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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.

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COMPETING INTERESTS

The authors of this manuscript do not have any relevant financial, personal, or professional conflicts of interests to disclose.

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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.

Keywords

acute myocardial infarction; blood; microarray; transcriptome; pathway analysis

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 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 pleiotropic 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, this data fosters 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 bioanalyzer and OD260/OD280 ratio. Biotinylated cRNA was prepared according to the standard Affymetrix protocol from 100ng total RNA. Following fragmentation, 120ug of cRNA was hybridized for 16 hours at 45°C on Affymetrix GeneChip Human Genome U133 Plus 2.0, which includes 54,675 probsets (<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 was 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>).

2.4. Enrichment analysis

Transcriptional differential expression profiles from circulating cells were functionally analyzed using the GeneGo Metacore pathway analysis software. Intersecting gene lists consisting of concordantly up- or downregulated transcripts were subsequently analyzed. Based on the curated Metacore database and using the hypergeometric distribution test, overrepresented functions and pathways associated with intersecting gene lists were identified and the significance of association (p-value) was calculated based on hypergeometric distribution.

2.5. Univariate statistical analysis of clinical characteristics

Comparisons of baseline demographic, clinical and laboratory characteristics were conducted using the Mann-Whitney-U test or Pearson's chi-square test for continuous and binary categorical variables, respectively. All hypothesis tests were conducted with a type I error rate of 0.05. Analyses were conducted using JMP 9 software (version 9.0.0.).

3. RESULTS

3.1. Patients' baseline demographic and clinical characteristics

Clinical characteristics of the 21 control subjects and 31 AMI patients whose samples were used for genome-wide gene expression microarray experiments are described in Table 1. As expected, there were significant differences in known clinical risk factors for CAD between control subjects and AMI patients that included BMI (27.2 vs. 32.3 kg/m², p = 0.001), CVRF score (3 vs. 4, p = 0.001), positive history of arterial hypertension (26 vs. 74%, p = 0.001), and history of smoking (7 vs. 21, p = 0.015). In addition, controls had higher left ventricular ejection fractions than AMI patients (64 vs. 57%, p=0.007). However, other risk factors such as total, HDL and LDL cholesterol, diabetes mellitus, family history of CAD and drug therapy were not different between the two groups. These findings indicate some baseline differences between AMI patients and controls as would be expected of patients suffering an acute coronary event compared to those without known heart disease.

3.2. Transcriptome of circulating cells reflects inflammatory response to AMI

To establish the feasibility of identifying differentially expressed gene patterns in circulating cells following myocardial infarction, we conducted a whole-genome microarray analysis of circulating cells from AMI patients and controls. Clustering dendrogram and PCA analysis did not identify significant global differences between groups on visual inspection (Figure 1A&B). However, differential analyses using empirical Bayesian method (R limma package) identified 158 probesets divergently expressed between controls and AMI patients that had a fold change > 1.2 or < -1.2 and met a false discovery rate cut off of 5% (Figure 1C). Functional analysis of this differential gene set using the MetaCore GeneGo platform revealed enrichment of apoptosis, immune response and blood coagulation pathways including granzyme B signaling, CD16 signaling in NK cells, G-protein coupled receptors in platelet aggregation, IL-12 signaling pathway, and IL-15 signaling via JAK-STAT cascade (Figure 1D). Consistent with pathological changes following ischemic cardiac injury, four of the top five pathways enriched in this dataset were associated with inflammation and immune response. These findings provide evidence that profiling of circulating cells in

patients following acute ischemic myocardial injury is capable of identifying distinct, biologically relevant gene expression changes and modulated networks and pathways.

Subset of AMI patients suffered a recurrent adverse cardiovascular event within 18 months following initial AMI despite an indistinguishable clinical phenotype from those without events—A total of 27 patients of the original 31 AMI patients had follow-up data. Four patients were excluded from this secondary analysis as they did not complete follow-up at 18 months. Of these 27, 5 patients had recurrent cardiovascular events (Figure 2). All five patients presented with anginal pain and were found to have significant restenosis of one or more coronaries and underwent percutaneous coronary intervention at a later clinical appointment. None of the patients developed more serious complications such as second myocardial infarction, stroke, amputation for peripheral vascular disease, or hospitalization for heart failure; highlighting the mild to moderate severity of the disease recurrence for patients with first-time myocardial infarction in this cohort. Analysis of the clinical characteristics of these AMI patients in comparison to those that did not have a documented second event in the 18-month follow-up period revealed that patients with complications were much more likely to have been on beta blockers prior to the AMI (80 vs. 22%, $p=0.014$; Table 2). In contrast, there were no differences in classical cardiovascular risk factors or in severity of MI as assessed by ejection fraction, peak troponins or prevalence of STEMI versus NSTEMI. These findings highlight a cohort of first-time AMI patients predisposed to recurrent long-term events but without distinguishable clinical characteristics at baseline that would enable meaningful prognostic stratification from amongst all first-time AMI patients.

3.3. Baseline circulating cell transcriptome identifies differential gene expression pattern distinguishing AMI patients with or without recurrent cardiovascular events

Based on this prospectively identified cohort of AMI patients with and without recurrent events at 18 months that at baseline were clinically indistinguishable, a secondary analysis was conducted to determine if a unique expressome signature could be detected between these two groups. Hierarchical clustering and principle component analysis of circulating cell transcriptome profiling did not distinguish between the two sub-groups (Figure 3A&B). Application of empirical Bayesian method identified 559 genes that were divergently expressed between AMI patients with or without recurrent events (Figure 4). This suggests that sub-group analysis based on 18-month follow-up within a cohort with indistinguishable initial clinical presentation may provide a sensitive means of identifying dysregulated pathways and genes underlying poor outcome following AMI.

Gene expression dysregulation in patients with recurrent events following AMI is enriched for cholesterol transport and epithelial to mesenchymal transition pathways—GeneGo pathway and network analysis tools were used to interrogate the functional significance of the 559 genes divergently expressed between patients with and without recurrent events at the completion of an 18-month follow-up. An integrated representation of this data is shown in figure 4 with the official gene symbols for the 559 genes listed in the outermost circle followed by each gene's statistical significance (stars), gene expression profile in no events (outer heatmap for 22 samples) and events

(inner heatmap for 5 samples), top 5 enriched pathways (gray circles with color bars for genes from the data set found in those pathways), hub genes (gene names in red have 10 interactions with other genes in the dataset), and gene-gene interactions (with circle size proportional to the number of interactions). This circle plot illustrates the interconnected interactome of the prioritized gene list.

Functional analyses revealed epithelial-to-mesenchymal transition (EMT) and intracellular cholesterol transport as the most statistically significant dysregulated pathways in event and no event AMI patients at baseline. Genes within these pathways were predominantly downregulated in the event group compared to the no event group when comparing initial blood transcriptomes retrospectively. Genes including apolipoprotein A1 (*APOA1*), low density lipoprotein receptor (*LDLR*), fibroblast growth factor 1 (*FGFR1*) and smooth muscle alpha-actin (*ACTA2*) showed increased expression in patients with recurrent events compared to those without recurrent events at baseline. Examination of individual genes within the EMT regulation and induction pathways showed network hub genes, including epidermal growth factor (*EGF*), IL-1 beta (*IL1 β*), cAMP responsive element binding protein 1 (*CREB1*), Vimentin (*VIM*) and *FGFR1*, that have at least 10 known gene-gene interactions with other genes in the differentially expressed dataset. Moreover, these genes, including *VIM* and *CREB1* also overlapped with the *TGF* beta-receptor (*TGFBR*) and protein kinase A (*PKA*) signaling pathways. Modulation of *TGFBR* expression, although not identified as a hub gene, was also a recognized driver of the EMT pathways. Despite conducting a broad, whole-genome screening of cells in the peripheral circulation, we identified specific dysregulated pathways which reaffirm suspected pathological mechanisms and highlight novel disease causing regulatory changes in AMI patients at this initial clinical presentation that went on to develop recurrent cardiovascular events.

3.4. Increasing severity of AMI is associated with disruption of cholesterol transport characterized by distinct patterns of gene expression dysregulation

Abnormalities in cholesterol metabolism are thought to play an important role in the formation and progression of atherosclerotic plaques [24]. A closer examination of the genes constituting the cholesterol transport pathway from our dataset revealed three distinct gene expression patterns when AMI patients with or without recurrent events and control subjects were compared at time of diagnosis (Figure 5). Apolipoprotein A1, a primary constituent of high density lipoprotein and critical for reverse cholesterol transport, as well as the low density lipoprotein receptor, a ubiquitous receptor required for low density lipoprotein (LDL) uptake in the liver and regulation of serum LDL levels, were upregulated in patients with recurrent events compared to those without recurrent events or when compared to individuals in the normal control cohort. In contrast, ATP binding cassette 1 (*ABCA1*) and cholesterylester transfer protein (*CETP*), which enable loading and unloading of cholesterol from high density lipoprotein (HDL), respectively, were downregulated in the transcriptome of patients that subsequently developed recurrent events. Additionally, genes such as disabled homolog 2 (*DAB2*) and calveolin 2 (*CAV2*) demonstrated a dose response relationship between the three groups that ranged from controls, AMI patients without recurrent events, to AMI patients with clinically significant recurrent events in 18-months (Figure 5). Increasing disease severity in myocardial infarction is associated with disruptions

in the cholesterol transport pathway at the time of first-time AMI presentation and is characterized by three distinct patterns of gene expression dysregulation.

4. DISCUSSION

A comprehensive whole-genome screen of the transcriptome has previously suggested that disease-specific mechanisms are distinguishable and may offer diagnostic and prognostic value [8–12]. Herein, we compared circulating cell transcriptome profiles from healthy controls and first-time AMI patients to demonstrate the sensitivity of cell-based gene expressome analysis, which revealed the anticipated modulation of inflammation and immune response networks and pathways in patients following acute myocardial injury. Unique to this application was the interrogation of dysregulated pathways underlying poor outcome in a prospective cohort of AMI patients at 18-month follow-up compared to initially indistinguishable patients that did not develop recurrent events during the follow-up period. This analysis revealed specific disruption of intracellular cholesterol transport and epithelial-to-mesenchymal-transition at the time of the event that was associated with first-time AMI patients developing additional cardiovascular events. These findings indicate that cell-based gene expression profiling at initial presentation is a sensitive, non-invasive platform and suggests that differential gene analysis may be sufficient to anticipate molecular dysregulation that are associated with high-risk patients affected by ischemic heart disease.

In order to assess the feasibility and sensitivity of divergent gene expression profiling of circulating cells we initially conducted a comprehensive, whole-genome microarray analysis of the blood of a carefully selected cohort of young, first-time AMI patients without severe co-morbidities who underwent percutaneous coronary intervention and healthy controls. Our analysis revealed a divergent gene expression of 158 probesets that were functionally enriched for inflammation, immune response and apoptosis networks and pathways in AMI patients compared to matched, healthy controls. These findings are consistent with myocardial tissue-based microarray studies of animal models of ischemia and reperfusion injury which also demonstrated divergent gene expression profiles enriched for immune response, inflammation, and apoptosis pathways, raising the possibility that inflammatory transcriptional changes in circulating cells may be a reflection of myocardial pathological changes. Indeed, targeted gene expression studies of the blood transcriptome in AMI patients have shown that following myocardial infarction modulation of leukocyte transcriptional machinery and over-expression of inflammatory markers by circulating leukocytes occurs independent of patient risk factors or plasma levels of inflammatory marker CRP, providing further evidence that myocardial ischemia results in transcriptional gene expression changes in the peripheral blood [25, 26]. In this context, microarray gene expression profiling of peripheral blood coupled with enrichment analysis techniques as demonstrated here appears to provide a feasible and non-invasive approach for identifying divergent, biologically relevant gene expression profiles, networks and pathways from circulating cells at the time of acute myocardial infarction.

Notably, the sub-group analysis of AMI patients with or without recurrent events at 18-month follow-up was particularly sensitive at distinguishing differential gene expression

patterns, identifying over 550 differentially expressed genes. Enrichment analysis of the divergent gene expression between these two cohorts at clinical presentation revealed that intracellular cholesterol transport pathway, critical for normal cholesterol metabolism, was prioritized amongst this dataset. Moreover, distinct patterns of gene expression changes within the cholesterol transport pathway within circulating cells were associated with increasing severity of disease upon follow-up. Knockout of *ABCA1*, a cellular lipid transport protein that was down regulated in AMI patients with a recurrent event, is associated with decreased cholesterol efflux from macrophages and decreased levels of HDL [27, 28]. In contrast, decreased expression of *CETP* as seen here in patients with recurrent events compared to the other two cohorts is associated with decreased risk of ischemic disease [29]. Disequilibrium of gene expression between specific pre-disposing and protective factors therefore appears to underlie dysregulation of cholesterol transport. The findings identified through a whole-genome screening of the peripheral blood transcriptome, were consistent with the established role of cholesterol metabolism dysregulation in acute coronary syndrome. This supports the utility of large-scale gene expression analysis of the blood transcriptome at initial diagnosis of AMI as a sensitive platform for identification of relevant mechanisms of disease progression to assist in risk stratification.

Epithelial to mesenchymal transition describes reversible changes in gene expression and cell structure that lead to a transition from an epithelial to mesenchymal phenotype and is a process naturally involved in embryogenesis and tissue repair and regeneration, and is frequently hijacked during neoplastic transformation [30]. In this study, three of the five most highly enriched pathways identified including EMT regulation, *TGFB*-dependent induction of EMT, and *TGFBR* signaling were associated with EMT and were primarily down regulated within circulating cells at clinical presentation in patients with recurrent AMI. Amongst these pathways, modulation of *TGFBR* expression was the common link. It has recently been demonstrated that EMT occurs in the post-MI epicardium, serving as a primary source of fibroblasts, myofibroblasts, endothelial cells and smooth muscle cells that arise in response to tissue injury and suggests an active role for EMT in myocardial repair and regeneration [31]. This EMT response following MI appears to be driven in part by *TGFB* signaling, an important regulator of EMT during cardiac development, and recently implicated as a modulator of fibroblast formation from endothelium and a driver of post-natal cardiac fibrosis [32, 33]. Moreover, a recent transcriptional screening of peripheral blood in AMI patients identified *TGFBR* as a prognostic marker of LV dysfunction at 4 months [34]. In this context, our findings provide further evidence for the role of *TGFB* signaling mediated EMT in cardiac remodeling and poor outcome following AMI, suggesting a central role for the regenerative response in determining the trajectory of disease progression.

Our findings also reveal that *TGFBR* dysregulation in AMI does not occur in isolation. Inspection of the gene-gene interaction and enrichment data as highlighted in a circle plot, a novel graphic display method used to integrate and visualize the relationship between heat map, revealed that the significance of the 3 EMT pathways is driven by a handful of hub-genes that were shared between pathways and include *EGF*, *IL1B*, *CREB1*, *VIM* and *FGFR1*. Genes such as *FGFR1* and *IL1B* are known to play an important role in positive and

negative regulation of epicardial EMT [35]. Moreover, our data suggest that the remaining genes, that have yet to be associated with epicardial EMT, may also be involved in the regulation of cardiac regeneration following AMI. These findings provide preliminary evidence that impairment of EMT regulation and induction of transforming growth factor receptor signaling during myocardial infarction characterize a mal-adaptive response that predisposes these individuals to a poor outcome. Moreover, specific dysregulation of several critical hub-genes appears to drive changes in EMT impairment, providing meaningful targets for future study.

While AMI patients with and without recurrent events were clinically indistinguishable at baseline based on traditional risk factors, AMI patients with recurrent events had significantly higher beta blocker use prior to MI. Amongst patients with recurrent events, all patients were on beta blockers for anti-hypertensive therapy prior to AMI. Although not a first-line agent for treatment of systemic, arterial hypertension, beta blockers are commonly used due to significant variation in treatment response between antihypertensive agents for a given patient. Therefore, it is possible that differences in beta blocker use represent a sampling bias, especially given the small sample size of the recurrent events groups. On the other hand, the difference may be a reflection of the increased prevalence of hypertension, as MI patients with recurrent events all had hypertension prior to MI while only 60% of those without events were hypertensive at the time of AMI. Although not a statistically significant difference, this suggests a worse cardiovascular risk profile and an important risk factor for disease progression amongst patients with recurrent events. Moreover, given the small sample size, larger and focused confirmatory studies are required to validate these findings and to examine the specificity of these gene expression changes for recurrent adverse events following AMI.

5. CONCLUSION

In summary, circulating cells as a 'surrogate tissue' in AMI patients for whole-genome transcriptome profiling at clinical presentation is sufficient to identify differentially regulated genes and modulated pathways that may predict recurrent cardiovascular outcomes. This feasible strategy may offer a new technology to stratify the long-term risk for patients after a first-time myocardial infarction based on inherent mechanisms of cellular dysfunction. This pilot study warrants further investigation with larger cohorts in the setting of coronary heart disease to determine the generalizability along with the sensitivity and specificity of this unbiased systematic expressome analysis based on blood-derived transcriptome profiling. Furthermore, this approach may represent a novel, non-invasive platform to anticipate potentially modifiable pathways and possible regenerative therapeutic targets to optimize the cardiovascular outcome for patients after first-time myocardial infarction.

Abbreviations

AMI	acute myocardial infarction
EMT	epithelial-to-mesenchymal transition

<i>APOA1</i>	apolipoprotein A1
<i>LDLR</i>	low density lipoprotein receptor
<i>FGFR1</i>	fibroblast growth factor 1
<i>ACTA2</i>	smooth muscle alpha-actin
<i>EGF</i>	epidermal growth factor
<i>IL1β</i>	IL-1 beta
<i>CREB1</i>	cAMP responsive element binding protein 1
<i>VIM</i>	Vimentin
<i>TGFBR</i>	TGF beta-receptor
<i>PKA</i>	protein kinase A
<i>ABCA1</i>	ATP binding cassette 1
<i>CETP</i>	cholesterylester transfer protein

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Highlights

- Blood transcriptome post-MI reveals modulation of immune response and inflammation pathways
- MI patients with recurrent events have a distinguishable expressome profile at baseline
- Cholesterol transport and TGF-beta signaling dysregulation is associated with recurrent events
- Cholesterol transport pathway expression profiles are associated with disease severity
- Blood transcriptome can detect dysregulated expressomes distinguishing poor outcome post-MI

Fig. 1. Transcriptome of circulating cells reflects inflammatory response to AMI
Principal component analysis (A), hierarchical clustering dendrogram (B), and heat map (C) are based on the whole-genome expression profile between 31 AMI patients (orange) and 21 control subjects (blue). The top 5 most enriched gene ontology processes, process networks and pathways (D) amongst the differentially expressed genes shown in the heat map in panel C. Columns of heat map represent individual samples with AMI patients on left-hand side and controls on right-hand side while rows represent all differentially expressed probe-sets. Heat map color gradient represents relative expression, with up-regulated genes shown in orange and down-regulated genes in blue. Abbreviations: ESR-1, estrogen receptor 1; GPCR, G-protein coupled receptor

Fig. 2. Subset of first-time AMI patients suffered an adverse cardiovascular event within 18-months following initial AMI

Abbreviations: MI, myocardial infarction; PCI, percutaneous coronary intervention; TIA, transient ischemic attack; PVD, peripheral vascular disease; UA, unstable angina; CHF, congestive heart failure

Fig. 3. Circulating cell transcriptome analysis identifies differentially expressed genes between AMI patients with or without recurrent cardiovascular events
Principal component analysis (A) and hierarchical clustering dendrogram (B) based on the whole-genome expression profiles between AMI patients with (n=5, red) and without (n=22, green) an adverse recurrent event at 18 months.

Fig. 4. Comprehensive analysis of circulating cell transcriptome identifies dysregulated pathways in patients with recurrent events after AMI

A circle plot integrating the gene expression profiles, pathway enrichment analysis, and gene interactome information for the 559 differentially expressed genes between AMI patients with and without recurrent events at 18 month follow-up. Gene symbols are listed in the outermost circle followed by statistical significance (stars), gene expression profile in no events (outer heatmap for 22 samples) and events (inner heatmap for 5 samples), top 5 enriched pathways (gray circles with color bars for genes from the data set found in those pathways), hub genes (gene names in red have 10 interactions with other genes in the dataset), and gene-gene interactions (with circle size proportional to the number of interactions). The abbreviated pathway titles stand for regulation of epithelial-to-mesenchymal transition (EMT), intracellular cholesterol transport, TGF-beta-dependent induction of EMT, TGF-beta receptor signaling, PKA signaling as listed from inner to outer circle in the legend provided below the plot.

Fig. 5. In patients with recurrent event after AMI, dysregulation of cholesterol transport is characterized by upregulation of *APOA1* and *LDLR* and downregulation of *ABCA1* and *CETP* Of the 559 divergently expressed genes between AMI patients with (AMIe; n=5) and without (AMIne; n=22) an adverse recurrent at 18-month follow-up, gene expression of the top six that constituted the canonical intracellular cholesterol transport pathway as identified by enrichment analysis on MetaCore GeneGO platform are shown. Gene expression in controls is shown for comparison (CTRL, n=21). Outlier box plots have been overlaid to show the distribution of the data.

Table 1

Baseline clinical characteristics of all subjects with whole-genome blood gene expression microarray analysis

Variable ^a	Control (n=21)	AMI (n=31)	p-value ^b
Age, years	56 (33–65)	53 (38–65)	0.575
Sex, male, n (%)	14 (67)	18 (58)	0.532
Body mass index, kg/m ²	27.2 (21.7–35.0)	32.3 (22.9–49.5)	0.001*
Cardiovascular risk factor score	3 (2–6)	4 (1–6)	0.001*
Cardiovascular history, n (%)			
Arterial hypertension	6 (26)	23 (74)	0.001*
Smoking	7 (33)	21 (68)	0.015*
Diabetes mellitus	1 (5)	4 (13)	0.329
Family history of coronary artery disease	15 (71)	18 (58)	0.326
Lipid Profile, mg/dL			
Total cholesterol	190 (136–328)	189 (110–325)	0.859
LDL cholesterol	110 (41–400)	110 (41–254)	0.678
HDL cholesterol	41 (27–113)	37 (24–172)	0.278
Medications, n (%)			
Statin therapy	6 (29)	10 (33)	0.718
Aspirin	7 (33)	17 (55)	0.127
ACE inhibitor	3 (14)	6 (20)	0.598
Beta blocker	6 (29)	11 (35)	0.602
Severity of AMI			
Ejection fraction, %	64 (57–70)	57 (35–71)	0.007*
Troponin ^c		0.53 (0.04–16.43)	-
NSTEMI/STEMI, n/n ^d	-	19/12	-

Abbreviations: AMI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST elevation myocardial infarction

^a All continuous variables are reported as median (range)^b p-values < 0.05 were considered statistically significant and are indicated by an asterisk (*)^c Troponins were not collected for control subjects.^d Diagnosis of NSTEMI/STEMI is not applicable to control subjects

Table 2

Baseline clinical characteristics of AMI patients with or without recurrent events following initial AMI that underwent whole-genome blood gene expression microarray analysis

Variable ^a	Event (n=5)	No event (n=22)	p-value ^b
Age, years	51 (41–53)	56.5 (48–65)	0.110
Sex, male, n (%)	3 (60)	13 (59)	0.972
Body mass index, kg/m ²	36.5 (25.0–46.5)	31.5 (22.9–48.4)	0.140
Cardiovascular risk factor score	5 (4–6)	4 (1–6)	0.266
Cardiovascular history, n (%)			
Arterial hypertension	5 (100)	13 (59)	0.080
Smoking	2 (40)	16 (73)	0.161
Diabetes mellitus	0 (0)	3 (14)	0.381
Family history of coronary artery disease	5 (100)	12 (55)	0.057
Lipid Profile, mg/dL			
Total cholesterol	128 (110–219)	191 (126–325)	0.190
LDL cholesterol	65 (41–152)	115 (70–254)	0.169
HDL cholesterol	31 (25–47)	38 (25–72)	0.165
Medications, n (%)			
Statin therapy	3 (60)	7 (33)	0.271
Aspirin	4 (80)	12 (55)	0.296
ACE inhibitor	1 (20)	4 (19)	0.961
Beta blocker	4 (80)	5 (22)	0.014*
Severity of AMI			
Ejection fraction, %	55 (43–65)	57 (35–71)	0.240
Troponin ^c	2.24 (0.11–9.51)	0.47 (0.04–16.43)	0.142
STEMI, n (%) ^d	2 (40)	7 (32)	0.726

Abbreviations: AMI, myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; STEMI, ST elevation myocardial infarction

^a All continuous variables are reported as median (range)

^b p-values < 0.05 were considered statistically significant and are indicated by an asterisk (*)

^c Troponins were not collected for control subjects.

^d Diagnosis of NSTEMI/STEMI is not applicable to control subjects