly dependent on efficient fractionation methods with low-level protein carry-over from fraction to fraction. On this respect, Egisto Boscheti (Bio-Rad) presented an alternative fractionation approach involving the use of solid-state buffers associated with ion exchangers to separate proteins of different pI ranges with a low level of protein overlapping, prior to extensive proteomic analysis.

Three presentations in this section were related to biomarker identification concerning human reproduction issues. The first was entitled "Finding Preeclampsia Biomarkers In Chorion Villus Biopsies By Mass Spectrometry" and was presented by Teo Luider (Erasmus MC, Rotterdam, The Netherlands). The study included application of MS analysis after enzymatic digestion of proteins from trophoblasts and stroma cells, collected by laser-catapulting microdissection, from three chorion villus biopsies of women with pregnancies subsequently complicated by preeclampsia and eight obtained from controls. Luider mentioned that significant differences in peptide patterns were found in both trophoblast and villous stroma cells in pregnancies with early-onset preeclampsia compared with uncomplicated and preterm pregnancies. Furthermore, the presentation was focused on two proteins

(LMAN1 and ITTGA6) that were found expressed only in the chorion villus biopsies of women with uncomplicated pregnancies and were absent in the chorion villus biopsies of pregnancies complicated by preeclampsia.

The second presentation, related to human reproduction, was given by Suresh J Gadher (Academy of Sciences of the Czech Republic, Czech Republic). In order to identify biomarkers for ovarian hyperstimulation syndrome, this group studied the protein profile of follicular fluid and plasma from women undergoing *in vitro* fertilization and those developing ovarian hyperstimulation syndrome. Gadher presented a group of seven proteins differentially expressed in the studied materials that are possible potential diagnostic and prognostic markers of the syndrome.

The third presentation on human reproduction was given by Athanasios Anagnostopoulos (BRF, University of Athens, Athens, Greece). He presented the 'Proteomic Analysis of Amniotic Fluid in Pregnancies with Klinefelter Fetuses'. The aim of the study was to identify possible biomarkers for the prenatal diagnosis of the specific chromosome abnormality, which is characterized by a male phenotype with an extra X chromosome (47, XXY). Anagnostopoulos announced that three proteins were found to be upregulated and four downregulated in the amniotic fluid of pregnancies carrying fetuses with Klinefelter syndrome compared with normal ones.

In the same section, two of the presentations discussed cancer biomarkers and two presented information on urine analysis. Hana Kovarova (Academy of Sciences of the Czech Republic) and Spiros Garbis (BRF, Academy of Athens) presented issues relating to cancer, and Harald Mischak (Mosaiques Diagnostics and Therapeutics AG, Germany) and Michalis Aivaliotis (BRF, Academy of Athens) presented issues relating to urine proteomic analysis. The presentation by Kovarova focused on the identification of valuable prognostic and/or diagnostic markers for the response to anticancer drugs and to the developed chemoresistance. Garbis presented a quantitative, liquid chromatography MS-MS stable-isotope-based method for the analysis of breast cancer tissue biopsies. He mentioned that the application of that method resulted in the identification of six proteins with altered expression in metastatic primary tumors and 19 proteins with altered expression in lymph node metastasis compared with non-metastatic primary tumors.

Finally, the section included one presentation from Ioannis Xenarios (Swiss Institute of Bioinformatics, Switzerland) and one from George Thireos (IMBB FORTH, Crete, Greece), while the section was completed with six presentations given by the sponsors of the conference.

Xenarios focused on the integration of biological knowledge through graph-based approaches, which allow representation of complex information and interrogation, while the topology and flux of such produced networks allow the dynamic behavior of the systems under study to be addressed. Thireos, under the title 'Systems Biology: Just a Slogan or a Reality', mentioned that each cell can be viewed as a system composed of subsystems, while each one of them has a given set of interacting and interconnected molecular components. In this regard, systems biology emerges not as a new independent field, but rather, as a unifying concept overlaying above the typical biological technologies, engineering, mathematical and computer science tools to solve biological problems.

The sponsors' presentations within this section referred to novel techniques for biomarker discovery, including new systems for 2DE (Ambrogio Sacchi, Bio-Rad, Milan, Itlay), the application of capillary electrophoresis–sodium dodecyl sulfate microchip in proteomics (Bahram Fathollahi, Caliper Life Science, MA, USA), the characterization of post-translational modifications (Michaela Scigelova, Thermo Fisher Scientific, UK) and a novel mass spectrometer combining ultra-high mass resolution and accuracy (Marcus Macht, Bruker Dalt, Leipzig, Germany). Additionally, two interesting presentations related to targeted proteomics, biomarker discovery and validation in combination with MS were given by David Potts (University of Hull, UK) and by Mark McDowall (Waters Corporation, MA, USA).

The second section of that day was entitled '*Biomarker Validation*' and included presentations from Mischak and Jadranka Koehn (Medical University of Vienna, Austria) and a 'Point–counterpoint Challenging the Expert' session with the theme 'The Peptide/Protein Quantification'. In his presentation, Mischak mentioned that the field of biomarkers has lost its credibility. This happens mainly for the reason that a huge number of potential biomarkers has been announced but in most cases their credibility cannot be validated in clinical practice. Mischak analyzed this fact in depth and mentioned that while a small data set is required for the biomarker identification, for its thorough validation, the biological variability requires large datasets and different classification performances using different size training sets. For the validation process, the statistical analysis is important, and Mischak noted that any statistical analysis must be adjusted for multiple tests while the application of machine-learning algorithms seems to be required, but any result of a machine-learning algorithm must be tested by an independent dataset. Koehn presented in details the US FDA's requirements for biomarker validation. Biomarkers are the foundation of evidence-based medicine, but how should they be treated, and with what? "Without markers, advance in a more targeted therapy will be limited and treatment will remain largely empirical" summarizes the FDA's point of view on biomarkers, said Koehn.

The last section of the second day of the meeting was an extensive discussion regarding peptide and protein quantification. This section was performed by Richetti, who spoke about ProteoMiner™; Rabilloud, who reported on 2DE staining techiques; Mischak, who spoke about the peptide profiling, and Domon, who informed the audience about SRM technology. The conclusion of the afternoon section was that, in the arena of biomarker discovery, the optimal result can only be achieved if complementarily analytical techniques are used. Furthermore, on the validation level, mistakes and erroneous results could be eliminated by employing well-characterized analytical platforms, adequate samples, controls and statistics. Finally, in order for one to prove experimental significance, one must validate the findings on an independent test set of the appropriate size.

The last day of the conference was committed to eight scientific presentations and to an educational section on 'Tips and Tricks and Reproducibility of 2D PAGE' by Hans Voschol (Novartis Institutes for Biomedical Research). In the scientific section, Tassos Economou (IMBB) presented ProFI, an a newly established proteomic facility in the Institute of Molecular Biology and Biotechnology. E. Balfoussia (BRF, Academy of Athens) presented the results of the proteomic analysis of superathletes' plasma as a model of severe stress, Elisa Fasoli (Politecnico di Milano, Milan, Italy) presented results about the analysis of maize cytoplasmic proteins with the goal of the identification of allergens, Alessia Farinazzo (Politecnico di Milano) presented the findings on the proteome analysis of chicken egg white and yolk. Following, Jérôme Zoidakis (BRF, Academy of Athens), developed an approach for the analysis of metal-binding proteins in urine. Dimitris Glotsos (Technological Educational Institute of Athens, Athens, Greece) presented a pattern-recognition strategy for processing MS spectra in order to identify potential biomarkers and a reliable and effective separation of normal from cancer tissues. Eugenia Giannopoulou (BRF, Academy of Athens) presented an interactive visualization tool that combines visually protein or peptide features into synthetic proteomic feature maps resembling 2DE images and, finally, Panagiotis Tsakanikas (Athens University) presented an efficient 2DE gel image-analysis pipeline for reliable spot segmentation and quantification. Finally, it must be noted that a number of posters were also presented. The majority were related to low-abundance protein analysis, identification of biomarkers by the analysis of various body fluids and biological materials, as well as the development of bioinformatics tools and approaches for data analysis.

Conclusion

The Third International Conference of the Hellenic Proteomic Society was dedicated to the proteomic research and its application to clinical practice. The presentations and discussions that took place during the conference demonstrated that, for biomarker discovery, all available proteomic methods should be complementary and the biomarker-validation step needs a more extensive, time-consuming and multiparametric approach. Application of such approaches is in progress for the validation of the already existing, great number of biomarkers. It must be noted that the increasing number of studied samples results in decrease of the number of biomarkers, yet strengthening their relevance.

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