Rev Port Cardiol. 2016;35(5):265–273

Revista Portuguesa de Cardiologia
Portuguese Journal of Cardiology
www.revportcardiol.org

ORIGINAL ARTICLE

Proteomic analysis associated with coronary artery dilatation caused by Kawasaki disease using serum exosomes

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Received 15 August 2015; accepted 22 November 2015
Available online 23 April 2016

KEYWORDS
Kawasaki disease;
Coronary artery dilatation;
Exosome;
Proteomics

Abstract
Introduction: The aim of this study was to investigate the serum exosome proteome profile of coronary artery dilatation (CAD) caused by Kawasaki disease (KD).
Methods: Two-dimensional electrophoresis was implemented on proteins of serum exosomes obtained from children with CAD caused by KD and from healthy controls. Differentially expressed proteins were identified by matrix-assisted laser desorption/ionization time-of-flight/time-of-flight mass spectrometry analysis.
Results: We identified 38 differentially expressed proteins (13 up-regulated and 25 down-regulated) from serum exosomes of patients with CAD caused by KD compared with healthy controls. Expression levels of three differentially expressed proteins (leucine-rich alpha-2-glycoprotein, sex hormone-binding globulin, and serotransferrin) were validated using western blot analysis. Classification and protein-protein network analysis showed that they are associated with multiple functional groups involved in the acute inflammatory response, defense response, complement activation, humoral immune response, and response to wounding. The majority of the proteins are involved in the inflammation and coagulation cascades.
Conclusions: These findings establish a comprehensive proteome profile of CAD caused by KD and increase our knowledge of scientific insight into its mechanisms.

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http://dx.doi.org/10.1016/j.repc.2015.11.016

PALAVRAS-CHAVE
Doença de Kawasaki;
Dilatação da artéria coronária;
Exossoma;
Proteómica

Análise proteómica associada a dilatação da artéria coronária causada pela doença de Kawasaki com utilização de exossoma sérico

Resumo
Introdução: O objetivo deste estudo consistiu em investigar o perfil proteico do exossoma sérico da dilatação da artéria coronária (DAC) causada pela doença de Kawasaki (DK).
Métodos: Implementámos a tecnologia 2-DE nas proteínas do exossoma proveniente do soro de crianças com DAC causada pela DK e de controlos saudáveis. Foram identificadas proteínas com diferente expressão, analisadas através de espectrometria de massa com técnica de ionização e dessorção a laser assistida por matriz com análise de tempo de voo de aceleração.
Resultados: Identificámos 38 proteínas diferenciais (13 reguladas positivamente e 25 negativamente) do exossoma sérico da DAC causada pela DK comparadas com os controlos saudáveis. Os níveis de expressão de três proteínas diferenciais (glicoproteína alfa 2 rica em leucina, globulina ligada às hormonas sexuais, serotransferrina) foram validados com a utilização de *western blot*. A análise da classificação e a rede proteína-proteína mostraram que estão associadas a múltiplos grupos funcionais, incluindo a resposta inflamatória aguda, a resposta de defesa, a ativação do complemento, a resposta imune humoral e a resposta a lesões. A maioria das proteínas estava envolvida no processo da inflamação e cascata de coagulação.
Conclusões: Estes achados estabeleceram um perfil do proteoma abrangente da DAC causado pela DK e aumentam os nossos conhecimentos de base sobre a visão científica dos mecanismos da DAC causada pela DK.

Introduction

Kawasaki disease (KD) is an acute systemic vasculitis of children that presents with prolonged fever and mucocutaneous inflammation, including inflammation of the oral mucosa, non-exudative conjunctivitis, rash, extremity changes and cervical lymphadenopathy that is usually unilateral.[1,2] Delays in accurate diagnosis lead to increased mortality and morbidity from complications. In particular, without timely treatment, as many as 25% of patients may develop coronary artery dilatation or aneurysms, with associated risk of long-term morbidity or death. Importantly, no pathognomonic test exists for the early identification and diagnosis of KD. Therefore, it is crucial to understand the molecular pathogenesis of CAD in KD so as to improve diagnosis and therapy.

Exosomes are secreted by multiple cell types and are found in virtually all body fluids.[3] They are nano-sized, cell membrane-surrounded structures harboring a broad range of biomolecules, containing mRNAs, miRNAs and proteins linked to cell type-associated functions.[4] Previous reports have demonstrated that exosomes play an important role in cell-to-cell communication and influence both physiological and pathological processes.[5,6] The protein composition of exosomes can be investigated using mass spectrometry. This approach allows for an unbiased assessment of the proteome without the need for prior knowledge of protein identities.

In the present study, we employed two-dimensional electrophoresis (2-DE) and matrix-assisted laser desorption ionization (MALDI) time-of-flight mass spectrometry (TOF MS) to compare proteomes in serum exosomes from healthy children and KD patients with CAD, seeking to identify these pathophysiological alterations on a proteomic scale. To our knowledge, this is the first serum exosome proteomic analysis in the context of pathogenesis of CAD caused by KD, providing a comprehensive atlas of the serum exosome proteome of CAD in KD.

Methods

Preparation of serum samples

Ethical approval was obtained for human sample collection from the Ethics Committee at Guangzhou Women and Children's Medical Center (trial no. 077 2013), and written informed consent was obtained from all guardians. Blood samples from six KD patients with CAD were randomly selected according to the American Heart Association and the Japanese Ministry of Health and Welfare criteria. The patients underwent systematic examination in the hospital and were confirmed to have no other diseases. Blood samples from six healthy children were used as the control group. Blood samples were separated by centrifugation at 1000 *g* for 10 min. Serum aliquots were collected and stored at −80 °C.

ExoQuick precipitation of serum exosome

Exosomes were isolated from two pooled serum samples consisting of equal amounts of each of six experimental samples from KD patients and healthy children using ExoQuick precipitation (System Biosciences Inc, Mountain View, CA) following the manufacturer's instructions.[7,8]