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Fang Bian, Morehouse School of Medicine
Roger Simon, Emory University
Yun Li, Morehouse School of Medicine
Larry David, Oregon Health Science University
Jolita Wainwright, Morehouse School of Medicine
Casey L. Hall, Emory University
Michael Frankel, Emory University
An Zhou, Morehouse School of Medicine

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Nascent proteomes in peripheral blood mononuclear cells as a novel source for biomarker discovery in human stroke

Fang Bian, PhD¹, Roger P. Simon, MD¹,²,*, Yun Li, MD¹, Larry David, PhD³, Jolita Wainwright, BS¹, Casey L. Hall, MD⁴, Michael Frankel, MD²,⁴, and An Zhou, PhD¹,²

¹Morehouse School of Medicine, Atlanta, GA
²Grady Memorial Hospital, Atlanta, GA
³Oregon Health Science University, Portland, OR
⁴Emory University, Atlanta, GA

Abstract

Background and Purpose—The proteome of newly synthesized proteins (nascent proteome) in peripheral blood mononuclear cells (PBMC) can be a novel source of stroke biomarkers. Changes in the PBMC nascent proteome after stroke reflect the dynamic response-in-action not detectable in the total proteome (all existing proteins) in blood. Here we test the application of nascent proteomics as a novel approach for stroke biomarker discovery.

Methods—The PBMC nascent proteome in human blood was determined by metabolic labeling of fresh PBMC cultures with azidohomoalanine (AHA, an azide-containing methionine surrogate), followed by mass spectrometry detection and quantitation of AHA-labeled proteins. The PBMC nascent and total proteomes were compared between stroke patients and matched controls.

Results—Both PBMC nascent and total proteomes showed differences between stroke patients and controls. Results of hierarchical clustering analysis of proteomic data revealed greater changes in the nascent than in the total PBMC proteomes, supporting the usefulness of the PBMC nascent proteome as a novel source of stroke biomarkers.

Conclusions—Nascent proteomes in PBMC can be a novel source for biomarker discovery in human stroke.

Keywords

Stroke; Biomarker; Leukocyte; Proteomics; Nascent Proteomics; Peripheral Blood Mononuclear Cells
Introduction

The identification of stroke biomarkers has important diagnostic and treatment implications. Recent studies have demonstrated that gene expression profiles in peripheral blood leukocytes can be a source of stroke biomarkers\textsuperscript{1,2}. Here, we build on this insight and demonstrate that newly synthesized proteins from peripheral blood leukocytes (the nascent proteome) are a novel and revealing source of potential biomarkers.

The nascent proteome of peripheral blood mononuclear cells (PBMC) defines altered protein biosynthesis in the PBMC’s response to an acute event. Essentially, it provides a snapshot of reactive changes that may not be detectable by characterization of the total proteome (consisting of all existing proteins in PBMC). In parallel with analysis of PBMC total proteomes in stroke patients and controls, we determined nascent proteomes in the same PBMC preparations using a novel approach of metabolically labeling the PBMC fraction with a chemically tagged amino acid, the incorporation of which into newly synthesized proteins allows subsequent isolation and characterization of PBMC nascent proteomes. We found that, compared to the total proteome, the PBMC nascent proteome shows unique bioinformatic features and greater differences between stroke patients and controls in a sex-specific pattern. This is the first published study investigating the human PBMC nascent proteome as a novel source of stroke biomarkers.

Study Subjects and Methods

Study protocols were approved by Institutional Review Board of the Morehouse School of Medicine (MSM) and the Grady Memorial Hospital of Atlanta. This pilot study included four male and three female African American patients, admitted to the Marcus Stroke and Neuroscience Center at Grady Memorial Hospital with a clinical diagnosis of stroke, and five male and three female age-matched African American controls, recruited from the Clinical Research Center at MSM. Clinical diagnosis of stroke was later verified by review of history, further clinical exam, and neuroimaging.

From each study subject, 8 ml whole blood was drawn. The average time between known well and study blood draw was 22.9±4.5 (mean±S.E.) hours. Within 1 hour after blood draw, the PMBC fraction was isolated from the whole blood (method described in Supplementary Materials and Methods (SMM)), followed by a 2-hour incubation with azidohomoalanine (AHA, an azide-containing methionine surrogate) to metabolically label newly synthesized proteins. After incubation, proteins were extracted from individual PBMC preparations and pooled according to study groups as follows: female patients, male patients, female controls, and male controls. The AHA-labeled PBMC proteins (i.e., nascent proteome) were isolated from the total proteome by means of Click reaction\textsuperscript{3,4} as described in SMM.

Nascent and total proteome preparations were analyzed by quantitative mass spectrometry (MS) with technical replications\textsuperscript{5}. Technical details for MS analysis using Waters’ Synapt G2S mass spectrometer are introduced in SMM. Bioinformatic analyses of proteomic data were performed with the assistance of commercial bioinformatics tools, as noted in SMM.
Initial MS results were validated by re-analyzing a subset of samples using a second, independent MS system (described in SMM), or by Western blot analysis of selected proteins.

**Results**

**Greater differences between stroke patients and controls in the PBMC nascent proteome than in the total proteome**

In both male and female subjects, common and unique proteins were identified in both stroke and control groups, with more robust unique protein subsets found in PBMC nascent proteomes than in total proteomes, as demonstrated by Venn diagrams (Figures 1A and 1C). The results of hierarchical clustering analysis of all identified PBMC proteins (Supplementary Table (ST) I) revealed the greatest difference in proteomes occurring between the nascent proteomes of male and female stroke groups, and the smallest difference between the total proteomes of male and female control groups (Figures 1B and 1D). This presents more profound, sex-specific changes in PBMC nascent proteomes after stroke than in total proteomes. As examples, in the PBMC nascent but not the total proteome, increases were seen for Ras-related protein Rab-10 and integrin linked protein kinase (male patients only), and alpha enolase, endoplasmin, and protein S100 A9 (female patients only) (ST II).

**Distinct bioinformatic properties of the PBMC nascent proteome**

The PBMC nascent proteome, not the total proteome, was enriched with ribosomal and nuclear proteins (Supplementary Figure (SF) I; ST III), whose molecular functions (in gene ontology terms) are of DNA binding, or RNA binding, or translation regulator activity, or structural molecular activity. A number of these proteins showed an increase in stroke patients (ST II).

**Discussion**

In the present study, more substantial changes in PBMC proteomes were detected in stroke patients with the nascent proteomics approach than with analyzing the total proteome (Figure 1). Of interest are the afore-mentioned proteins that were increased or only detected in the PBMC nascent but not in total proteomes, yielding potential candidates for stroke biomarkers. The observed sex specificity and sex-associated differences in PBMC proteomes in stroke are consistent with prevalence and outcome data, as sexual dimorphism in genomic responses to stroke has been reported. Here, the nascent proteomics offers additional tools for investigating underlying mechanisms. Myeloperoxidase (MPO) has been used as a marker for inflammation and neutrophil infiltration in stroke. A significant association of MPO’s single-nucleotide polymorphism with stroke risk has been reported in human. Interestingly, in the present study, its increase was detected in both PBMC nascent and total proteomes and in both sexes of stroke patients (SFs II and III, ST-II). Such results present another potential application of the PBMC nascent proteomics – as a mean to understand the *response-in-action* of reactive protein synthesis in acute brain injury, and distinguish changes resulting from increased protein synthesis or degradation. Last but not
the least, proteins that were enriched in the PBMC nascent but not total proteome, notably those involved in translation machinery (SF I, ST III) and those showing an increase in stroke patients (ST II), offer novel leads for mechanistic studies on the PBMC’s response to stroke.

With limited numbers of study subjects, we are cautious in making definite conclusions regarding specific proteins as putative stroke biomarkers. However, our quantitative MS analyses are of high reproducibility and reliability, with results confirmed by independent analyses using a second MS system at another facility and by Western blotting (SFs II and III).

**Conclusion**

Our results demonstrate the effectiveness and usefulness of using the nascent proteomics approach to identify PBMC proteins whose changes in stroke have not been reported before, or would otherwise not be detected if only the total proteome was analyzed. The methodology established here is being applied in our on-going studies with expanded numbers of study subjects and inclusion of different stroke subtypes and outcomes.

**Supplementary Material**

Refer to Web version on PubMed Central for supplementary material.

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**References**


A. Total proteomes, Venn diagrams

B. Total proteomes, hierarchical clustering

C. Nascent proteomes, Venn diagrams

D. Nascent proteomes, hierarchical clustering

Figure 1. Differences in PBMC proteomes
Venn diagrams illustrate the extent of overlapping of identified proteins between control and stroke groups (A and C, total and nascent proteomes, respectively). Numbers are the number of proteins identified by MS. Heat maps present results of hierarchical clustering analysis, showing distances among four study groups in PBMC total (B) or nascent (D) proteomes. Ctr: Control; Str: Stroke; M: Male; F: Female. Color codes in heat maps: Red and Green - low and high protein abundances, respectively; Gray - no protein identification.