JOURNAL OF PROTEOMICS 112 (2015) 190–209

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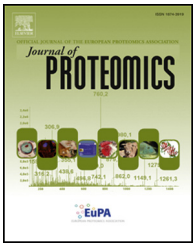

Proteomics of apheresis platelet supernatants during routine storage: Gender-related differences

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ARTICLE INFO

Article history:
Received 10 June 2014
Accepted 31 August 2014

Keywords:
Donor
Transfusion medicine
Proteomics
Human
Label-free quantitation
Mass spectrometry

ABSTRACT

Proteomics has identified potential pathways involved in platelet storage lesions, which correlate with untoward effects in the recipient, including febrile non-haemolytic reactions. We hypothesize that an additional pathway involves protein mediators that accumulate in the platelet supernatants during routine storage in a donor gender-specific fashion.

Apheresis platelet concentrates were collected from 5 healthy males and 5 females and routinely stored. The 14 most abundant plasma proteins were removed and the supernatant proteins from days 1 and 5 were analyzed via 1D-SDS-PAGE/nanoLC-MS/MS, before label-free quantitative proteomics analyses. Findings from a subset of 18 proteins were validated via LC-SRM analyses against stable isotope labeled standards.

A total of 503 distinct proteins were detected in the platelet supernatants from the 4 sample groups: female or male donor platelets, either at storage day 1 or 5. Proteomics suggested a storage and gender-dependent impairment of blood coagulation mediators, pro-inflammatory complement components and cytokines, energy and redox metabolic enzymes. The supernatants from female donors demonstrated increased deregulation of structural proteins, extracellular matrix proteins and focal adhesion proteins, possibly indicating storage-dependent platelet activation.

Routine storage of platelet concentrates induces changes in the supernatant proteome, which may have effects on the transfused patient, some of which are related to donor gender.

Biological significance

The rationale behind this study is that protein components in platelet releasates have been increasingly observed to play a key role in adverse events and impaired homeostasis in transfused recipients.

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http://dx.doi.org/10.1016/j.jprot.2014.08.016

In this view, proteomics has recently emerged as a functional tool to address the issue of protein composition of platelet releasates from buffy coat-derived platelet concentrates in the blood bank. Despite early encouraging studies on buffy coat-derived platelet concentrates, platelet releasates from apheresis platelets have not been hitherto addressed by means of extensive proteomics technologies. Indeed, apheresis platelets are resuspended in donors' plasma, which hampers detection of less abundant proteins, owing to the overwhelming abundance of albumin (and a handful of other proteins), and the dynamic range of protein concentrations of plasma proteins.

In order to cope with these issues, we hereby performed an immuno-affinity column-based depletion of the 14 most abundant plasma proteins. Samples were thus assayed via GeLC-MS, a workflow that allowed us to cover an unprecedented portion of the platelet supernatant proteome, in comparison to previous transfusion medicine-oriented studies in the literature.

Finally, we hereby address the issue of biological variability, by considering the donor gender as a key factor influencing the composition of apheresis platelet supernatants. As a result, we could conclude that platelet supernatants from male and female donors are not only different in the first place, but they also store differently. This conclusion has been so far only suggested by classic transfusion medicine studies, but has been hitherto unsupported by actual biochemistry/proteomics investigations.

In our opinion, the main strengths of this study are related to the analytical workflow (immunodepletion and GeLC-MS) and proteome coverage, the translational validity of the results (from a transfusion medicine standpoint) and the biological conclusion about the intrinsic (and storage-dependent) gender-related differences of platelet supernatants.

1. Introduction

Platelets (PLTs) are key mediators of hemostasis, and PLT concentrates (PCs) are an important component of supportive care for thrombocytopenic patients. Despite decades of improvements, preparation of PLT concentrates still represents one of the major challenges to the blood bank, in light of standard platelets storage limitations [1–3]. Indeed, routine storage results in decreased PLT morphology scores (loss of disk shape) and responses to agonist (shape change), increased hypotonic shock response, volume and density heterogeneity, PLT activation marker expression (e.g. CD62P, PAC-1 binding/expression of the fibrinogen binding site on the $\alpha IIb\beta_3$ complex), release of PLT α-granules, cytosolic proteins and inflammatory mediators, increased pro-coagulant activity, and altered supernatant pH and glycoprotein expression [1–4].

In most countries, PCs are stored for 5–7 days, depending on the national guidelines. This short lifespan results in a significant amount of the PLT inventory becoming outdated and discarded. Lifespan limitations also impose constraints regarding the narrow time window to complete donor testing, shipment of PCs, and leaves even less time for the recipient hospitals to transfuse these units.

Proteomics is an ideal tool to investigate *in vitro* changes of stored PLTs [5]. Owing to their anucleate nature, platelets have limited protein synthesis capacity [6] and a rather stable proteome (~5,000 proteins) that, in comparison to other cell types, is less affected by biological variability issues [7–10]. Translational application of proteomics to PLT-related transfusion medicine issues has focused on many aspects of PLT product preparation, including the effects of preparation (cell processing and pathogen inactivation) on *in vivo* viability [11–18].

PLT supernatants are recognized mediators of hypercoagulability after injury [19], as they become increasingly enriched with PLT releasates [20], lipid [21] and oncogenic, angiogenic and invasion-promoting mediators [22,23]. In this view, it has become increasingly accepted that protein components in PLT releasates might play a non-secondary biological role in mediating untoward responses in the recipients [24]. Despite recent breakthroughs, the intrinsic limitations of the gel-based approaches [5,25] (limited coverage of the proteome, impaired detection of very high molecular weight and hydrophobic proteins) leave room for further improvements in this research endeavor, with evident benefit for the transfusion community.

In the present study we hypothesize that a significant number of proteins accumulate during routine PLT storage which are directly affected by donor gender and may in turn affect the recipient. The differences in male and female donors were addressed at the two extreme storage time points, as to assess whether the PLT proteomes of freshly-donated apheresis PLTs differed as a function of storage time, either in a storage or gender-dependent fashion.

2. Materials and methods

2.1. Sample collection

After obtaining informed consent under a protocol approved by the Combined Multiple Institution Review Board, 10 healthy donors (5 females – mean age: 54.0 ± 5.7 and 5 males – mean age 47.7 ± 1.2) donated one unit of apheresis platelets using a Cobe Trima apparatus with appropriate leukoreduction ($<5 \times 10^6$/unit per Bonfils Blood Center standard operating procedures and consistent with AABB guidelines, as determined by direct leukocyte counting by flow cytometry). Duration of the apheresis procedure was 92.0 ± 2 and 97.3 ± 1.5 min for female and