JOURNAL OF PROTEOMICS 73 (2010) 361–364

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Preface

In Caprarola in 1971 for Jeffries Wyman's birthday party, with Robert W. Noble

In everlasting memory of Eraldo Antonini: a brilliant scientist, a charming and captivating man, a pioneer in the field of blood biochemistry.

Since in the last few years clinicians and proteomicists have rapidly reached the rendez-vous point, we are currently in the middle of the docking manoeuvres. Two formerly-independent worlds have started to cooperate, with the declared intent to ameliorate the quality of the production and handling processes of blood components and plasma derivatives, with the shared goal to guarantee their safety and effectiveness for the whole health care system.

This special issue tries to add some little pieces to this yet complicated puzzle, since it represents an attempt to sail across the mare magnum of the actual knowledge in this research endeavour, to the end of summarizing the main advancements. In the light of what emerges, blood-related proteomics appears as much as promising as never before. In particular, proteomics has been recently transformed from a mere basic-research expensive toy into a dramatically-sensitive and high-throughput research tool which could be exploited to investigate the molecular mechanisms of blood and blood components and promises to establish quality parameters in the blood-banking production-chain totally anew.

The twenty eight accepted papers have been collected into three subgroups:

1. Proteomics of Blood Banking;
2. Blood-related proteomics;
3. Blood Proteomics and the dynamic range.

An editorial by worldwide experts in the relative field introduces each subgroup:

1. Hess J.R. and Grazzini G. for "Proteomics of Blood Banking";
2. Tissot J.D. for the "Blood-related proteomics" section;
3. Righetti P.G. and Boschetti E. for "Blood Proteomics and the dynamic range".

This special issue, moreover, is the first attempt to bridge the gap between independent laboratories and regroup recent findings in the promising field of blood-related proteomics, with the declared intent to regroup laboratories which have lately branched out from the same research root. To this regard it has been organized the first meeting in Viterbo (Italy), on the 12th–14th October 2009, with the hope to organize a second one in 2010 in some other location.

Although blood and blood components equally attract a great deal of attention, actual concern still arises and persists around the safety and effectiveness of long-stored labile blood components, especially of red blood cell concentrates, in the light of recent retrospective clinical observations. Therefore, a whole section has been devoted to red blood cell storage and proteomics. This section is introduced by the editorial co-signed by Hess and Grazzini.

Blood storage has the potential to reduce the efficacy of transfused components. What has been harder to show is that these known changes have significant clinical pitfalls, as stated by Hess in his article on red cell storage. The increasing power of proteomics and metabolomics will allow a deeper understanding of storage processes at a molecular level. This offers the potential for more insightful physiologic and clinical studies of the safety and efficacy of the present storage

1874-3919/$ – see front matter
doi:10.1016/j.jprot.2009.11.006

systems and the opportunity to build better storage systems in the future.

Greening et al. reviewed how blood and its derivatives play a critical role in worldwide health care systems, with blood components having direct clinical indications. As the areas of clinical validation of different disease states from blood-derived sources (i.e., disease biomarkers) move towards validation stages, the importance of controlled- and standardized protocols is imperative.

Lion et al. from Tissot's group reviewed the current state of knowledge about red blood cell storage lesions from a proteomic and biochemical integrated perspective. He concluded with suggesting a few possible proteomic studies that would provide further knowledge of the molecular alterations carried by RBCs stored in the cold for six weeks.

In parallel, Bosman et al. described red blood cell storage lesions from a strict proteomic perspective and reported that comparative proteomic analyses of erythrocytes and secreted micro-vesicles support the theory that aging is accompanied by increased binding of modified hemoglobins to band 3, disruption of the band 3-mediated anchorage of the cytoskeleton to the lipid bilayer, vesicle formation, and antigenic changes in band 3 conformation. This process involves chaperones, stress proteins, and proteasomes.

Pasini, Mann, Lutz and Thomas provided an in-depth portrait of red blood cell proteomics either under physiological or pathological conditions through a double review article. The authors reported that the high lipid content of the environment in which membrane proteins are found often causes a particular set of problems that must be overcome when isolating the required material before effective HPLC-MS approaches can be performed. A full understanding of this critical cell component will ultimately require, in addition to proteomics, lipidomics, glycomics, interactomics and study of post-translational modifications.

In the second part of their review, Pasini et al. provided a short overview of some of the main achievements obtained by comparative proteomics in the field of red blood cell-related local and systemic diseases. The authors suggested some additional areas of research to which comparative proteomic approaches could be fruitfully applied or extended in combination with biochemical techniques to a better understanding of disease mechanisms: from transfusion medicine to infectious diseases, from cardiovascular affections to diabetes.

Turrini et al. summarized the wide range of hereditary diseases affecting different red cell membrane functions and the membrane modifications induced by malaria parasite intracellular growth. This represents a unique opportunity to study PTM modifications in response of variable cellular stresses.

Goodman et al. profiled sickle RBC membranes from of HU-treated and untreated patients with the final aim to understand hydroxyurea-induced changes at the proteomic level. The proteins that significantly increased in abundance, such as palmitoylated membrane protein 55 (p55), were mostly membrane skeletal components involved in the regulation of red blood cell shape and flexibility, and those showing a significant decrease were components of the protein repair and degradation machinery.

Sliipers et al. used a novel proteome-wide approach to quantitatively analyze membrane proteins of healthy donor and patient erythrocytes in order to extend the current set of diagnostic tools. Blue-native PAGE was used to detect protein level changes in disordered erythrocyte membranes. They could successfully confirm that erythrocyte spectrin levels were dramatically decreased in hemolytic anemia-affected patients.

Proteomic tools are also routinely applied for the study of the storage issues related to other blood components, such as platelets, as in the paper hereby presented by Schubert and Devine. The authors focused on the application of proteomics to identify molecular mechanisms leading to the deterioration of blood platelets during storage — a critical aspect in the provision of platelet transfusion products. Once validated and placed in a transfusion context, their data will provide further understanding of the underlying molecular mechanisms leading to platelet storage lesion.

Each section is aimed at providing an in-depth analysis of the related topic, as it is for the Blood-related proteomics section, which is opened by the editorial by Jean-Daniel Tissot. In this section, the review of Liumbruno et al. provides a panoramic view of the current papers available in literature dealing with proteomics of blood components. Moreover, they have given a glance at close prospective studies which promise to shift the focus of proteomic attentions from the end product to its provider, the donor, in a sort of Kantian "Copernican revolution" in the field of transfusion medicine. A well-rounded portrait of the usefulness of proteomics in blood-related research is accurately given, with references to functional and applied prospective.

While a series of method and findings have been yet produced and accumulated in the field of blood-related proteomics, new technical innovations could pave the way for further advancements. In this scenario, Urbani et al. reported that although several MS-based bioinformatics methods have been proposed for the elaboration of certain MS data, the divergence of the findings by different research groups on the same MS data suggests that the definition of a reliable method has not yet been achieved. Thus, he proposed an integrated software platform, MASCAP, intended for comparative biomarker detection from MALDI- and SELDI-TOF MS data. The analysis approach implemented in MASCAP may simplify biomarker detection, by assisting the recognition of proteomic expression signatures of the disease.

Basic science research is still a funding pillar of blood-related proteomics, since we have not yet fully succeeded in mapping the whole proteome of blood and its components. In this special issue, Sintiprungrat et al. have provided some insights into molecular mechanisms of biological processes upon differentiation from monocytes to macrophages. Global protein network analysis demonstrated that these altered proteins were involved in cell death, lipid metabolism, cell morphology, cellular movement, and protein folding.

McLeish et al. showed how a comprehensive understanding of the regulation of neutrophil responses can be provided by high-throughput proteomic technologies and sophisticated computational analysis. The authors reviewed the application of expression, structural and functional proteomic studies to neutrophils.

D'Alessandro et al. summarized recent advancements in the field of umbilical cord blood-derived hematopoietic

stem cell transplantation. However, although cord blood is routinely used as an alternative source of hematopoietic stem cells, no substantial advances have been made in the field of clinical regenerative medicine, due to the low cell/dose limit within a single unit. A review is provided of the proteomic studies that have investigated the plasticity of cord blood-derived stem and progenitor cells, in particular of $CD34^+$ populations. Recent proteomic insights could pave the way for the final understanding of the molecular mechanisms regulating stem cell proliferation and differentiation, thus broadening the application range for this fruitful product.

Wasinger et al. used the Microflow MF10 to partition CD34+ haematopoietic stem and progenitor cells and CD4+/CD8+ T-cells in order to separate proteins restricted by cell number. Less than 10 μg total protein per fraction was used for comparative analysis, which revealed a series of differentially expressed proteins between the 3 cell populations.

Proteomics in general and, in particular, MS could be adopted to individuate early markers of certain haematological and non-haematological diseases, such as cancers.

Vlasova et al. reviewed extensive data about the identity of differential features detected on mass-spectra up to now in order to draw conclusions about potency and perspectives of MALDI-TOF mass-spectrometry for cancer biomarker search in blood serum/plasma. This because of MALDI-TOF mass-spectrometry, thanks to its high throughput and relative simplicity, has become a popular tool for cancer research during the last decade.

Gianazza et al. reported how it was possible to identify markers for the early detection of Diabetic nephropathy (DN) through proteomic analysis. Three peptides were found to be differentially expressed in serum of patients as compared to controls: the whole fibrinopeptide A peptide and two of its fragments, respectively. The two fragments were under-expressed in diabetic patients, while Fibrinopeptide A was overexpressed, suggesting that anomalous turnover of Fibrinopeptide A could be involved in the pathogenesis of DN.

Thongboonkerd summarized important findings obtained from recent proteomic studies applied to extracorporeal blood purification and peritoneal dialysis in settings of renal disease (ESRD) and acute kidney injury (AKI) and hepatic failure. Efforts have been made to characterize the toxins removed by diffusion (dialysis), convection (ultrafiltration), and/or adsorption (toxins are adsorbed onto the dialysis membrane and are thus removed) using different types of dialysis membrane.

Zamò and Cecconi reported how the application of proteomics to the study of neoplastic haematological diseases could help in the search for new diagnostic and prognostic markers as well as in the development of new therapeutic strategies. He focused on the results obtained in the field of body fluid, cell lines, and tissues proteomics, and discussed the improvement allowed by the newly developed proteomic strategies, such as nanofluidic systems, analysis of formalin-fixed tissues, and quantitative high-throughput techniques (SILAC, ICAT, and iTRAQ).

The proteomic analysis of blood and blood components is not an easy task. This is mainly due to the dynamic range of certain proteins, which often hinder detection of a whole hidden proteome. In red blood cells for example, haemoglobin accounts for approximately 90% of its dry weight, 98% of its cytoplasmic protein content. A series of methods have been recently accumulated and hereby reviewed by several authors in order to deliver an exhaustive portrait, as it is introduced in the brilliant editorial by Righetti and Boschetti.

Hortin examined the range of protein concentration for 18 proteins (secretory proteins or highly-expressed components of specific tissues) measured over a year in a clinical laboratory to provide data on pathophysiological extremes in protein concentrations.

Di Girolamo et al. reported a systematic analysis on the influence of pre-analytical factors [clotting times, temperature and time storage, and addition of protease inhibitor (PI)] on serum LMW proteome profiling. The use of standardized pre-analytical and storage procedures together with an automated peptide purification might minimize potential bias on serum LMW profiling results, thus allowing a better homogeneity and reproducibility in futures proteomic studies.

Greening and Richard Simpson developed an enrichment strategy for sub-proteome based on centrifugal ultrafiltration. By using four commercially available filter membranes they reported the identification of several proteins (e.g., rheumatoid factor D5, serine protease inhibitor A3N, and transmembrane adapter protein PAG) previously not reported in extant HUPO Plasma Proteome Project datasets.

Levin et al. presented the coupling of label-free SAX chromatography and IMAC to a data-independent nanoLC-MS/MS (nanoLC-MSE) platform for analysis of blood plasma and serum proteins. The combined approach provides complementary information on sub-proteomes, potentially useful for systems biology, biomarker discovery, and investigation of post-translational modifications of blood proteins.

Ostroff et al. studied three sample collection variables commonly encountered in archived sample sets: (1) three different sample tube types, PPT plasma, SST serum, and Red Top serum, (2) the time from venipuncture to centrifugation, and (3) the time from centrifugation to freezing. Remarkably, the results found no significant variation in the measurements for most proteins (~99%) when the two sample processing times tested were 2 h or less, regardless of the sample tube type.

Castagna et al. reviewed recent results obtained with SELDI-TOF-MS as well as with other mass-spectrometry-based and immunological methods. In particular, SELDI-TOF-MS being optimal for low-molecular-weight biomarkers, has emerged as a valid tool for hepcidin assay, a liver peptide hormone, that is the central regulator of iron homeostasis.

Urbani et al. developed and validated a linear MALDI-TOF-MS protein profiling method to explore the low protein molecular weight region (5–20 kDa) of serum samples. His data showed critical conditions for serum protein profiles depending on storage times and temperatures: Apolipoprotein A-IV and complement C3 protein fragment, transthyretin and the oxidized isoforms in different apolipoprotein species represent the major molecular features of such a degradation pattern.

Josic et al. by using anion-exchange chromatography and affinity chromatography with immobilized heparin, fractionated

the bulk of human serum albumin (HSA), immunoglobulin G, and other non-binding proteins from F IX. This methodology resulted very helpful for further process optimization, rapid identification of target proteins with relatively low abundance, and for the design of subsequent steps for their removal or purification.

While seemingly representing only one of the numerous attempts to start a network of information among multiple laboratories working on this delicate topic, this Blood Proteomics issue promises to start bridging the persisting gaps between the clinical and the academic milieu. For the foreseeable future, when a full collaboration between these two endeavours will be accomplished, proteomics will perhaps find a widespread diffusion in the clinical routine as well, in order to deliver blood components and plasma derivatives which are safe, efficient and effective for the whole health care system.

I would like to thank the Editor in Chief Professor Juan Calvete for the opportunity and my collaborators – a special mention goes to Angelo D'Alessandro – in helping me with the correspondence with the contributing Authors and with the update of the progresses of this, hopefully, successful Special Issue.

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