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MicroRNA Set : A Novel Way to Uncover the Potential Black Box of Chronic Heart Failure in MicroRNA Microarray Analysis

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Abstract

As the prime criminal among all the cardio-vascular diseases, chronic heart failure (CHF) is still far from being fully understood after decades of study by researchers. The booming bioinformatics studies, especially the microRNA (miRNA) microarray analysis, have significantly accelerated the uncovering of underlying mechanisms of human diseases. However, these miRNA researches mainly focus on single miRNA, paying less attention to the group characteristics of miRNAs, which may ignore the group characteristics of miRNAs. Here we introduce a novel miRNA set concept incorporation with a group analysis method of CHF miRNA microarray expression. Our results show great accordance with previous studies, and also reveal potential characteristics of miRNAs in CHF. Furthermore, this novel miRNA set approach may give us new insights into other diseases studies as well.

Keywords: Microarray; MicroRNA; Gene set; Heart failure; Cardiovascular disease

Introduction

MiRNA is a single-stranded RNA molecule of 21–25 nucleotides in length, which extensively regulates gene expression in many cellular and molecular processes (Calin et al., 2002). Basically, miRNAs act as negative regulators of gene expression by guiding a RNA-induced silencing complex to inhibit the translation or promote the degradation of their targets. The deregulation of miRNAs has been associated with the pathogenesis of several diseases such as inflammation (O'Connell et al., 2007), cancers (Gregory and Shiekhattar, 2005), cardiovascular diseases (van Rooij and Olson, 2007) and metabolic diseases like diabetes (Williams, 2008). Some of human miRNAs, like hsa-let-7e, hsa-let-125a and hsa-let-99b have important links to some specific cancers or carcinomas (Feitelson and Lee, 2007). One study found the miRNA function in the heart had revealed that they play a critical role during the Cardiac myocyte development (Chen et al., 2008). Each miRNA is considered to regulate lots of transcripts, but only a few targets have experimental validations (Korpal et al., 2008). We previously uncovered some important pattern of miRNAs in signaling network(Cui et al., 2006), cross-species gene expression diversity(Cui et al., 2007), transcription factor regulation(Cui et al., 2007), recombination rate (Zhao et al., 2009), and co-expression with host genes (Wang et al., 2009). The thorough study of miRNAs may provide us some undiscovered regula-

tory mechanisms and potential therapeutic targets for the future treatment of human diseases, especially cancers and cardiovascular diseases which are the leading causes of death.

Generally, some key regulators of gene expression and left ventricular remodeling may relate to CHF. Persistent pressure stimuli to the heart would probably lead to hypertrophy, and eventually result in CHF. Some miRNAs expression profiling studies demonstrates that expression levels of specific miRNA changes in diseased human hearts are closely connected to their involvement in cardiomyopathies (Tatsuguchi et al., 2007; Thum et al., 2007; van Rooij et al., 2006). Thum et al., (2007) found out some evidence that cardiac miRNAs and reactivation of a fetal miRNA program substantially contribute to the result of the failing human heart (Thum et al., 2007). They derived a conclusion of a novel mode of regulations for the transcriptional changes in CHF.

Microarray is a powerful tool for studying the evidence of thousands of genes (or miRNAs) simultaneously. Many algorithms and tools (Khatri and Draghici, 2005) are developed to predict the underlying mechanisms of the complex physiological and pathological conditions. The current microarray technology may reveal many significant discriminative expressed miRNAs. However, the statistically significant miRNAs from different groups mostly focus on single miRNA research, which means they lack the idea of group (or set) and unable to identify the miRNAs whose single statistical significance was not obivious but the biological significance might be great. Generally, miRNAs in the same cluster or miRNA family may have similar features. Some studies also show that differentially expressed miRNAs tend to work in groups (Hammond, 2006; Khatri and Draghici, 2005; Lu et al., 2008). Therefore, taking multiple miRNAs as a set to perform the significance analysis is useful for the identification. GSEA (Gene Set Enrichment Analysis) (Subramanian et al., 2005) is a popular method to identify set of genes (or miRNAs) that expresses or is regulated in one way or one direction statistically. The use of GSEA has already made contributions to biology and clinical research (Bourquin et al., 2006; Mootha et al., 2003). In this study, we firstly apply the cluster miRNA set and family miRNA set to a CHF miRNA microarray dataset, and identified some important miRNAs up or down-regulated, some of which were validated by others. Additionally, the result shows important relationship in miRNA group characteristics with CHF, which had not been identified as significant previously.

Materials and Methods

In our study, we manually collected the microarray data (Thum et al., 2007), the Ambion's chip data on the website (http://www.ambion.com/techlib/resources/miRNA_array), and used GSEA package (http://www.broad.mit.edu/gsea/) to identify the significant miRNAs.

Data Preparation

In Thum's paper (Thum et al., 2007), it was demonstrated that miRNA expression profiles between failing and fetal human heart tissue have a close relationship. According to their result, alterations in miRNAs expression in heart failure and fetal heart are similar. The 86.6% of induced miRNAs and 83.7% of repressed miRNAs were regulated in the same direction. The downloaded expression data which is about to use, mainly includes the up and down-regulated miRNAs classified by the Fold Change (Fold change means the ratio between the highest gene expression value and the lowest gene expression value).

The Defining of miRNA Set

Existing sets in GSEA databases based on biological pathways, chromosomal location, or the one in the common status of health or diseases. Here we defined two miRNA sets according to the miRNA family database and miRNA cluster information.

A gene family is a set of genes with a known homology, which generally have similar structures and biochemical functions. For example, the hsa-mir-133 family consists of 2 homologous hsa-mir-133a members including hsa-133a-1 and hsa-133a-2, and a nearly homologous hsa-mir-133b. Xiao et al reported hsa-mir-133 family to play a role in cardiac conductance abnormalities during diabetes (Xiao et al., 2007). The miRNAs in hsa-mir-133 family play an active role in the inhibition of cardiac hypertrophy (Care et al., 2007). Some other scientists also announced that variable deregulation of miRNA patterns of hsa-mir-133 expression were reported during cardiac hypertrophy (Cheng et al., 2007; Tatsuguchi et al., 2007; van Rooij et al., 2006). It is interesting to investigate the potential involvement of other family members in cardiac diseases when we have the knowledge of a specific miRNA which belongs to a famlily of related miRNAs. According to Sanger's database (http://miRNA.sanger.ac.uk), we manually constructed the miRNA family set. The miRNAs in one family consist one sub-set. All the sub-sets in the database will compose the miRNA family set.

As miRNAs tend to exist in clusters (Altuvia et al., 2005) and the miRNAs in the same clusters usually have the similar expression profiles and act together in cell processes (Baskerville and Bartel, 2005), we made our miRNA set. The miRNAs in one single cluster consist one subset. The whole clustered set is comprised by all the subsets.

Results and Discussion

By the GSEA method, we found two different results respectively. The detailed significant miRNAs are in the supplementary files 1 and 2.

Result by the miRNA Family Set

As to the gene family set, we find that 4 gene sub-sets (groups) are up-regulated with the significant FDR (false discovery rate) < 25% and nominal p-value < 1% (see Table 1, Figure 1). FDR is the estimated probability that a gene set with a given NES (normalized enrichment score) (Subramanian et al., 2005) represents a false positive find-

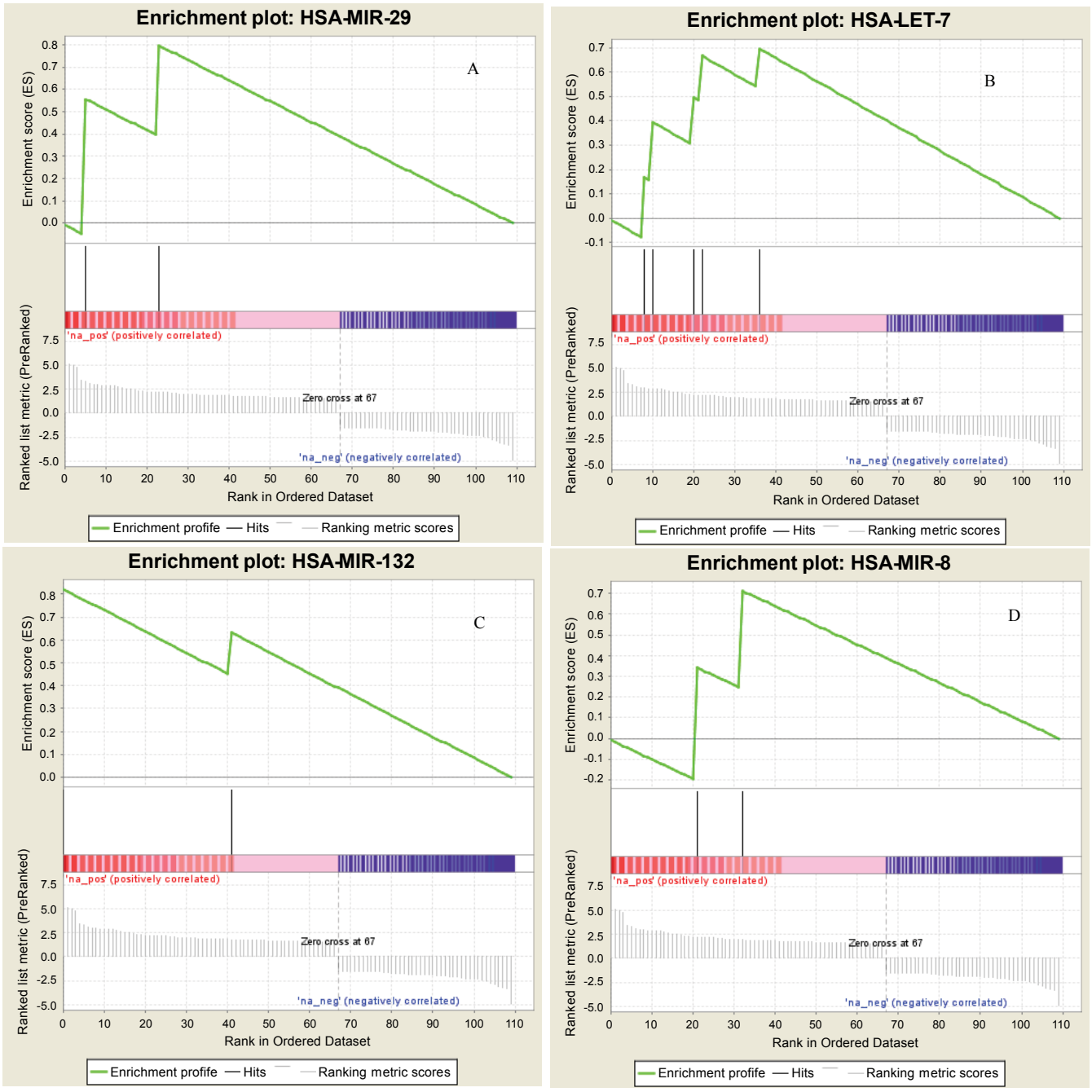

Figure 1: The four results of miRNA subsets. A, B, C, D show different enrichment score behaviors. These four subsets are significantly differentially expressed.

miRNA family	Size	ES	NES	P value	FDR
HSA-MIR-29	2	0.80	1.79	0.000	0.000
HSA-LET-7	5	0.70	1.32	0.000	0.140
HSA-MIR-132	2	0.82	1.27	0.000	0.093
HSA-MIR-8	2	0.71	1.21	0.000	0.227
HSA-MIR-515	4	-0.41	-1.00	0.333	0.507

Table 1: Four sub-sets are up-regulated significantly. And there is also a down-regulated sub-set (HSA-MIR-515) which is not significant enough.

ing. For example, an FDR of 25% indicates that the result is likely to be valid 3 out of 4 times. As to HSA-MIR-29 family, this sub-set consists of hsa-mir-29a and hsa-mir-29c after the filtering out process (Some miRNAs in a set will not be used because of set size, the minimal set size was set as 2). After the process, we get the result that hsa-mir-29a and hsa-mir-29c are significantly enriched, which means that they may have an important relationship with CHF. At the Same time, we should not neglect the HSA-MIR-515 sub-set. This sub-set is not significantly down-regulated, but it may also have important effects. It is one of the most useful statistical values.

Result by the Clustered miRNA Set

As to the clustered miRNA set, we find one miRNA sub-set is up-regulated with the significant FDR < 25% and nominal p-value < 1% (see Table 2, Figure2). Two sub-sets are down-regulated but not significant enough. As to CLUSTER 5, this sub-set consists of hsa-let-7e and hsa-mir-125a after the filtering out. hsa-let-7e and hsa-mir-125a may have the most important effects and highly correlated with CHF. Similarly, we should not neglect the other two sub-sets.

Discussion

More than 700 human miRNAs have been identified so far and the total number may be around 1000 (Berezikov et al., 2005). Although the mechanism of CHF is not presently clear, the relationship between miRNAs and heart diseases has advanced rapidly. In our results, there are some miRNAs which may have very important relationships and close correlations with CHF. The subsequent problem is that whether these miRNAs really have links to CHF. Here we searched them in scientific journals to confirm our findings.

With the results predicted by miRNA family set, HSA-MIR-29 family (mir-29 family: hsa-mir-29a, hsa-mir-29c) and HSA-LET-7 (let-7 family: hsa-let-7b, hsa-let-7c, hsa-let-7d, hsa-let-7e, hsa-mir-98) may have the links to CHF. According to Thum's paper (Thum et al., 2007), has-mir-29a was up-regulated in CHF. Additionally, the dysregulation of hsa-mir-29a in the development of cardiac hypertrophy has been validated by Northern blot and/or qRT-PCR (Cheng et al., 2007; Sayed et al., 2007).

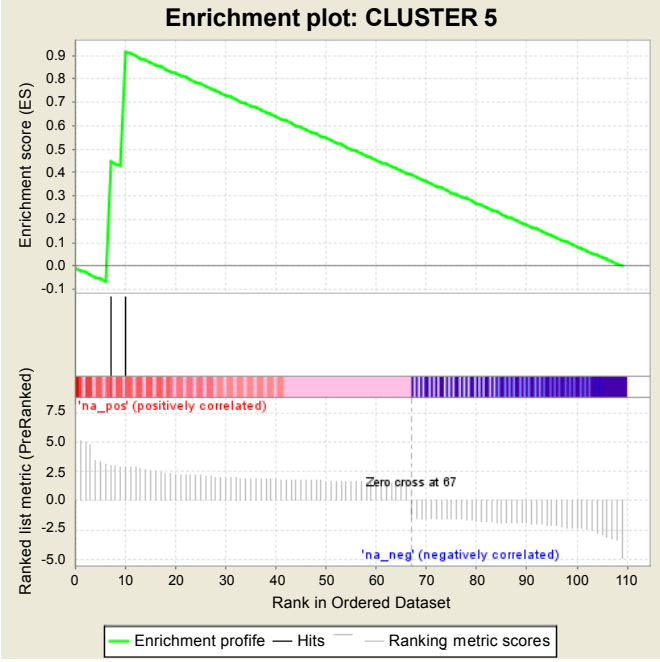

Figure 2: The enrichment score of CLUSTER 5 (hsa-let-7e, hsa-mir-125a) shows the significance of differentially expressed miRNAs.

Clusterd miRNA	Size	ES	NES	P value	FDR q-val
CLUSTER 5	2	0.92	2.20	0.000	0.000
CLUSTER 13	2	-0.59	-1.06	0.500	0.383
CLUSTER 47	2	-0.46			1.000

Table 2: One sub-set is up-regulated significantly. And there are also two down-regulated sets, which¡¡are not significant enough.

Besides mir-29 family, Feitelson et al reported that let-7 family is related to many diseases and is down-regulated in hepatocellular carcinoma, lung neoplasm, colonic neoplasm (Feitelson and Lee, 2007; Zhao et al., 2007). As we know, c-myc is one of the critical oncogenes. A study of c-Myc protein shows that miRNA has an effect on the development of cancer (He et al., 2005). The expression of the c-myc gene is modulated by let-7 (Koscianska et al., 2007). Since the proto-oncogene expression might mediate the hypertrophic mechanism in heart failure (Kai et al., 1998), we may get the idea of the close relationship of has-let-7 and CHF. Moreover, hsa-mir-98, which exist in

the same HSA-LET-7 set is up-regulated (Thum et al., 2007).

Furthermore, some of the miRNAs in other significant sub-sets also have been reported. Most of these miRNAs we predicted are important and have links to CHF.

With the results we predicted by miRNA clustered set, hsa-let-7e and hsa-mir-125a may have links to CHF. As for hsa-let-7e, it is also included in hsa-let-7 sub-set with miRNA gene family set analysis as in the previous analysis. As for hsa-mir-125a, it is down-regulated in hepatocellular carcinoma, breast Neoplasms, and heart failure (Williams, 2008; Dalmay and Edwards, 2006; Thum et al., 2007). When it comes to the other miRNAs, we predicted most of them like hsa-mir-432, hsa-mir-136, hsa-mir-302a, and hsa-mir-367 have also been reported with similar effects in Thum's paper and other papers, although they were not enough significant in our research (Thum et al., 2007).

Through the differentially we only used secondary data from Thum's paper. Additionally, the novel analysis method may have the limitations like the defining of the set, which leaves us challenges in the future improvement. Nevertheless, the result is surprising that the predicted miRNAs still give us hints that we may put more emphasis on them in the future biology explorations in order to make sure whether they have real functions. To better understand the biology of these miRNAs, we may further study them by doing biological experiments like knocking down the expression of the some miRNAs in a specific pathological model.

Conclusion

Traditional differential miRNA identification methods in miRNA microarray analysis always focus on testing one miRNA at one time. They may fail to detect miRNAs that don't have very significant fold change but really have significant associations. In our study, we introduced the miRNA set concept and analyzed the miRNA microarray expression data of CHF at the level of groups by the tool of GSEA rather than that of the individual gene. In the study, we put forward a novel way to find the potential relationships between miRNAs and CHF. Some of the miRNAs have been reported and validated in the discussion. Although the clear mechanism of CHF has not been fully understood, our results may help to further understand the relationship between miRNAs and CHF or even other diseases. Future identification and validation of miRNA functions will substantially improve our understanding of CHF and other human diseases.

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