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Transcriptome from circulating cells suggests dysregulated pathways associated with long-term recurrent events following first-time myocardial infarction

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ABSTRACT

Background: Whole-genome gene expression analysis has been successfully utilized to diagnose, prognosticate, and identify potential therapeutic targets for high-risk cardiovascular diseases. However, the feasibility of this approach to identify outcome-related genes and dysregulated pathways following first-time myocardial infarction (AMI) remains unknown and may offer a novel strategy to detect affected expressome networks that predict long-term outcome.

Methods and results: Whole-genome expression microarray on blood samples from normal cardiac function controls (n = 21) and first-time AMI patients (n = 31) within 48-hours post-MI revealed expected differential gene expression profiles enriched for inflammation and immune-response pathways. To determine molecular signatures at the time of AMI associated with long-term outcomes, transcriptional profiles from sub-groups of AMI patients with (n = 5) or without (n = 22) any recurrent events over an 18-month follow-up were compared. This analysis identified 559 differentially-expressed genes. Bioinformatic analysis of this differential gene-set for associated pathways revealed 1) increasing disease severity in AMI patients is associated with a decreased expression of genes involved in the developmental epithelial-to-mesenchymal transition pathway, and 2) modulation of cholesterol transport genes that include *ABCA1*, *CETP*, *APOA1*, and *LDLR* is associated with clinical outcome.

Conclusion: Differentially regulated genes and modulated pathways were identified that were associated with recurrent cardiovascular outcomes in first-time AMI patients. This cell-based approach for risk stratification in AMI could represent a novel, non-invasive platform to anticipate modifiable pathways and therapeutic targets to optimize long-term outcome for AMI patients and warrants further study to determine the role of metabolic remodeling and regenerative processes required for optimal outcomes.

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1. Introduction

Despite significant advances in pharmacotherapy, revascularization strategies, organ transplantation and cardiac rehabilitation algorithms, coronary heart disease remains the leading cause of death in adults over 35 years of age in the United States [1]. Assessment of classic cardiovascular risk factors – including hypertension, diabetes, and smoking – has a critical role in disease prevention and predicting outcomes but is not sufficient to fully predict risk of recurrent events [2–4]. Molecular markers such as BNP, CRP, and other serum inflammatory markers have gained increased attention in this regard but have only provided modest increases in predictive capacity, mandating the search

Abbreviations: AMI, acute myocardial infarction; EMT, epithelial-to-mesenchymal transition; *APOA1*, apolipoprotein A1; *LDLR*, low density lipoprotein receptor; *FGFR1*, fibroblast growth factor 1; *ACTA2*, smooth muscle alpha-actin; *EGF*, epidermal growth factor; *IL1β*, IL-1 beta; *CREB1*, cAMP responsive element binding protein 1; *VIM*, vimentin; *TGFBR*, TGF beta-receptor; *PKA*, protein kinase A; *ABCA1*, ATP binding cassette 1; *CETP*, cholesterylester transfer protein.

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for more sensitive disease markers to reveal initial dysregulation and underlying mechanisms of disease [5]. Such biomarkers could illuminate novel molecular deficiencies that may reveal the determinants for diagnostic platforms and motivate hypotheses for cell-based therapeutic targets to prevent disease progression.

Current cardiac biomarkers have been developed based on targeted physiological studies of known inflammation and homeostasis pathways. As a result, these biomarkers provide information correlated with what is already known or being measured, limiting their contribution to increasing predictive value of current models. In contrast, emerging technologies are beginning to allow the systematic, unbiased characterization of variation in genes, RNA, proteins and metabolites associated with disease conditions and disease outcomes [6]. Gene expression profiling by microarray offers a comprehensive tool to interrogate underlying mechanisms of disease and to identify disease associated genes and dysregulated pathways that may not have been previously linked to cardiovascular diseases [7]. Indeed, application of tissue-based microarray gene expression profiling in a number of cardiomyopathies has helped improve classification of ischemic vs. nonischemic etiologies [8–10], predict outcomes in non-ischemic cardiomyopathy [11], and identify novel pathogenic mechanisms in giant cell myocarditis [12]. However, utility of blood-based whole-genome gene expression to interrogate disease pathogenesis and disease progression in patients following first-time myocardial infarction was previously uncharted.

Initial studies in animal models of myocardial infarction demonstrated that microarray platforms are sufficient to identify both established and novel molecular mechanisms of disease [13,14]. Translation of this approach to patients, however, has been limited by lack of myocardial tissue samples from patients with an acute event. Recently, evidence for the utility of whole blood analysis as a "sentinel" of disease was demonstrated [15]. Using microarray and expressed sequence tags, comparison of the transcriptome of blood with genes expressed in nine different human tissue types, including the heart, revealed that expression of over 80% was shared with any given tissue. Moreover, environmental conditions affecting transcriptional regulation of insulin were detected in the peripheral blood, suggesting that circulating cells may serve as a convenient surrogate for interrogation of other tissue types [15]. Therefore, the practical and cost-effective microarray platform may provide meaningful analysis of patients with acute myocardial infarction (AMI) that are vulnerable to a pleotrophic range of disease mechanisms including myocardial ischemia, plaque rupture, and inflammatory response.

We herein hypothesized that a whole-genome microarray transcriptional analysis using circulating cells as a "surrogate tissue" could identify novel genes and dysregulated pathways associated with disease pathogenesis and progression in a prospective cohort of first-time AMI patients. This comprehensive screen of the transcriptome yielded specific gene expression changes at the time of the initial event between clinically indiscernible first-time AMI patients that developed long-term complications from those that did not. Moreover, the modulated pathways enriched within this dataset revealed both established and novel mechanisms of disease severity and long-term outcome. These findings provide evidence for the role of whole-blood microarray gene-expression profiling as a non-invasive strategy in acute myocardial infarction to anticipate recurrent disease as a function of dysregulated pathways. Furthermore, these data foster novel hypotheses for possible regenerative therapeutic targets to optimize treatment options for ischemic cardiovascular patients at risk for developing clinical complications due to underlying cellular dysfunction.

2. Methods

2.1. Patient population and follow up

This study was approved by the Mayo Clinic Rochester Institutional Review Board. The study samples consisted of whole blood collected from first-time AMI patients within 48-hours post-MI and controls with a normal echocardiogram. Patients that were previously enrolled in a cardiac rehabilitation program, had history of cardiovascular disease, or had clinical or biochemical evidence of other comorbidities such as cancer, rheumatoid arthritis, liver disease, myeloproliferative disorders or were unable to provide consent were excluded. Controls were recruited from the Mayo Clinic Rochester echocardiography laboratory and were frequency matched by age and sex to AMI subjects. Controls had no previous history of cardiac diseases or other comorbidities as listed above, and had a normal echocardiogram.

Blood samples from 52 patients (31 AMI and 21 controls) were analyzed for transcriptome analysis. All participants provided a signed informed consent. A chart review of all AMI patients' records was conducted at 18 months following index event to determine incidence of adverse recurrent events, defined as recurrent myocardial infarction, revascularization, evidence of restenosis, hospitalization for unstable angina or heart failure, cardiovascular death, stroke or transient ischemic attack, or amputation due to peripheral vascular disease.

2.2. Microarray experiment

There were a total of 52 samples (31 AMI and 21 controls) available for transcriptome analysis. Blood samples were collected in EDTA tubes within 48-hours of AMI or following recruitment into the study. Nucleated cells were fractionated from 5 mL of heparinized blood. Total RNA was extracted from cell populations using a combination of gDNA Eliminator and RNeasy columns (Qiagen, Valencia, CA) and was assessed for quality and quantification using Agilent bioanalzyer and OD260/OD280 ratio. Biotinylated cRNA was prepared according to the standard Affymetrix protocol from 100 ng total RNA. Following fragmentation, 120 ug of cRNA was hybridized for 16 hours at 45 °C on Affymetrix GeneChip Human Genome U133 Plus 2.0, which includes 54,675 probe sets (http://www.affymetrix.com/estore/), with one sample per array. GeneChips were washed and stained in the Affymetrix Fluidics Station 450 and subsequently scanned using the GeneChip Scanner 3000 7G.

2.3. Statistical analysis of microarray data

Raw microarray image data were analyzed using several statistical R/Bioconductor packages and customized R scripts for quality assessment (QA) and quality control (QC), background correction, and normalization across arrays [16–19]. QA/QC process included the assessment of the raw microarray images, MA plot, normalized unscaled standard error, residual images from the RMA model, relative log expression, RNA degradation based on all the probes on microarray using our own R scripts and R/Bioconductor packages [16,20,21]. Standard Affymetrix quality metrics were also assessed, such as 3'/5' ratios, background, scaling factor, control probes, GAPDH, and percent present (PP) calls.

Gene filtering was performed using R genefilter package [22]. Differential analysis was performed using empirical Bayesian method implemented in R limma package [23] with FDR control at 0.05 and fold change of 1.2 from AMI patients and control subjects. Due to the limited number of AMI patients with recurrent events, differential analysis between AMI patients with a recurrent event (events groups) and those that were event free (no events group) at 18 months was conducted to determine all genes with a fold change of 1.2 or greater before FDR control. The raw microarray data (.CEL files), processed gene expression matrix and sample information are deposited to NCBI Gene Expression Omnibus (GEO) database with accession number GSE48060 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE48060).

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