Bioinformatics Analyses Identified Phase-Specific Heart Biomarkers and Blood Surrogate Markers for a Mouse Model of Viral Myocarditis

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Abstract

[Objective] Myocarditis is an inflammatory disease of the heart. The discovery of myocarditis in 1 to 9% of autopsies showed that myocarditis is an underdiagnosed cause of sudden death. Infection with viruses, particularly picornavirus, is a major cause of myocarditis. Viral myocarditis has been proposed to be divided into three phases, acute, subacute, and chronic. Although each phase requires specified treatment, single biomarker that can distinguish the three phases does not exist. We aimed to identify 1) phase-specific heart biomarkers and 2) blood surrogate markers that reflect changes in the heart, using a novel mouse model of myocarditis.

[Materials and Methods] We infected mice with Theiler's murine encephalomyelitis virus (TMEV), which belongs to the genus *Cardiovirus*, family *Picornaviridae*, to induce myocarditis. We conducted bioinformatics analyses, using microarray transcriptome data of heart and blood samples from TMEV-infected mice at days 4 (acute), 7 (subacute), and 60 (chronic) post infection.

[Results] Principal component analysis (PCA) of heart transcriptome data separated clearly between the three phases. Innate and acquired immunity-related genes, such as natural killer cell-related gene (*Nkg7*) and T cell-related gene (*Cd3g*), contributed to the separation between acute and subacute phases, while cardiac remodeling-related genes, such as matrix metallopeptidase (*Mmp12*) and osteoactivin (*Gpnmb*), contributed to the separation between subacute and chronic phases. Pattern matching analysis between brain PCA and blood transcriptome data identified blood surrogate marker candidates, such as interferon-stimulated genes (*Irf7*; $r = 0.97$) in acute phase.

[Discussion] Future translational application of this approach will lead to discovery of not only phase-specific markers in the heart, but also the biomarkers in the periphery (surrogate markers), which will be useful to diagnose myocarditis without heart biopsy.

Introduction

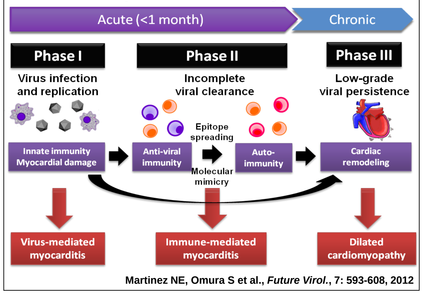
Theiler's murine encephalomyelitis virus (TMEV)

- Non-enveloped, single-stranded RNA virus, the genus *Cardiovirus*, family *Picornaviridae*
- Can cause myocarditis in susceptible mice
- Pathogenesis of TMEV-induced myocarditis is unclear

Myocarditis

- Inflammatory disease of the heart
- Estimated prevalence is 1%
- Can result in sudden death (20%) or dilated cardiomyopathy
- Viral infections are major causes of myocarditis (70% of patients)

Three phases of viral myocarditis



Martinez NE, Omura S et al., *Future Virol.*, 7: 593-608, 2012

Conventional markers for viral myocarditis

	Day 4 (Phase I)	Day 7 (Phase II)	Day 60 (Phase III)
Echocardiography	–	+	+
Serum troponin I	+	++	–
Viral replication	++	+	–

–: undetectable, +: above the sensitivity limit, ++: moderately positive

Materials and Methods

C3H/HeN mice

Intraperitoneal infection of TMEV (2×10^7 PFUs)

Heart and blood samples

↓

Microarray analysis

↓

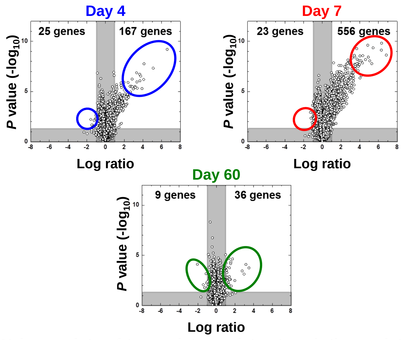
Bioinformatics

Microarray analysis: Microarray analysis was conducted, using GeneChip Mouse Gene 1.0ST Arrays (Affymetrix). Data were normalized by Robust Multi-array Average.

Bioinformatics analyses: Bioinformatics analyses were conducted, using a software 'R' version 3.3.2 and the packages: 'gplots' and 'genefilter' for heat map, 'cclust' for *k*-means clustering, and 'prcomp' for principal component analysis (PCA) (Omura et al., 2014). A radar chart was drawn, using Microsoft Excel. Pattern matching analysis was also conducted, using R.

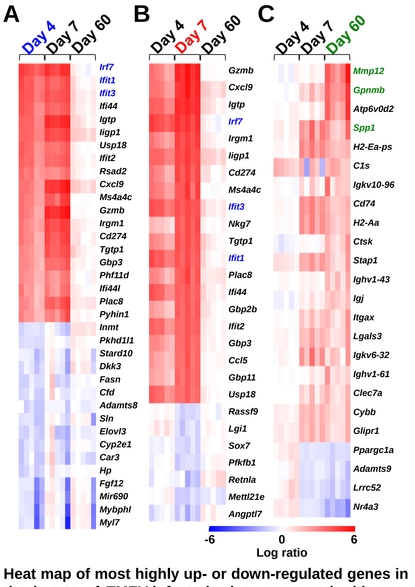
Results

Volcano plot visualizes cardiac gene expression changes



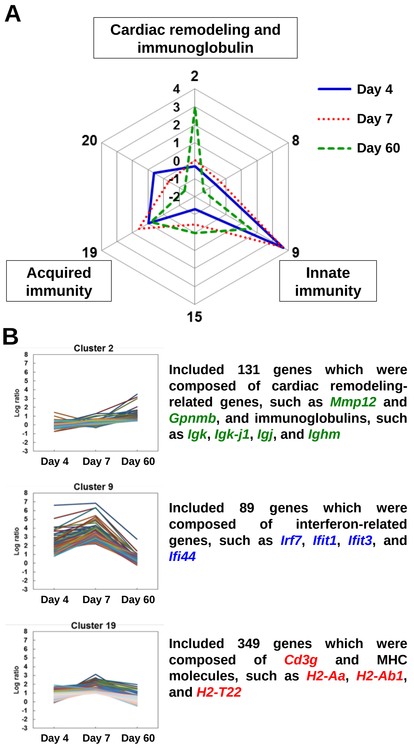
Volcano plots of transcriptome data at each time point. White areas indicated that the expression ratios were less than 0.5-fold or more than 2-fold and *P* values were less than 0.05. The number of upregulated genes was increased on day 7 compared with day 4 and decreased on day 60.

Heat map shows the gene expression differences between the phases



Heat map of most highly up- or down-regulated genes in the hearts of TMEV-infected mice, compared with age-matched control mice based on the microarray data from days 4 (A), 7 (B), and 60 (C). Each column represents the data from one mouse. On days 4 and 7, innate immunity-related genes, such as *Irf7*, *Ifit1*, and *Ifit3*, were upregulated, while cardiac remodeling-related genes, such as *Mmp12*, *Gpnmb* (osteoactivin), and *Spp1* (osteopontin), were upregulated on day 60. Heat map showed similar patterns between days 4 and 7 as well as days 7 and 60, indicating that heat map could not separate the samples clearly.

K-means clustering shows the distinct expression patterns among the genes



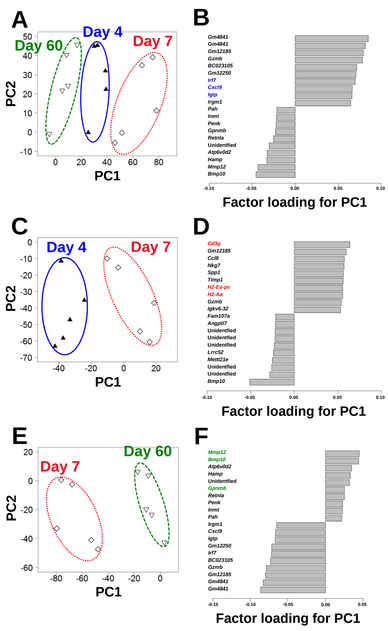
Included 131 genes which were composed of cardiac remodeling-related genes, such as *Mmp12* and *Gpnmb*, and immunoglobulins, such as *Igk*, *Igk-j1*, *Igj*, and *Ighm*

Included 89 genes which were composed of interferon-related genes, such as *Irf7*, *Ifit1*, *Ifit3*, and *Ifi44*

Included 349 genes which were composed of *Cd3g* and MHC molecules, such as *H2-Aa*, *H2-Ab1*, and *H2-T22*

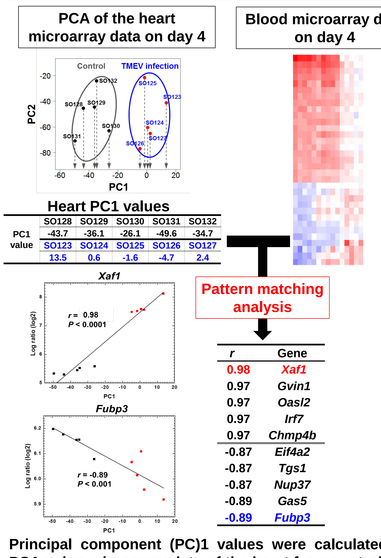
K-means clustering of heart transcriptome data. Among 20 clusters, 6 clusters showed significant expression changes. A) A radar chart of 6 clusters. B) Expression patterns of 3 clusters in which genes were upregulated.

Principal component analysis (PCA) separates the heart microarray data between the phases



A,B) PCA of the heart transcriptome data from days 4, 7, and 60. Innate immunity-related genes, such as *Irf7*, *Cxcl9*, and *Igtp*, were associated with disease activity.
C,D) PCA of the data from days 4 and 7. Acquired immunity-related genes, such as *Cd3g* and MHC class II molecules, contributed to the principal component (PC)1 distribution.
E,F) PCA of the data from days 7 and 60. Cardiac remodeling-related genes, such as *Mmp12*, *Bmp10*, and *Gpnmb*, contributed to the variance between days 7 and 60.

Pattern matching analysis identifies surrogate markers in the blood, which reflect the changes in the heart



r	Gene
0.98	*Xaf1*
0.97	*Gvin1*
0.97	*Oasl2*
0.97	*Irf7*
0.97	*Chmp4b*
-0.87	*Eif4a2*
-0.87	*Tgs1*
-0.87	*Nup37*
-0.89	*Gas5*
-0.89	*Fubp3*

Principal component (PC)1 values were calculated by PCA using microarray data of the heart from control and TMEV-infected mice on day 4. Correlation coefficients (*r*) between the heart PC1 values and blood microarray data were calculated by a pattern matching analysis. *Xaf1* and *Fubp3* in the blood showed the strongest positive and negative correlation with the heart PC1 values, respectively.

Conclusions

- The set of molecules, but not a single molecule, was useful as phase-specific biomarkers of viral myocarditis.
- Pattern matching analysis, using heart PC1 values and blood microarray data, identified the blood surrogate markers which reflect the changes in hearts.
- Future translational application of this approach will lead to discovery of not only phase-specific markers in the heart, but also the biomarkers in the periphery (surrogate markers), which will be useful to diagnose myocarditis without having to biopsy the heart.

References

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Conflict of Interest (COI)

We have no conflict of interest.