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Review Article

Identifying microRNA biomarkers and constructing microRNA-regulated networks in coronary artery diseases: a meta-analysis

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Abstract: Background: Coronary artery disease (CAD) places a large burden on society. Thus, exploration of novel early biomarkers and therapeutics is of great importance. MicroRNAs (miRNAs) are likely to represent early biomarkers of CAD that can be used to detect and monitor the progression of this disease. Materials and methods: In this study, a comprehensive meta-analysis of 28 independent miRNA expression studies relating to CAD was performed. These studies consisted of 1,337 cases and 2,303 control samples involving 263 differentially expressed miRNAs (202 up-regulated miRNAs and 109 down-regulated miRNAs). Of these, 48 miRNAs were reported in both up-regulated and down-regulated groups. Results: Seventy-one significantly dysregulated miRNAs were identified. Subsequently, miRNA target gene and enrichment analysis was performed to determine the biological and functional relevant genes involved in the meta-signature miRNA regulation. Finally, 5 pathways with the most representation and with *P*-values ≤0.05 were identified as "Pathways in cancer", "MicroRNAs in cancer", "PI3K-Akt-signaling pathway", "Proteoglycans in cancer", and "MAPK-signaling pathway" and are thus suggested to be associated with these miRNAs. Conclusions: Based on this research, the identified meta-signature miRNA can be used to develop a series of specific diagnostic and prognostic biomarkers for CAD that meta-signature miRNA can be used in the clinic.

Keywords: Meta-analysis, miRNA, CAD, enrichment analysis

Introduction

Cardiovascular diseases are the main cause of death and disability worldwide. Coronary artery disease (CAD) is characterized by occlusive epicardial coronary artery stenosis and is the principal clinical manifestation of atherosclerosis. CAD imposes a great burden on society and therefore the exploration of novel early biomarkers and therapeutics for CAD is of great importance. MicroRNAs (miRNAs) are likely to represent early biomarkers of CAD that can be used to detect and monitor the progression of this disease [1-7]. miRNAs are small (approximately 22 nucleotides), endogenous, highly stable non-coding RNAs that regulate gene and protein expression at the post-transcriptional levels. These proteins are involved in many biological processes, including cellular differentiation, metabolism, and cancer development [8-11]. The regulatory effects of microRNAs are exerted by binding to the 3'-untranslated regions (UTRs) of target mRNAs, which leads to the degradation or translational repression of target genes. Previous reports have predicted that 1/3 of human genes are regulated by microRNAs [9]. Thus, microRNAs interfere with many physiological and pathological processes, and their modes of dysregulation are linked to many diseases [10].

In studies attempting to identify novel early biomarkers of CAD miRNA expression [12, 13], profiling has often been performed in peripher-

al blood mononuclear cells (PBMCs), blood, or plasma. Although many miRNAs have been identified as being either over- or under-expressed, few may actually be important signatures or therapeutic targets for CAD. Profiling studies have employed different profiling platforms and methods. Therefore, determining which miRNAs are potential biomarkers or are tissue-specific and which are specific for various CAD phenotypes is challenging. Because existing literature reviews on miRNAs in CAD are purely narrative without any meta-analysis, the results may be inconsistent. A survey on data reproducibility in the biomedical sciences also found that data non-reproducibility is an issue [2, 14-17]. For example, miR-21-5p has been reported in different papers as acting in different regulatory directions [2, 14, 15]. The same is true for miR-24-3p [16, 17] and miR-92a-3p [2, 17]. In this study, the results of other studies were evaluated through a meta-analysis in order to fill this gap and identify consistently dysregulated miRNAs that have been shown in reproducible profiling results to be potential biomarkers for CAD. Furthermore, these dysregulated miRNAs were visualized as a miRNA-target network and gene set enrichment analyses on statistically significant objects of our meta-analysis was performed to predict the targeted functions of dysregulated miRNAs in CAD.

Materials and methods

Meta-analysis was conducted totally following the guidelines of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2009 Checklist (http://www.prisma-statement.org/). The PRISMA 2009 Checklist for our study are shown in supplementary materials (Checklist S1).

Publication search strategy

PubMed was searched for CAD miRNA expression profiling studies without publication date limitations using the following terms: ('miRNA' OR 'microRNA') AND ('expression' OR 'profiling' OR 'profile') AND ('CAD' OR 'CHD' OR 'coronary heart disease' OR 'coronary artery disease') in the title/abstract. References found in the extracted studies were reviewed.

Study selection

The eligible studies that were included in our meta-analysis complied with the following criteria: (a) included miRNA expression profiling studies on patients with CAD phenotypes; (b) included comparisons between CAD and non-CAD control samples; (c) the cut-off criteria of differentially expressed miRNAs were reported; (d) sample sizes were reported; and (e) the detection and quantification methods were reported. Exclusion criteria were set as follows: (a) the publications were unrelated to CAD; (b) duplicated or incomplete data were included; (c) the publications were letters, editorials, commentaries, reviews, or case reports; and (d) the studies were not conducted in humans.

Data extraction

From the full text and supplementary information of each expression profiling study, the following eligibility items were collected and recorded: first author, year of publication, region of study, tissue types, sample size of the case/control group, detection platform, cut-off criteria, and numbers of up- and down-regulated miRNAs. All miRNAs were mapped to miRBase [18] to merge the aliases.

Quality assessment

MIAME guideline version 2.0 was used to assess microarray quality, and the MIQE guideline was used to evaluate the studies involving quantitative PCR (qPCR)-based miRNA arrays [19].

Meta-analysis

All statistical analyses were performed using R-3.2.1 software on the extracted data; the additional statistical package Meta was also used. Metabin was used as the main function. The outcomes are presented as $\log_{10}$ odds ratios (logORs) based on the number of dysregulation events in both CAD and non-CAD control samples together with their 95% confidence intervals (CIs) using the random-effect model. *P*-values were corrected using the Bonferroni correction, and adjusted *P*-values of less than 0.05 were considered significant. Potential circulating biomarkers should be significantly up- or down-regulated and should be detectable in human blood or in both blood and tissues. Potential tissue biomarkers should be significantly up- or down-regulated and should be highly tissue-specific. Differentially expressed miRNAs in the CAD and non-CAD control samples were ranked according to the following order of importance: (1) *P*-values; (2) the num-

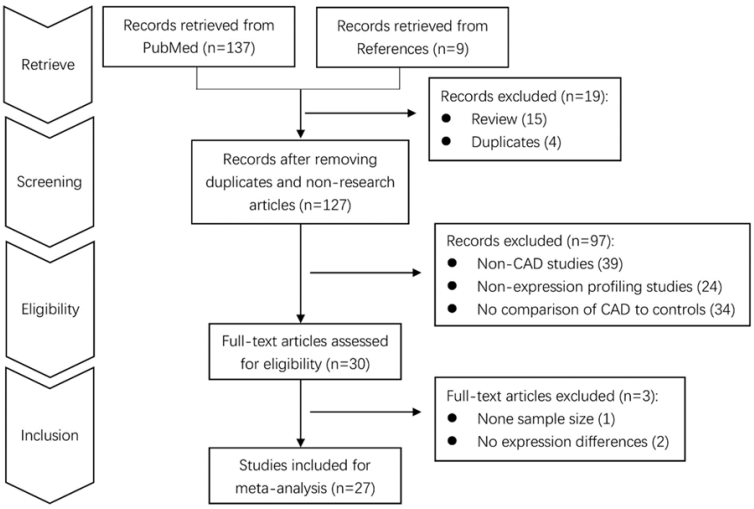

Figure 1. Flow diagram of the study selection process. The study selection process, including retrieval, screening, eligibility extraction and inclusion, is depicted in the flow diagram.

ber of consistent reports; and (3) logOR values.

Subgroup analysis

miRNAs are differentially expressed among tissue types and CAD phenotypes, with corresponding overall effects and heterogeneities. Subgroup analyses split the extracted data according to tissue type and CAD phenotype to compare miRNA expression profiles among tissue types and CAD phenotypes.

Sensitivity analysis

Northern blot, microarray, miRNA in-situ hybridization (ISH), and RT-PCR are common miRNA detection technologies, and sensitivity depends on the detection method used. PCR detection yields higher sensitivity than other methods. Sensitivity analysis was performed on the detection method to test the robustness of the findings. Detection method is a dominant factor that affects precision in determining the overall effects. Thus, the meta-analysis was repeated after excluding studies using detection methods other than RT-PCR.

Network building and gene set enrichment analysis

In humans, each function is not realized by a single gene, but rather, miRNAs and genes regulate the activities of the organism through networks. Gene network construction is based on a set of genes and can present and give an understanding of life phenomena or pathogenesis at the systemic level [20]. Because each miRNA can have multiple target genes and a gene can be regulated by multiple miRNAs, miRNA-target network maps are more useful for illustrating disease-related gene-regulation pathways at a high level [21].

Understanding regulation of miRNA target genes is usually achieved using target prediction software and is then experimentally verified. To establish a more reliable regulation network, this study collected three experimentally supported target gene databases: miRTarBase [22], miRecords [23], and miR2Disease [24]. All target genes were manually validated based on strong evidence (i.e., Western blot, qPCR, reporter assay); the following were removed: non-human genes, duplicated genes, and genes with no evidence-based support. The gene sets were imported into the DAVID platform (https://david.ncifcrf.gov/) [25, 26] and the MetaCore database (MetaCore™ version 6.26 build 68-498, Thomson Reuters) for enrichment and pathway analysis.

Results

Search results and characteristics of the eligible studies

Figure 1 shows the Preferred Reporting Items for Systemic Reviews and Meta-Analyses (PRISMA) flow chart of the literature search process. After searching the database, 137 potentially relevant studies were found. After carefully screening these studies according to our selected criteria, duplicate studies and reviews were excluded. The remaining 27 studies (including 72 sub-studies) met our eligibility criteria and were included in the meta-analysis (Table S1). The studies were all published between 2010 and 2015, and most originated from East Asia. A total of 1,337 cases and 2,303 control samples were enrolled in our meta-analysis and involved 359 differentially expressed miRNAs (246 of which were up-regu-

Table 1. Characteristics of miRNA expression profiling studies (CAD vs non-CAD controls)

Study	First Author	Year	Region	Tissue	Control	CAD (SA/UA/MI)	Detection Method	Cut-off Criteria	Total	Up-regulated	Down-regulated
1	GK Wang	2010	China	Heart, skeletal muscle, plasma	30	33 (0/0/33)	qRT-PCR	P<0.01, Ct=40	4	4	0
2	Hoekstra	2010	Norway	PBMCs	20	50 (25/25/0)	qRT-PCR	P<0.01, Ct=40	2	1	1
3	Fichtlscherer	2010	Germany	Plasma	8	8 (8/0/0)	Geniome Biochips	P<0.01, FC>1.2	66	20	46
				Plasma	17	36 (36/0/0)	TaqMan qPCR	P<0.05, FC>1.2	7	1	6
				Serum	14	31 (31/0/0)	TaqMan qPCR	P<0.05, FC>1.2	6	0	6
4	Weber	2011	United States	Blood	15	10	qRT-PCR	P<0.05	11	0	11
5	Sondermeijer	2011	Netherlands	Platelet	12	12	Illumina Human v2 MicroRNA Beadarrays	P<0.05	7	6	1
				Platelet	40	40	qRT-PCR	P<0.05	3	3	0
				Platelet	40	27	qRT-PCR	P<0.05	2	2	0
6	F Wang	2013	China	Plasma	27	13 (0/0/13)	qRT-PCR	P<0.01	1	1	0
7	K Li	2013	China	Platelet	12	12	Illumina Human v2 MicroRNA Beadarrays	SAM, FDR<0.01	1	1	0
8	Alessandra	2013	Italy	Plasma	20	53 (34/19/0)	TaqMan Human microRNA Card A Arrays	FC>2, P<0.05	10	10	0
				Plasma	20	53 (34/19/0)	qRT-PCR	FC>2, P<0.05	3	3	0
9	JY Ren	2013	China	Plasma	13	13 (0/13/0)	Taqman low-density miRNA array	FC>8, FDR<0.0001%	34	34	0
				Plasma	37	76 (31/45/0)	RT-PCR	p<0.05	7	7	0
				MPs	5	5 (0/5/0)	RT-PCR	p<0.05	7	7	0
10	GF Zhu	2014	China	PBMCs	31	79 (24/31/24)	RT-PCR	p<0.01	1	0	1
				Plasma	31	55 (0/31/24)	RT-PCR	p<0.01	1	0	1
11	F Chen	2014	China	Platelet	12	12 (6/0/6)	Illumina Human v2 MicroRNA expression beadchip	FC>1.5, FDR<0.05	10	6	4
12	SF Li	2014	China	Plasma	9	9 (0/9/0)	TaqMan low density array	FC>2, FDR<0.0001%	36	36	0
				Plasma	20	30 (0/30/0)	RT-PCR	FC>20, Ct<30	5	5	0
				MPs	6	6 (0/6/0)	RT-PCR	FC>20, Ct<30	5	5	0
13	Satoh	2015	Japan	Plasma	20	41	3D-Gene Human miRNA Oligo chips	P<0.05	7	0	7
				Plasma	20	41	RT-PCR	P<0.05	4	0	4
14	FQ Liu	2014	China	Plasma	20	20	microarray	P<0.05, FC>2	53	36	17
				Plasma	50	81	qRT-PCR	P<0.05, FC>2	4	1	3
15	D Liu	2015	China	Plasma	30	30	qRT-PCR	P<0.05	1	0	1
16	TX Huan	2015	USA	Blood	186	186	qRT-PCR	FDR<0.2	15	15	0
17	C Chen	2015	China	Plasma	10	10	miRCURY LNA Array	P<0.05	75	57	18
				Plasma	20	20	qRT-PCR	P<0.05	9	5	4
				Plasma	50	50	qRT-PCR	P<0.05	5	5	0
18	YM Chen	2015	China	$CD4^+$ T cells	31	20 (0/20/0)	qRT-PCR	FC>1.5, P<0.05	6	4	2
1919	XD Li	2015	China	Plasma	8	16 (8/0/8)	Agilent miRNAs microarray Version 16.0	FC>2, P<0.05	7	7	0
					21	60 (30/30/30)	qRT-PCR	FC>2, P<0.05	6	6	0
					51	334 (43/257/77)	qRT-PCR	FC>2, P<0.05	5	5	0
20	X Chen	2014	China	Plasma	30	73 (0/20/53)	qRT-PCR	P<0.01	1	1	0

21	Y Tang	2015	China	EPCs	3	3	microarray	P<0.05	6	4	2
				EPCs	34	53	qRT-PCR	P<0.05	1	1	0
22	ZL Xu	2015	China	Serum	5	13	miRCURY LNA Array	P<0.05	36	26	10
				Serum	5	13	qRT-PCR	P<0.05	11	9	2
				Serum	45	77	miRCURY LNA Array	P<0.05	13	10	3
				Serum	45	77	qRT-PCR	P<0.05	2	2	0
23	SJ Yang	2014	China	Plasma	10	56 (0/39/17)	qRT-PCR	P<0.05	1	1	0
24	SH Li	2015	China	PBMCs	27	69 (24/21/24)	qRT-PCR	P<0.05	1	1	0
				PBMCs	24	45 (0/21/24)	qRT-PCR	P<0.05	1	1	0
25	S. Niculescu	2015	Romania	Serum	16	95 (30/39/26)	TaqMan miRNA Assays	P<0.05, FC>4	48	48	0
26	W Liu	2015	China	Plasma	70	90	qRT-PCR	P<0.05	4	4	0
27	JQ Zhou	2015	China	Plasma	67	67	qRT-PCR	P<0.05, FC>5	2	2	0

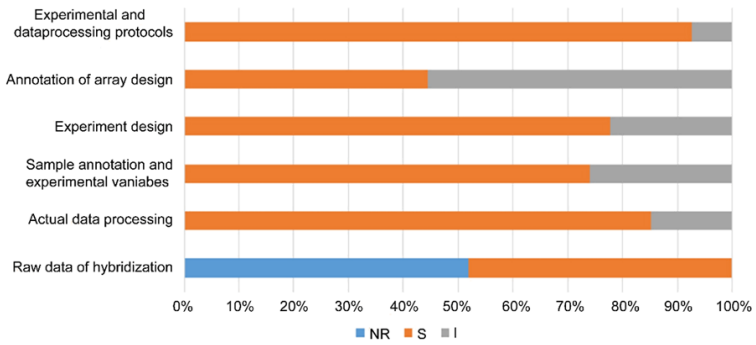

Figure 2. Quality assessment according to the MIAME guidelines. Blue bars, orange and grey and indicate items that were not reported (NR), sufficiently annotated (S) and not sufficiently annotated (I), respectively.

lated and 113 of which were down-regulated). The detailed characteristics of these studies are presented in **Table 1**.

Quality assessment of studies

The quality of the studies was assessed according to the Minimum Information About a Microarray Experiment (MIAME) 2.0 and Minimum Information for the Publication of Quantitative Real-time PCR Experiments (MIQE) guidelines. **Figure 2** and Table S2 summarize the quality assessment process. Of the included studies, 52% did not report raw data regarding hybridization, and 22% did not provide sufficient information regarding experimental design and sample data relationships. For more than 50% of the studies, array designs were not fully reported.

Protect differentially expressed miRNAs

The 359 obtained differentially expressed miRNAs were mapped to miRBase; 6 miRNAs were excluded from our study due to mismapping to the database, including the up-regulated miRNAs miR-199, miR-545: 9.1, ebv-miR-BART7-5p, and miR-130 and the down-regulated miRNAs let-7 and miRPlus-J212*; 353 miRNAs remained (242 up-regulated and 111 down-regulated miRNAs). Forty-four miRNAs were reported in the both up- and down-regulated groups, leaving only 309 differentially expressed miRNAs. After alias merging according to the miRBase, 46 miRNAs had two aliases; therefore, 263 miRNAs were finally retained (the study found 202 up-regulated miRNAs and 109 down-regulated miRNAs; 48 miRNAs were reported in both the up- and down-regulated groups, leaving only 263 differentially expressed miRNAs).

Among these 263 differentially expressed miRNAs, which were reported in 27 studies comparing CAD samples with non-CAD control samples, 118 miRNAs (44.87%) were reported in at least two sub-studies. Among the 118 differentially expressed miRNAs, 71 (61.21%) significantly differed in the direction of their dysregulation (65 were significantly up-regulated, and 6 were significantly down-regulated). In meta-analysis of dysregulated miRNAs, dysregulation of these 71 miRNAs was significant, as shown in Table S3. The most significantly up-regulated miRNA was miR-451a, which was reported in 10 sub-studies (with an adjusted P=3.59*10-24). miR-379-5p (adjusted P=1.09*10-11) was the most significantly down-regulated miRNA and was reported in 4 sub-studies.

Subgroup analysis

miRNA expression among different tissue types: Thirty-eight of the 72 sub-studies investigated miRNAs in plasma, 11 investigated peripheral blood mononuclear cells (PBMCs), 8 investigated serum, 6 investigated platelets, 2 investigated whole blood, 2 investigated microparticles (MPs), 2 investigated endothelial progenitor cells (EPCs), and 1 each investigated $CD4^+$ T cells, heart, and skeletal muscle. Meta-analysis was conducted on miRNA profiles in plasma, PBMCs, sera, and platelets (the details are shown in **Table 1**). Significant miRNA dysregulation in the different tissue types is shown in Tables S4, S5, S6, S7. Among the dysregulated miRNAs, 10 up-regulated miRNAs (miR-451a, miR-122-5p, miR-125a-5p, miR-146a-5p, miR-20b-5p, miR-21-5p, miR-223-3p, miR-26b-5p, miR-30a-5p, and miR-486-5p) were consistently reported in at least two tissue types, and one down-regulated miRNA (miR-155-5p) was consistently reported in at least two tissue types; 77 miRNAs (70 up- and 7 down-regulated miRNAs) were reported in only one tissue (Table S8), suggesting that their regulation might be tissue-specific.

miRNA expression in various CAD phenotypes: Forty of the 72 sub-studies investigated miRNAs that were differentially expressed between different CAD phenotypes and healthy controls; of these, 14 investigated miRNAs that were

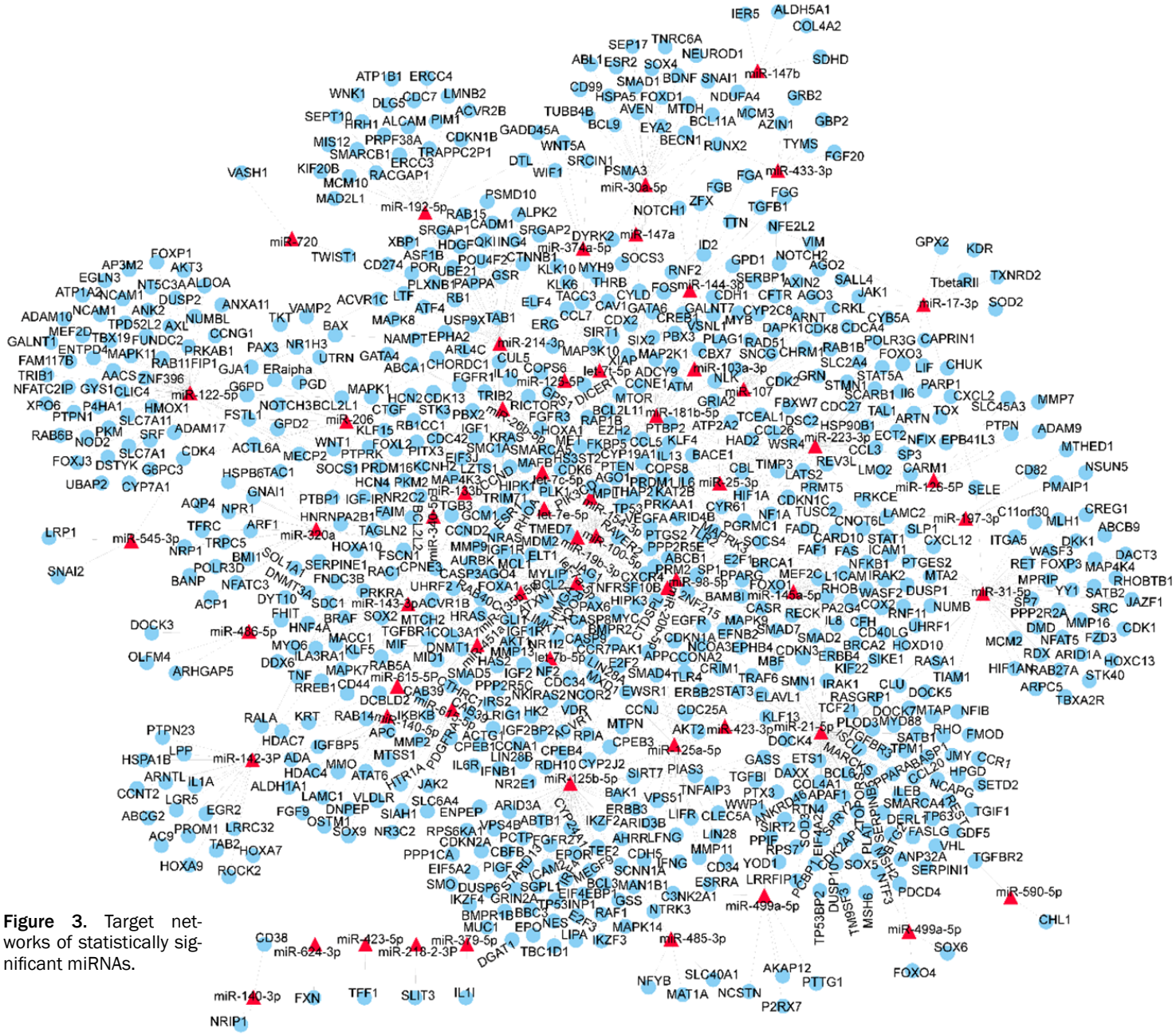

Figure 3. Target networks of statistically significant miRNAs.

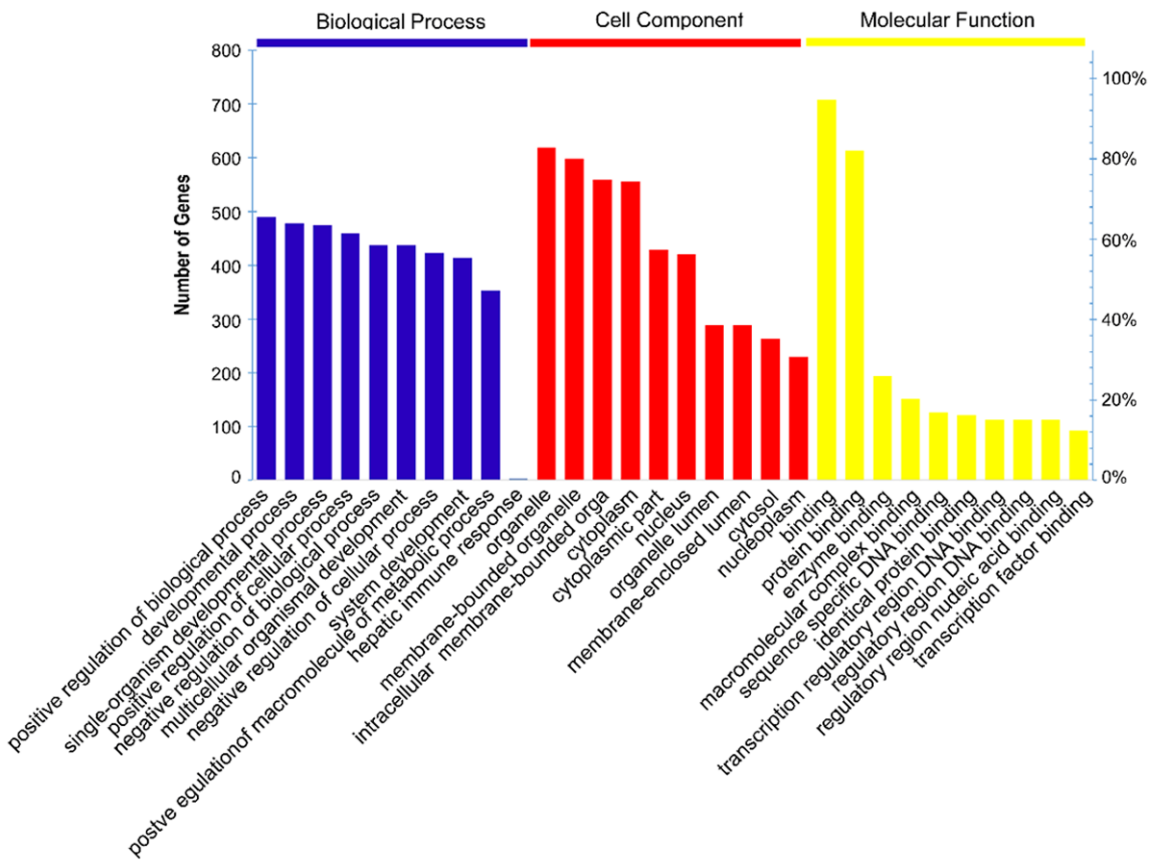

Figure 4. Top 10 GO terms in the BP, CC, and MF categories of target gene GO enrichment analysis.

differentially expressed between myocardial infarction (MI) patients and healthy controls, 15 investigated unstable angina (UA) patients, and 11 investigated stable angina (SA) patients. Significant miRNA dysregulation in different CAD phenotypes is shown in Tables S9-S11. Among the dysregulated miRNAs, miR-122-5p and miR-133a-3p were up-regulated in MI and SA patients but not in UA patients, miR-145-5p and miR-155-5p were down-regulated only in the SA group, and 23 up-regulated miRNAs occurred in only one CAD phenotype (Table S12), possibly suggesting that their regulation is phenotype-specific.

Sensitivity analysis

Sensitivity analysis was conducted to examine the robustness of the findings and to determine what effect detection method had on the overall analysis. Forty-two of the 72 sub-studies used the RT-PCR detection method and found 31 differentially expressed miRNAs. Sub-studies were collected using RT-PCR as the detection method. Analyzing these 42 sub-studies, 21 miRNAs were identified that were significantly differentially expressed, 17 of which were up-regulated and 4 of which were down-regulated (Table S13). Seventeen (16 up-regulated and 1 down-regulated) of the 21 miRNAs were significantly differentially expressed both in the sensitivity analysis and the overall analysis, and the remaining 4 were only significantly differentially expressed in the sensitivity analysis. This result indicates that the detection method used in miRNA profiling studies can affect the results of miRNA profiling studies.

MicroRNA-regulated networks

Seventy-one statistically significant miRNAs have been identified by meta-analysis. All targets of these miRNAs were retrieved from miRTarBase, miRecords, and miR2Disease. In total, 785 unique human genes were obtained (File S1). Statistically significant miRNAs and the corresponding target genes were imported into Cytoscape3.3.0, and a miRNA-Target Gene network was constructed (**Figure 3**). Gene On-

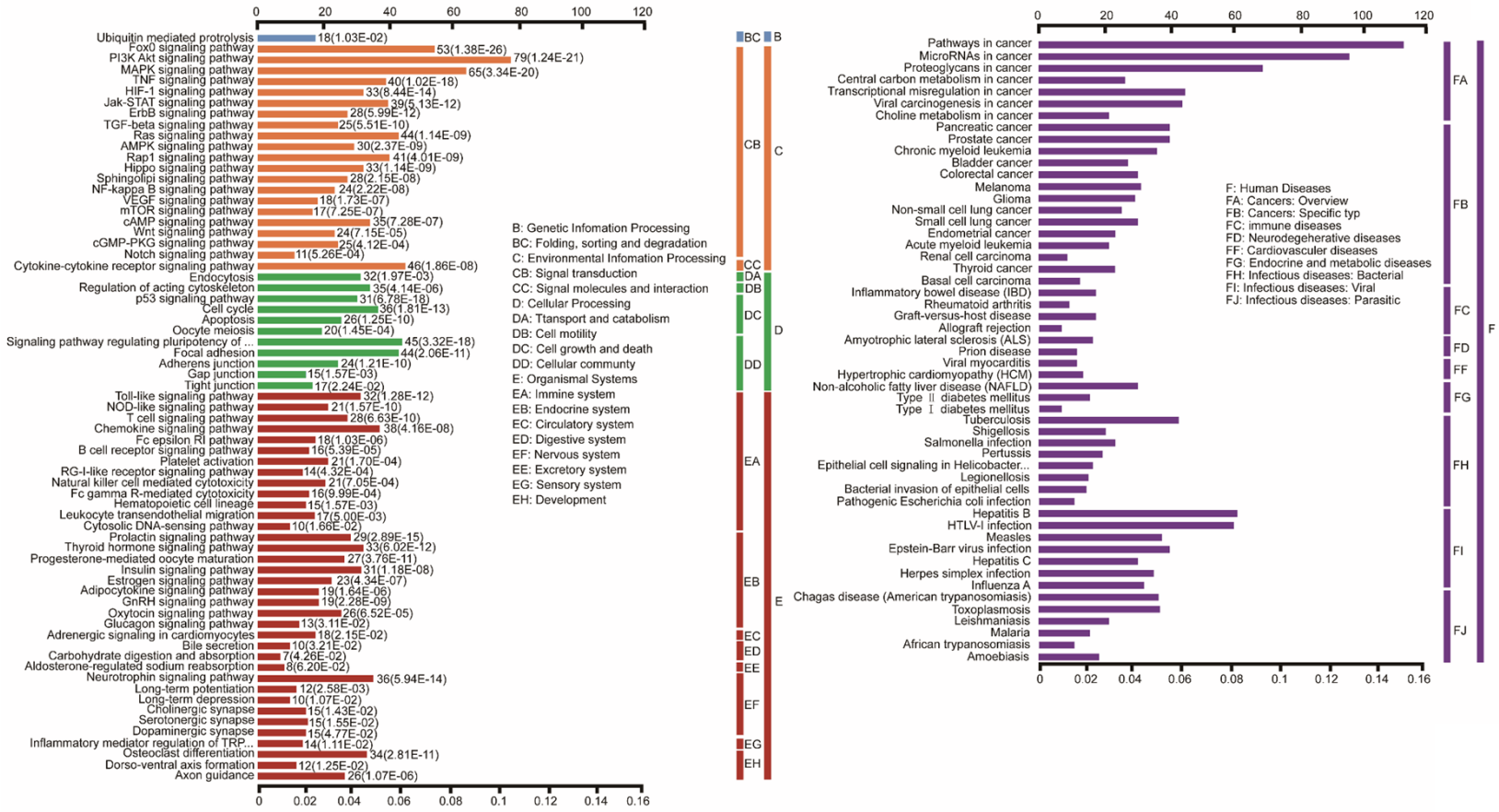

Figure 5. KEGG pathway enrichment analysis and classification of KEGG terms.

tology (GO) analysis was conducted using the DAVID platform **Figure 4**. (File S2 shows the top 100 GO terms in the "Biological Process" [BP], "Cellular Component" [CC], and "Molecular Function" [MF] categories, respectively) shows an overview of the gene ontology analysis, including up to 10 significantly enriched terms in the BP, CC, and MF categories, respectively. The cut-off *P*-value was set to 0.05. Terms in the same category are ordered according to *P*-value, and terms on the left are more significant. The percentage and number of involved genes in a term are shown in the left and right y-axes; 785 genes were mapped to 40 GO classification terms. Among these terms, "positive regulation of biological process" (490, level 2) and "developmental process" (478, level 3) were dominant in the BP category. For the CC category, the dominant subcategories were "organelle" (619, level 2) and "membrane-bounded organelle" (598, level 3). For the MF category, "bind" (708, level 2) and "catalytic" (613, level 3) were highly represented.

The pathways were clustered into sub-categories according to the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. As shown in **Figure 5** (File S3 shows the top 100 KEGG pathway names), all pathways were included in five categories: B) Genetic Information Processing, C) Environmental Information Processing, D) Cellular Processes, E) Organismal Systems, and F) Human Diseases. The 5 pathways with the most representation and with *P*-values ≤0.05 were "Pathways in cancer", "MicroRNAs in cancer", "PI3K-Akt-signaling pathway", "Proteoglycans in cancer", and "MAPK-signaling pathway".

All target genes were mapped to the MetaCore database, and enrichment analysis was performed (including Pathway Maps Enrichment, Process Networks Enrichment, and Diseases Enrichment). Detailed results are shown in File S4. Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling was the top ranked map. These target genes or proteins are associated with pathological processes, conditions, signs and symptoms, carcinoma, and neoplasms.

Discussion

miRNAs are considered promising biomarkers for detecting disease at an early stage and enable accurate prognosis after medical therapy [27-29]. However, miRNA profiles have always shown inconsistent results in studies. This meta-analysis of miRNAs was conducted in CAD to identify the most appropriate miRNA biomarkers that provide a precise and accurate diagnosis. The advantages of miRNAs as useful biomarkers include their accurate diagnostic value and stable existence in humans and the noninvasive nature of their use [30, 31]. However, conflicting results have been reported in studies of various miRNAs for screening and diagnosing CAD. Consequently, meta-analysis was performed to address this issue and to verify the intrinsic diagnostic value of miRNAs for CAD detection.

In total, 263 differentially expressed miRNAs were reported in the 27 examined studies, and 118 miRNAs (44.87%) were reported in at least two sub-studies. Among these 118 miRNAs, 71 (61.21%) were significantly differentially expressed and 47 (38.79%) were not. The meta-analysis results of the differentially expressed miRNAs can be influenced by many factors, including publication bias, biological complexity (gene susceptibility), and heterogeneity among the various types of specimens and conditions used.

Circulating miRNAs often hold much promise as biomarkers of various diseases [31-33]. In our study, miRNAs were mainly obtained from plasma, serum, peripheral blood mononuclear cells and platelet samples; thus, almost all of the extracted miRNAs are circulating miRNAs [34]. In the subgroup analysis based on tissue type, 77 (70 up- and 7 down-regulated) miRNAs were reported in only one tissue. Ten up-regulated miRNAs (miR-451a, miR-122-5p, miR-125a-5p, miR-146a-5p, miR-20b-5p, miR-21-5p, miR-223-3p, miR-26b-5p, miR-30a-5p, and miR-486-5p) and one down-regulated miRNA (miR-155-5p) were consistently reported in at least two tissue types. In this manner, the physiological state at the tissue level was revealed based on circulating miRNA levels. miRNAs that circulate in the blood can remain stable under harsh conditions, such as freezing temperatures, and can be detected easily. Circulating miRNAs are also specific to disease states. As shown in the results of the subgroup analysis based on phenotypes, two miRNAs (miR-122-5p and miR-133a-3p) were up-regulated in MI and SA patients but not in UA patients; only two miRNAs were down-regulated (miR-145-5p, miR-155-5p), both of which were in the SA

group. The remaining 23 up-regulated miRNAs were statistically significant in only one phenotype group each. These miRNAs represent minimally invasive biomarkers that might be useful for CAD diagnosis and monitoring [35-37].

The top ten miRNAs (miR-451a, miR-122-5p, miR-223-3p, miR-30a-5p, miR-26b-5p, miR-125a-5p, miR-486-5p, miR-146a-5p, miR-433-3p, and miR-485-3p) can serve as potential biomarkers of CAD because the results were statistically significant up-regulated. Circulating miRNAs are preferred biomarkers due to their ease of sampling and testing.

The most frequently reported miRNA (reported in 20 sub-studies) in profiling studies was miR-21-5p (adjusted P=4.96*10-2), which plays an important role in the development of heart disease [38]. This miRNA is among those whose expression is increased in human heart [38, 39]. miR-21 has been hypothesized mediate the effects of air pollution that lead to endothelial dysfunction and eventually to cardiac disease. Expression of miR-21 is negatively associated with exposure to PM10 air pollution and may mediate its effect on small blood vessels [40]. miR-21-5p is also among the most frequently up-regulated miRNAs in solid tumors [17]. miR-451a is the most significantly up-regulated miRNA [17]. Masaki et al. [41] found that miR451a expression was increased approximately 270-fold during the differentiation of purified normal human erythroid progenitor cells over 12 days in culture. Dore et al. [42] showed that miR451 expression was up-regulated during induction of erythroid maturation in human CD34-positive cells and in murine erythroleukemia cells.

In sub-group analysis, miR-451a was the most commonly up-regulated miRNA that was significantly enriched in platelet, serum, and plasma groups [43]. miR-122-5p was the most up-regulated miRNA in plasma; miR-135b-5p was the most up-regulated in serum; miR-624-5p was the most up-regulated in platelets, and miR-21-5p was the most up-regulated in PBMCs. The obtained results show that these miRNAs can be identified as biomarkers in specific tissues. miR-133a-3p was the most up-regulated miRNA in MI patients compared with healthy controls [44]; miR-30d-5p was the most up-regulated miRNA in UA patients, and miR-451a was the most up-regulated miRNA in SA patients. These findings suggest that the above mentioned miRNAs can serve as biomarkers to distinguish patients in different CAD groups and can be used to provide an accurate diagnosis.

When using miRNAs as biomarkers, several factors should be considered. (a) The expression profiles of miRNAs in different tissues should be known in detail, as these profiles differ between tissues. (b) The clinical testing procedure should be practical. (c) It is difficult to detect a single miRNA with low specificity. Studies have shown that the reproducibility of network marking is higher than that for single biomarkers. A target network for the miRNAs was constructed, and GO and KEGG pathway enrichment analyses were performed. The enrichment results show that the target genes of these miRNAs are related to many significant pathways [45], such as the PI3K-Akt, TGF-beta, Jak-STAT, NF-kappa B, and Notch signaling pathways. These genes are also involved in many cellular processes, such as focal adhesion, the cell cycle, regulation of the actin cytoskeleton, the p53-signaling pathway, and apoptosis, along with certain diseases, such as various cancers, immune diseases, and cardiovascular diseases [1, 4, 6, 9, 15].

Conclusions

In conclusion, this study provides evidence that multiple miRNAs obtained from various samples exhibit relatively high diagnostic accuracy for CAD. This approach is considered more appropriate for use in Asian populations. Therefore, miRNAs can serve as potential biomarkers for CAD detection.

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Disclosure of conflict of interest

None.

Abbreviations

CAD, coronary artery diseases; miRNA, microRNA; PBMCs, peripheral blood mononuclear cells; PRISMA, Preferred Reporting Items for

Systematic Reviews and Meta-Analyses; ISH, in-situ hybridization.

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Checklist S1. PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	2
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	2
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	2
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	3
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	2
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	3
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	3
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	3
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	3
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	3
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	3
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	3
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	3
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	4
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	4
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	6
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	4
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	5
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	6
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	5
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	7
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	7
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	7
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	9

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097. For more information, visit: www.prisma-statement.org.

Table S1. List of eligible studies

Number	PMID	First Author	Year	Reference
1	20159880	GK Wang	2010	[1]
2	20230787	Hoekstra	2010	[2]
3	20595655	Fichtlscherer	2010	[3]
4	21785714	Weber	2011	[4]
5	22022480	Sondermeijer	2011	[5]
6	24053180	F Wang	2013	[6]
7	24247647	K Li	2013	[7]
8	24260372	Alessandra	2013	[8]
9	24339880	JY Ren	2013	[9]
10	24525789	GF Zhu	2014	[10]
11	24913032	F Chen	2014	[11]
12	24998411	SF Li	2014	[12]
13	25385173	Satoh	2015	[13]
14	25415674	FQ Liu	2014	[14]
15	25466836	D Liu	2015	[15]
16	25657313	TX Huan	2015	[16]
17	25728840	C Chen	2015	[17]
18	25760478	YM Chen	2015	[18]
19	25933289	XD Li	2015	[19]
20	26101645	X Chen	2014	[20]
21	26175229	Y Tang	2015	[21]
22	26184978	ZL Xu	2015	[22]
23	26248417	SJ Yang	2014	[23]
24	26383248	SH Li	2015	[24]
25	26485305	S. Niculescu	2015	[25]
26	26537670	W Liu	2015	[26]
27	26685009	JQ Zhou	2015	[27]

Table S2. Quality assessment table according to the MIAME and MIQE guideline

Number	Source of bias	Raw data of hybridization	Actual data processing	Sample annotation and experimental variables	Experiment design	Annotation of array design	Experimental and data processing protocols
1	GK Wang	NR	S	S	S	I	S
2	Hoekstra	NR	S	S	S	I	I
3	Fichtlscherer	NR	S	I	I	S	S
4	Weber	NR	S	S	S	I	S
5	Sondermeijer	S	S	S	S	S	S
6	F Wang	NR	S	I	S	I	S
7	K Li	NR	I	S	I	I	S
8	Alessandra	NR	S	S	S	S	S
9	JY Ren	S	S	S	I	S	S
10	GF Zhu	NR	S	S	S	I	S
11	F Chen	NR	I	I	S	I	S
12	SF Li	S	S	S	S	S	S
13	Satoh	NR	S	I	S	I	S
14	FQ Liu,	S	S	S	S	I	S
15	D Liu	S	S	S	S	I	S
16	TX Huan	S	S	S	S	I	S
17	C Chen	S	S	S	S	S	S

18	YM Chen	S	S	I	I	I	S
19	XD Li	S	S	S	S	S	S
20	X Chen	NR	I	S	I	I	S
21	Y Tang	S	S	I	S	S	S
22	ZL Xu	S	S	S	S	S	S
23	SJ Yang	NR	I	S	I	I	I
24	SH Li	S	S	I	S	I	S
25	S. Niculescu	NR	S	S	S	S	S
26	W Liu	NR	S	S	S	S	S
27	JQ Zhou	S	S	S	S	S	S

Table S3. Statistically significant regulation of miRNAs in the overall meta-analysis

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
UP-Regulated	miR-451a	10 (9$*3, 11*2, 12, 5, 25*3)	325	804.65	[227.0671; 2851.429]	3.591551E-24
	miR-122-5p	8 (19*2, 8*2, 17, 25*3)	323	1404.20	[344.0536; 5731.025]	4.438850E-23
	miR-223-3p	8 (9, 25*3, 12*3, 17)	269	920.41	[224.3162; 3776.636]	2.135212E-20
	miR-30a-5p	7 (9, 25*3, 12, 17*2)	247	1095.82	[243.1085; 4939.464]	5.735256E-19
	miR-26b-5p	7 (9, 25*2, 12*3, 17)	227	838.63	[185.1471; 3798.612]	1.718009E-17
	miR-125a-5p	5 (25*3, 14, 17)	203	1475.87	[249.4895; 8730.594]	4.288881E-15
	miR-486-5p	5 (25*3, 12, 14)	201	1420.82	[239.9847; 8411.958]	6.220239E-15
	miR-146a-5p	5 (9, 25*3, 12)	187	1202.72	[202.7042; 7136.287]	2.933477E-14
	miR-433-3p	4 (8*4)	186	2128.52	[293.4078; 15441.35]	1.388248E-13
	miR-485-3p	4 (8*4)	186	2128.52	[293.4078; 15441.35]	1.388248E-13
	let-7b-5p	4 (25*3, 14)	183	1982.32	[272.9296; 14397.88]	2.795217E-13
	miR-499a-5p	3 (1*2, 20)	209	4778.57	[488.8824; 46708.17]	9.762365E-13
	miR-20b-5p	5 (9, 12, 22*3)	202	823.18	[137.1744; 4939.902]	1.046520E-12
	miR-140-5p	4 (9, 25*3)	169	1612.86	[221.3910; 11749.97]	1.244082E-12
	miR-103a-3p	4 (25*3, 17)	163	1428.40	[195.5843; 10432.08]	3.210614E-12
	miR-126-5p	5 (9*3, 12, 17)	142	597.18	[99.2474; 3593.291]	1.463160E-11
	miR-135b-5p	4 (22*4)	280	2136.53	[240.5846; 18973.67]	2.379413E-11
	miR-499a-3p	4 (22*4)	280	2136.53	[240.5846; 18973.67]	2.379413E-11
	miR-19b-3p	5 (9, 12*3, 17)	126	555.71	[92.466; 3339.833]	2.471719E-11
	miR-340-3p	3 (5*3)	171	2666.47	[270.4662; 26288.20]	4.242679E-11
	miR-624-5p	3 (5*3)	171	2666.47	[270.4662; 26288.20]	4.242679E-11
	miR-337-5p	3 (8*3)	147	2340.65	[237.7093; 23047.83]	8.890144E-11
	miR-100-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.759721E-10
	miR-423-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.759721E-10
	let-7a-5p	3 (25*2, 14)	141	2066.69	[209.4689; 20390.72]	1.895395E-10
	let-7c-5p	3 (25*2, 14)	141	2066.69	[209.4689; 20390.72]	1.895395E-10
	let-7e-5p	3 (25*2, 14)	141	2066.69	[209.4689; 20390.72]	1.895395E-10
	let-7f-5p	3 (25*2, 14)	141	2066.69	[209.4689; 20390.72]	1.895395E-10
	miR-720	4 (19*3, 12)	393	2011.43	[186.6697; 21674.03]	1.431668E-09
	miR-143-3p	3 (25*2, 17)	121	1334.64	[134.1280; 13280.42]	2.494006E-09
	miR-2861	3 (19*2, 26)	304	5061.34	[315.3606; 81231.51]	5.142966E-09
	miR-107	3 (25*2, 17)	108	1166.49	[117.0920; 11620.83]	5.204055E-09
	miR-125b-5p	3 (25*2, 17)	108	1166.49	[117.0920; 11620.83]	5.204055E-09
	miR-206	4 (27, 14, 21*2)	267	1959.37	[159.0424; 24139.19]	1.316418E-08
	miR-590-5p	3 (9*3)	104	780.54	[77.0719; 7904.877]	5.161576E-08
	miR-186-5p	3 (12, 14, 17)	78	649.67	[64.5170; 6542.134]	1.164368E-07
	miR-365b-3p	2 (25*2)	101	2291.09	[138.9464; 37778.11]	1.257426E-07
	miR-133b	2 (8*2)	93	2128.52	[129.1209; 35088.14]	1.669327E-07
	miR-135a-5p	2 (2*2)	90	2091.00	[126.8832; 34459.09]	1.782451E-07
	miR-138-1-3p	3 (22*3)	158	1116.91	[86.8406; 14365.41]	2.168528E-07
	miR-147b	3 (22*3)	158	1116.91	[86.8406; 14365.41]	2.168528E-07
	miR-218-2-3p	3 (22*3)	158	1116.91	[86.8406; 14365.41]	2.168528E-07

	miR-302f	3 (22*3)	158	1116.91	[86.8406; 14365.41]	2.168528E-07
	miR-181b-5p	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.776175E-07
	miR-142-3p	3 (9, 12, 17)	64	488.67	[48.2973; 4944.535]	4.723176E-07
	miR-425-5p	3 (9, 12, 17)	64	488.67	[48.2973; 4944.535]	4.723176E-07
	miR-454-5p	3 (11*2, 5)	60	405.19	[39.7590; 4129.423]	1.198337E-06
	miR-340-5p	3 (11*2, 17)	56	360.05	[35.2295; 3679.914]	2.077915E-06
	miR-214-3p	2 (17*2)	60	867.48	[51.6509; 14569.46]	5.192325E-06
	miR-144-3p	2 (19, 25)	62	775.44	[45.8637; 13110.96]	7.991914E-06
	miR-423-5p	2 (14, 16)	412	15444.1	[203.8519; 1170066]	2.505374E-05
	miR-505-5p	2 (14, 16)	412	15444.1	[203.8519; 1170066]	2.505374E-05
	miR-192-5p	2 (9, 12)	44	514.34	[30.2566; 8743.639]	3.138086E-05
	miR-374a-5p	2 (9, 12)	44	514.34	[30.2566; 8743.639]	3.138086E-05
	miR-615-5p	2 (3, 5)	40	426.47	[24.9332; 7294.645]	5.828535E-05
	miR-197-3p	2 (12, 17)	38	399.09	[23.3076; 6833.685]	7.170344E-05
	miR-1264	2 (22, 17)	38	362.46	[21.0083; 6253.646]	1.000779E-04
	miR-140-3p	2 (19, 12)	34	323.10	[18.7276; 5574.511]	1.399500E-04
	miR-545-3p	2 (11*2)	36	325.00	[18.7679; 5627.97]	1.406206E-04
	miR-585-3p	2 (11*2)	36	325.00	[18.7679; 5627.97]	1.406206E-04
	miR-624-3p	2 (11*2)	36	325.00	[18.7679; 5627.97]	1.406206E-04
	miR-3149	2 (19*2)	144	2203.01	[43.2116; 112314.35]	2.485980E-04
	miR-25-3p	9 (9*3, 25*3, 3, 12, 17)	301	235.70	[8.0677; 6886.3715]	1.359954E-02
	miR-320a	8 (25*3, 3, 12, 17*3)	337	312.82	[6.5026; 15049.1416]	2.916522E-02
	miR-21-5p	20 (24*3, 25*3, 12, 22*3, 17*3, 23*2, 18, 3, 9*3)	876	91.55	[4.9094; 1707.2806]	4.958330E-02
Down-Regulated	miR-379-5p	4 (14, 22*3)	198	1246.79	[168.9913; 9198.663]	1.092222E-11
	miR-31-5p	2 (13*2)	122	3403.00	[207.5701; 55790.36]	2.413008E-08
	miR-147a	2 (2*2)	90	2091.00	[126.8832; 34459.09]	1.782451E-07
	miR-17-3p	2 (11*2)	36	325.00	[18.7679; 5627.97]	1.406206E-04
	miR-154-5p	2 (11*2)	36	325.00	[18.7679; 5627.97]	1.406206E-04
	miR-98-5p	2 (22*2)	140	2106.17	[47.9131; 92583.43]	1.470635E-04

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S4. Statistically significant dysregulation of miRNAs in the plasma

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-122-5p	5 (19$*2, 8*2, 17)	180	1102.00	[185.3742; 6551.132]	6.6847E-14
	miR-433-3p	4 (8*4)	186	2128.52	[293.4078; 15441.35]	1.3882E-13
	miR-485-3p	4 (8*4)	186	2128.52	[293.4078; 15441.35]	1.3882E-13
	miR-337-5p	3 (8*3)	147	2340.66	[237.7093; 23047.83]	8.8901E-11
	miR-126-5p	4 (9*2, 12, 17)	132	872.96	[118.5405; 6428.614]	1.1922E-10
	miR-19b-3p	4 (9, 12*2, 17)	114	740.59	[100.4203; 5461.745]	3.6350E-10
	miR-26b-5p	4 (9, 12*2, 17)	114	740.59	[100.4203; 5461.745]	3.6350E-10
	miR-223-3p	4 (9, 12*2, 17)	114	740.59	[100.4203; 5461.745]	3.6350E-10
	miR-30a-5p	4 (9, 12, 17*2)	104	668.72	[90.5853; 4936.629]	7.1721E-10
	miR-720	4 (19*3, 12)	393	2011.44	[186.6697; 21674.03]	1.4317E-09
	miR-206	2 (27, 14)	174	5590.41	[342.1162; 91351.06]	2.8299E-09
	miR-499a-5p	2 (1, 20)	146	5166.53	[316.8575; 84243.00]	3.8723E-09
	miR-2861	3 (19*2, 26)	304	5061.35	[315.3606; 81231.51]	5.1430E-09
	miR-451a	3 (9*2, 12)	112	1092.97	[109.3713; 10922.23]	7.6920E-09
	miR-186-5p	3 (12, 14, 17)	78	649.68	[64.5170; 6542.134]	1.1644E-07
	miR-133b	2 (8*2)	93	2128.52	[129.1209; 35088.14]	1.6693E-07
	miR-590-5p	2 (9*2)	94	1875.01	[113.2016; 31056.47]	2.8525E-07
	miR-142-3p	3 (9, 12, 17)	64	488.68	[48.2973; 4944.535]	4.7232E-07
	miR-425-5p	3 (9, 12, 17)	64	488.68	[48.2973; 4944.535]	4.7232E-07
	miR-22-3p	2 (14, 17)	60	867.48	[51.6509; 14569.46]	5.1923E-06

	miR-125a-5p	2 (14, 17)	60	867.48	[51.6509; 14569.46]	5.1923E-06
	miR-214-3p	2 (17*2)	60	867.48	[51.6509; 14569.46]	5.1923E-06
	miR-486-5p	2 (12, 14)	58	787.19	[46.7145; 13264.99]	7.4024E-06
	miR-20b-5p	2 (9, 12)	44	514.35	[30.2566; 8743.639]	3.1381E-05
	miR-146a-5p	2 (9, 12)	44	514.35	[30.2566; 8743.639]	3.1381E-05
	miR-192-5p	2 (9, 12)	44	514.35	[30.2566; 8743.639]	3.1381E-05
	miR-374a-5p	2 (9, 12)	44	514.35	[30.2566; 8743.639]	3.1381E-05
	miR-197-3p	2 (12, 17)	38	399.10	[23.3076; 6833.685]	7.1703E-05
	miR-140-3p	2 (19, 12)	34	323.11	[18.7276; 5574.511]	1.3995E-04
	miR-3149	2 (19*2)	144	2203.02	[43.2116; 112314.35]	2.4860E-04
	miR-21-5p	9 (9*2, 3, 12, 17*3, 23*2)	364	326.50	[10.1376; 10515.2467]	9.7672E-03
Down-regulated	miR-155-5p	3 (10*2, 3)	170	3152.83	[321.2375; 30943.79]	1.4184E-11
	miR-31-5p	2 (13*2)	122	3403.00	207.5701; 55790.36]	2.4130E-08

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S5. Statistically significant dysregulation of miRNAs in the serum

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-135b-5p	4 (22$*4)	280	2136.53	[240.5846; 18973.67]	2.3794E-11
	miR-499b-3p	4 (22*4)	280	2136.53	[240.5846; 18973.67]	2.3794E-11
	let-7b-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	let-7d-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-15b-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-16-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-23a-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-23b-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-24-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-25-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-30a-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-30d-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-30e-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-93-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-100-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-103a-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-122-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-125a-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-140-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-146a-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-195-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-221-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-223-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-320a	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-423-3p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-424-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-451a	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	miR-486-5p	3 (25*3)	143	2094.18	[212.2395; 20663.43]	1.7597E-10
	let-7a-5p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	let-7c-5p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	let-7f-5p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	miR-26b-5p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	miR-27a-3p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	miR-143-3p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	miR-342-3p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	miR-365b-3p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.2574E-07
	let-7e-5p	2 (25*2)	101	2291.10	[138.9464; 37778.11]	1.3053E-07
	miR-20b-5p	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07

	miR-138-1-3p	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07
	miR-142-5p	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07
	miR-147b	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07
	miR-218-2-3p	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07
	miR-302f	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07
	miR-29b-3p	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.7762E-07
	miR-107	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.7762E-07
	miR-125b-5p	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.7762E-07
	miR-130a-3p	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.7762E-07
	miR-150-5p	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.7762E-07
	miR-181b-5p	2 (25*2)	88	1876.44	[113.5572; 31006.72]	2.7762E-07
Down-regulated	miR-379-5p	3 (22*3)	158	1116.92	[86.8406; 14365.41]	2.1685E-07
	miR-98-5p	2 (22*2)	140	2106.17	[47.9131; 92583.43]	1.4706E-04

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S6. Statistically significant dysregulation of miRNAs in the platelet

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-624-5p	3 (5$*3)	171	2666.471	[270.4662; 26288.20]	4.2427E-11
	miR-451a	3 (11*2, 5)	60	405.1936	[39.7590; 4129.423]	1.1983E-06
	miR-454-5p	3 (11*2, 5)	60	405.1936	[39.7590; 4129.423]	1.1983E-06
	miR-340-3p	3 (5*3)	171	2666.471	[270.4662; 26288.20]	1.0000E-04
	miR-340-5p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
	miR-545-3p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
	miR-585-3p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
	miR-624-3p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
Down-regulated	miR-17-3p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
	miR-154-5p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
	miR-199a-5p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04
	miR-339-5p	2 (11*2)	36	325	[18.7679; 5627.97]	1.4062E-04

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S7. Statistically significant dysregulation of miRNAs in the PBMCs

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-21-5p	3 (24$*3)	150	2580.28	[262.4922; 25364.01]	4.8530E-11
	miR-135a-5p	2 (2*2)	90	2091.00	[126.8832; 34459.09]	1.7825E-07
Down-regulated	miR-147a	2 (2*2)	90	2091.00	[126.8832; 34459.09]	1.7825E-07
	miR-155-5p	3 (10*3)	172	3357.16	[342.4389; 32912.53]	9.3394E-12

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S8. Statistically significant dysregulation of miRNAs in different tissues

	PBMCs	Platelet	Serum	Plasma
miR-451a		↑	↑	↑
miR-122-5p			↑	↑
miR-125a-5p			↑	↑
miR-146a-5p			↑	↑
miR-155-5p	↓			↓
miR-20b-5p			↑	↑
miR-21-5p	↑			↑
miR-223-3p			↑	↑
miR-26b-5p			↑	↑
miR-30a-5p			↑	↑
miR-486-5p			↑	↑
Let-7a-5p			↑	
Let-7b-5p			↑	
Let-7c-5p			↑	
Let-7d-5p			↑	
Let-7e-5p			↑	
Let-7f-5p			↑	
miR-100-5p			↑	
miR-103a-3p			↑	
miR-107			↑	
miR-125b-5p			↑	
miR-126-5p				↑
miR-130a-3p			↑	
miR-133b				↑
miR-135a-5p	↑			
miR-135b-5p			↑	
miR-138-1-3p			↑	
miR-140-3p				↑
miR-140-5p			↑	
miR-142-3p				↑
miR-142-5p			↑	
miR-143-3p			↑	
miR-147a	↓			
miR-147b			↑	
miR-150-5p			↑	
miR-154-5p		↓		
miR-15b-5p			↑	
miR-16-5p			↑	
miR-17-3p		↓		
miR-181b-5p			↑	
miR-186-5p				↑
miR-192-5p				↑
miR-195-5p			↑	
miR-197-3p				↑
miR-199a-5p		↓		
miR-19b-3p				↑
miR-206				↑
miR-214-3p				↑
miR-218-2-3p			↑	
miR-221-3p			↑	
miR-22-3p				↑
miR-23a-3p			↑	
miR-23b-3p			↑	
miR-24-3p			↑	
miR-25-3p			↑	
miR-27a-3p			↑	
miR-2861				↑
miR-29b-3p			↑	
miR-302f			↑	
miR-30d-5p			↑	
miR-30e-5p			↑	
miR-3149				↑
miR-31-5p				↓
miR-320a			↑	
miR-337-5p				↑
miR-339-5p		↓		
miR-340-3p		↑		
miR-340-5p		↑		
miR-342-3p			↑	
miR-365b-3p			↑	
miR-374a-5p				↑
miR-379-5p			↓	
miR-423-3p			↑	
miR-424-5p			↑	
miR-425-5p				↑
miR-433-3p				↑
miR-454-5p		↑		
miR-485-3p				↑
miR-499a-5p				↑
miR-499b-3p			↑	
miR-545-3p		↑		
miR-585-3p		↑		
miR-590-5p				↑
miR-624-3p		↑		
miR-624-5p		↑		
miR-720				↑
miR-93-5p			↑	
miR-98-5p			↓	

Table S9. Statistically significant dysregulation of miRNAs between MI patients and health controls

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-133a-3p	4 (1$*3, 6)	229	3180.142	[439.9026; 22989.87]	5.3701E-15
	miR-499a-5p	3 (1*2, 20)	209	4778.577	[488.8824; 46708.17]	9.7624E-13
	miR-1-3p	3 (1*3)	189	4087	[417.6918; 39990.18]	2.6850E-12
	miR-21-5p	3 (24, 25, 23)	120	1519.418	[153.2801; 15061.53]	1.1563E-09
	miR-122-5p	3 (19*2, 25)	109	1116.172	[111.7429; 11149.18]	6.8452E-09
	miR-208a-3p	2 (1*2)	126	4087	[250.17; 66768.87]	1.0791E-08
	miR-720	3 (19*3)	375	3516.286	[180.9323; 68336.41]	2.0702E-07
	miR-2861	2 (19*2)	144	2203.017	[43.2116; 112314.35]	2.4860E-04
	miR-3149	2 (19*2)	144	2203.017	[43.2116; 112314.35]	2.4860E-04

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S10. Statistically significant dysregulation of miRNAs between UA patients and health controls

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-30d-5p	2$ (9, 25)	81	1385.241	[83.2331; 23054.46]	9.2202E-07
	miR-93-5p	2 (9, 25)	81	1385.241	[83.2331; 23054.46]	9.2202E-07
	miR-140-5p	2 (9, 25)	81	1385.241	[83.2331; 23054.46]	9.2202E-07
	miR-320a	2 (25, 12)	73	984.6276	[58.5502; 16558.28]	3.3986E-06
	miR-486-5p	2 (25, 12)	73	984.6276	[58.5502; 16558.28]	3.3986E-06
	miR-106b-5p	3 (9*2, 12)	54	321.9263	[31.3295; 3307.956]	3.5614E-06
	miR-126-5p	3 (9*2, 12)	54	321.9263	[31.3295; 3307.956]	3.5614E-06
	miR-126-3p	2 (8, 12)	57	767.3145	[45.5151; 12935.73]	8.0909E-06
	miR-20a-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-20b-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-26a-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-29a-3p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-106a-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-142-3p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-192-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-374a-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-375	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-425-5p	2 (9, 12)	44	514.347	[30.2566; 8743.639]	3.1381E-05
	miR-590-5p	2 (9*2)	36	303.8289	[17.4316; 5295.657]	1.7710E-04

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S11. Statistically significant dysregulation of miRNAs between SA patients and health controls

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-451a	2 (9$, 25)	114	3089.71	[188.2698; 50705.50]	3.6233E-08
	miR-337-5p	2 (8*2)	108	2829.00	[172.2689; 46457.84]	5.2127E-08
	miR-433-3p	2 (8*2)	108	2829.00	[172.2689; 46457.84]	5.2127E-08
	miR-485-3p	2 (8*2)	108	2829.00	[172.2689; 46457.84]	5.2127E-08
	miR-133a-3p	2 (8, 3)	107	2688.63	[163.5309; 44203.9]	6.4752E-08
	miR-122-5p	2 (8, 25)	100	2387.15	[144.9746; 39306.78]	1.0544E-07

Down-regulated	miR-145-5p	2 (3*2)	98	2161.27	[130.9400; 35673.43]	1.5949E-07
	miR-155-5p	2 (3*2)	98	2161.27	[130.9400; 35673.43]	1.5949E-07

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: The paper IDs are from the "Number" column in Table S1.

Table S12. Statistically significant dysregulation of miRNAs in different phenotypes of CAD

	MI	UA	SA
miR-122-5p	↑		↑
miR-133a-3p	↑		↑
miR-106a-5p		↑	
miR-106b-5p		↑	
miR-126-3p		↑	
miR-126-5p		↑	
miR-1-3p	↑		
miR-140-5p		↑	
miR-142-3p		↑	
miR-145-5p			↓
miR-155-5p			↓
miR-192-5p		↑	
miR-208a-3p	↑		
miR-20a-5p		↑	
miR-20b-5p		↑	
miR-21-5p	↑		
miR-26a-5p		↑	
miR-2861	↑		
miR-29a-3p		↑	
miR-30d-5p		↑	
miR-3149	↑		
miR-320a		↑	
miR-337-5p			↑
miR-374a-5p		↑	
miR-375		↑	
miR-425-5p		↑	
miR-433-3p			↑
miR-451a			↑
miR-485-3p			↑
miR-486-5p		↑	
miR-499a-5p	↑		
miR-590-5p		↑	
miR-720	↑		
miR-93-5p		↑	

Table S13. Statistically significant dysregulation of miRNAs in sensitivity analysis based on RT-PCR detection method

	miRNA Name	No. of sub-studies (Paper#)	No. of samples	LogOR	[95% CI]	Adjusted *p* value
Up-regulated	miR-21-5p	10 (24@*3, 9*2, 18, 17*2, 23*2)	495	1914.31	[544.6310; 6728.590]	4.7597E-31
	miR-499a-5p	3 (1*2, 20)	209	4778.58	[488.8824; 46708.17]	9.7624E-13
	miR-2861	2 (19, 26)	288	20189.38	[1250.317; 326006.0]	5.7211E-12
	miR-720	2 (19*2)	359	11904.47	[612.6112; 231331.8]	1.1327E-09
	miR-340-3p	2 (5*2)	147	5407.92	[331.8715; 88123.11]	3.1498E-09
	miR-624-5p	2 (5*2)	147	5407.92	[331.8715; 88123.11]	3.1498E-09
	miR-320a	2 (17*2)	140	4167.68	[254.6057; 68221.28]	1.0188E-08
	miR-135a-5p	2 (2*2)	90	2091.00	[126.8832; 34459.09]	1.7825E-07
	miR-19b-3p	2 (12*2)	62	674.17	[39.4912; 11509.14]	1.3639E-05
	miR-24-3p$	2 (12*2)	62	674.17	[39.4912; 11509.14]	1.3639E-05
	miR-26b-5p	2 (12*2)	62	674.17	[39.4912; 11509.14]	1.3639E-05
	miR-223-3p	2 (12*2)	62	674.17	[39.4912; 11509.14]	1.3639E-05
	miR-206	3 (27, 21*2)	227	1999.63	[56.5359; 70725.17]	8.8329E-05
	miR-25-3p	2 (9*2)	78	788.27	[21.7400; 28582.07]	5.4378E-04
	miR-126-5p	2 (9*2)	78	788.27	[21.7400; 28582.07]	5.4378E-04
	miR-451a	2 (9*2)	78	788.27	[21.7400; 28582.07]	5.4378E-04
	miR-590-5p	2 (9*2)	78	788.27	[21.7400; 28582.07]	5.4378E-04
Down-regulated	miR-145-5p$	4 (3*2, 13, 4)	184	1802.33	[247.5237; 13123.55]	5.4043E-13
	miR-181a-5p$	2 (18, 13)	112	2965.17	[180.6269; 48676.16]	4.2952E-08
	miR-199a-5p$	2 (3*2)	98	2161.27	[130.9400; 35673.43]	1.5949E-07
	miR-147a	2 (2*2)	90	2091.00	[126.8832; 34459.09]	1.7825E-07

#: Presented in Paper *number of sub-studies reported in the Paper ("*1" is omitted). $: Statistically significant dysregulation of miRNAs only in sensitivity analysis. @: The paper IDs are from the "Number" column in Table S1.

Detailed results of the MetaCore analysis

Enrichment analysis

Enrichment analysis consists of matching gene IDs of possible targets for the “common”, “similar” and “unique” sets with gene IDs in functional ontologies in MetaCore. The probability of a random intersection between a set of IDs the size of target list with ontology entities is estimated in *p* value of hypergeometric intersection. The lower *p* value means higher relevance of the entity to the dataset, which shows in higher rating for the entity.

Pathway maps

Canonical pathway maps represent a set of signaling and metabolic maps covering human in a comprehensive way. All maps are created by Thomson Reuters scientists by a high quality manual curation process based on published peer reviewed literature. Experimental data is visualized on the maps as blue (for downregulation) and red (upregulation) histograms. The height of the histogram corresponds to the relative expression value for a particular gene/protein.

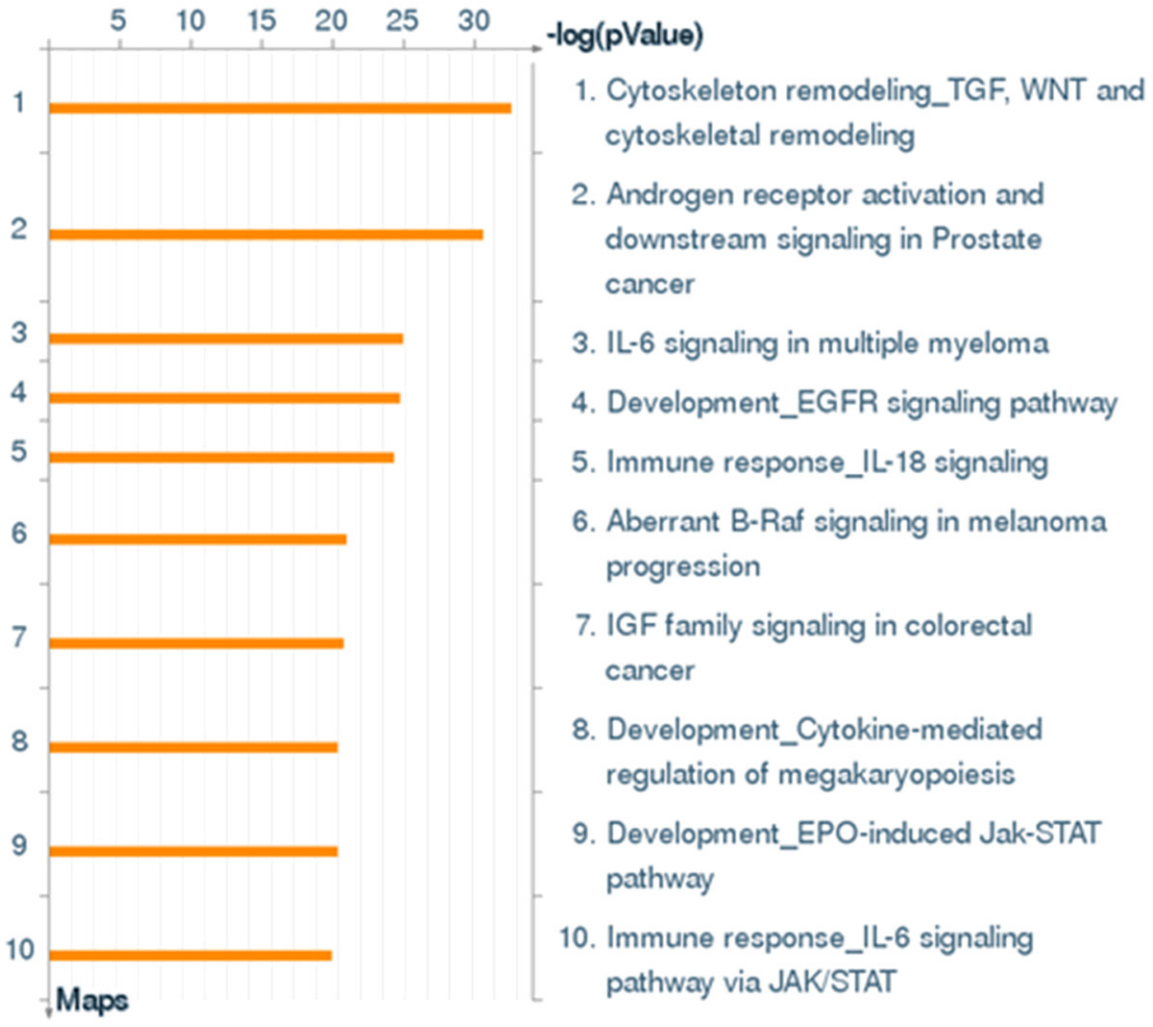

Figure S1. Pathway Maps. Sorting is done for the ‘Statistically significant Maps’.

Top 5 maps (sorted by Statistically Significant Maps)

(1). Map: Cytoskeleton remodeling_TGF, WNT and cytoskeletal remodeling.

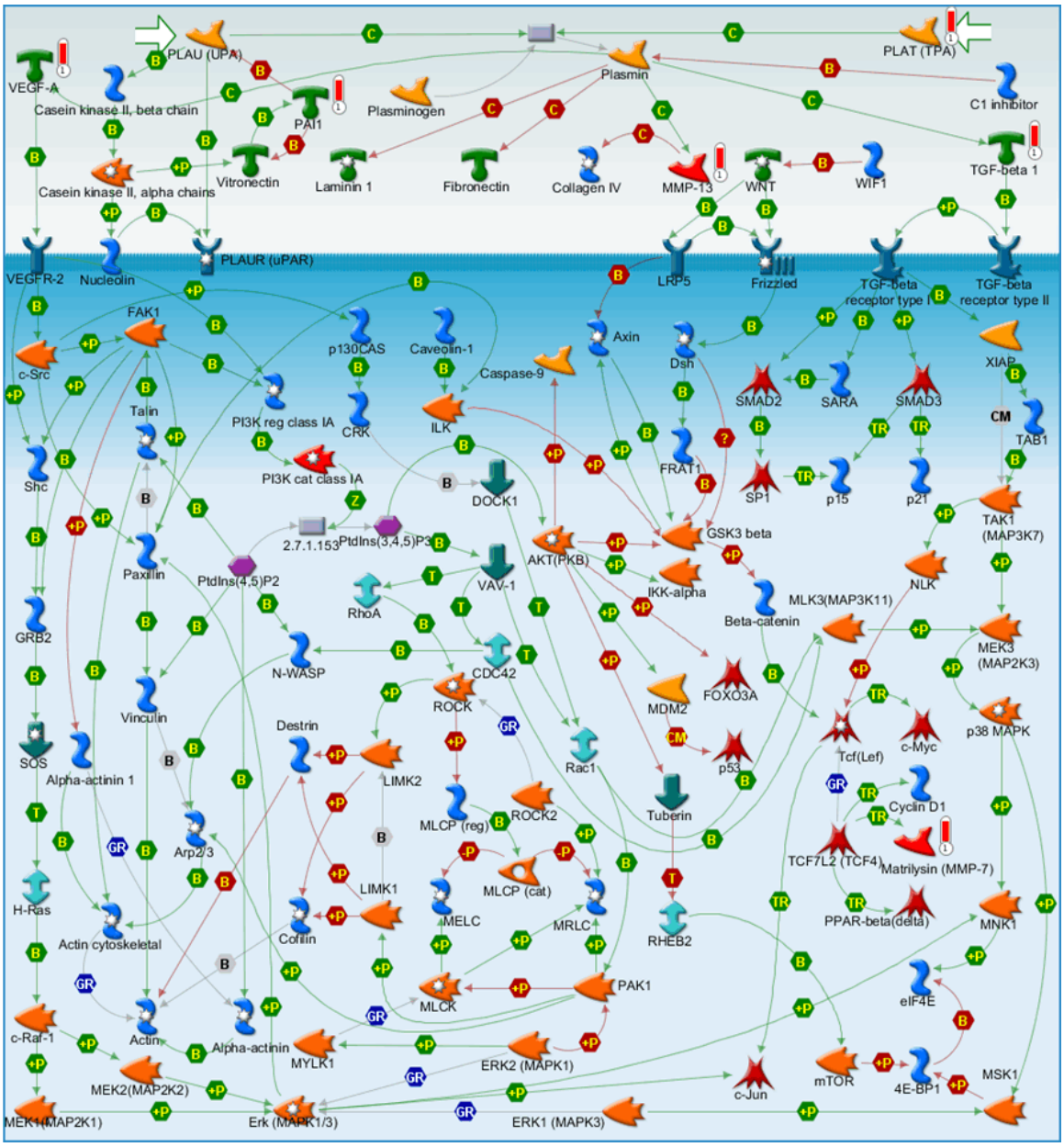

Figure S2. The top scored map (map with the lowest *p*-value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files is linked to and visualized on the maps as thermometer like figures.

(2). Map: Androgen receptor activation and downstream signaling in Prostate cancer.

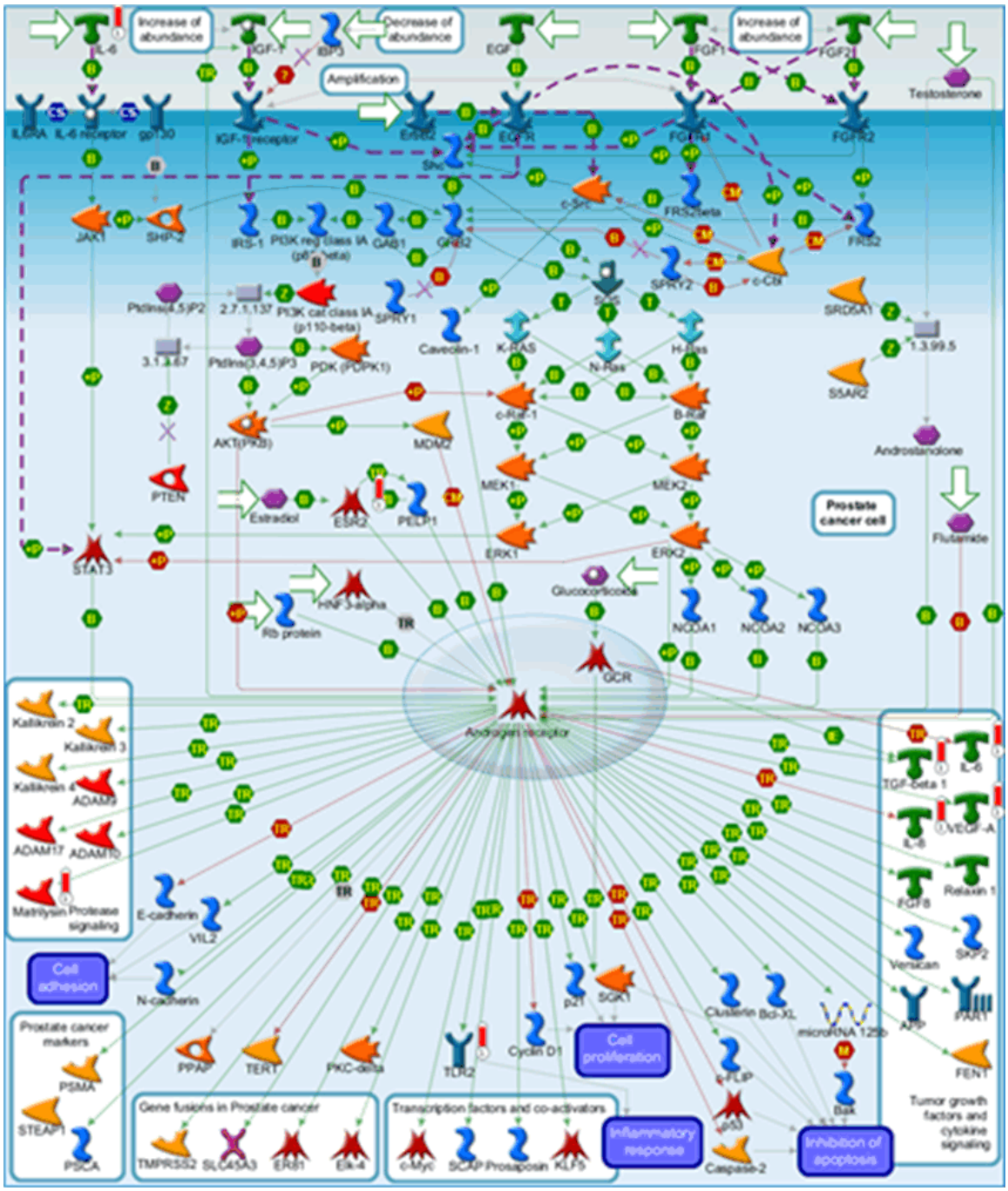

Figure S3. The second scored map (map with the lowest *p*-value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files is linked to and visualized on the maps as thermometer like figures.

(3). Map: IL 6 signaling in multiple myeloma.

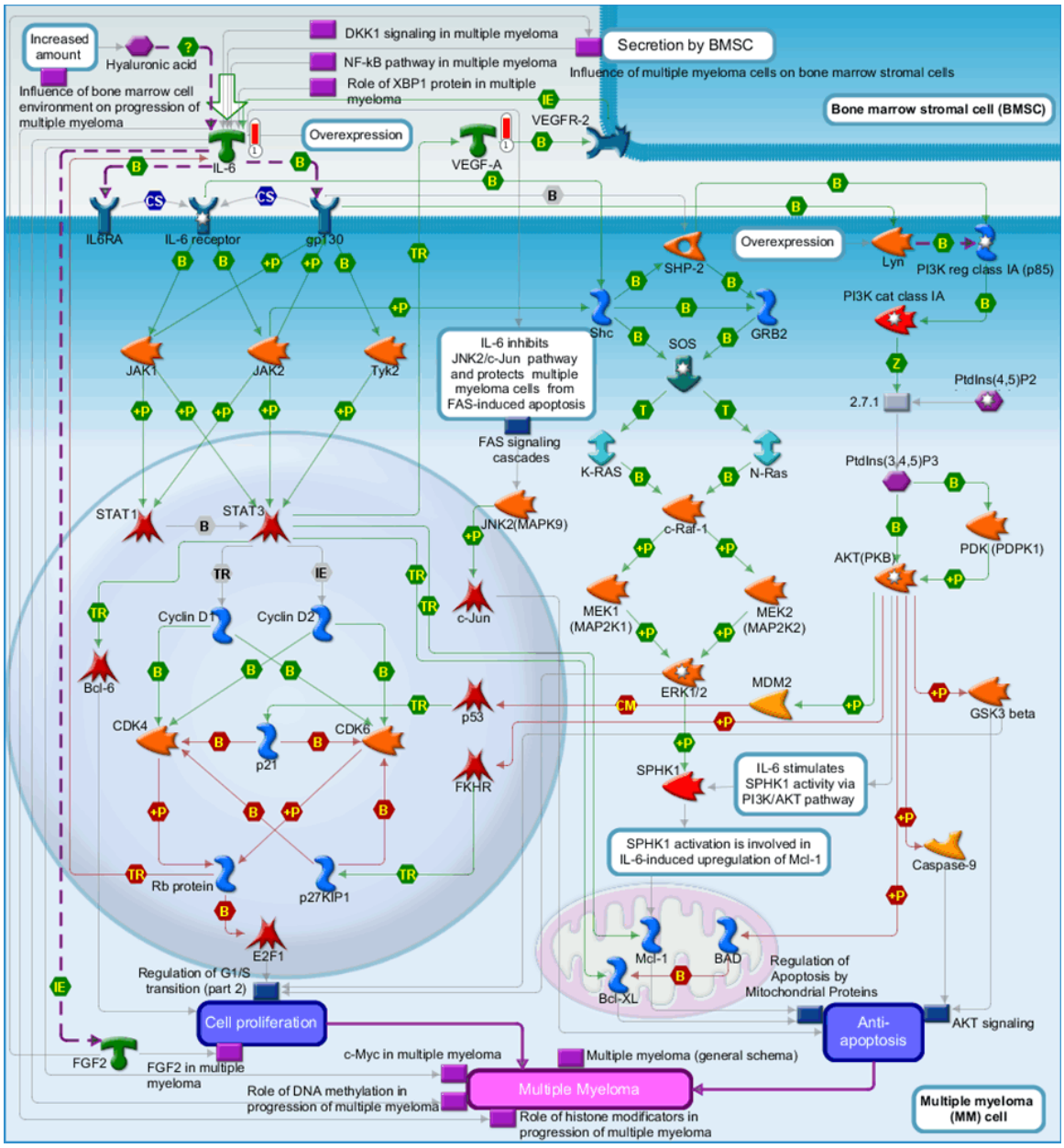

Figure S4. The third scored map (map with the lowest *p*-value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files is linked to and visualized on the maps as thermometer like figures.

(4). Map: Development_EGFR signaling pathway.

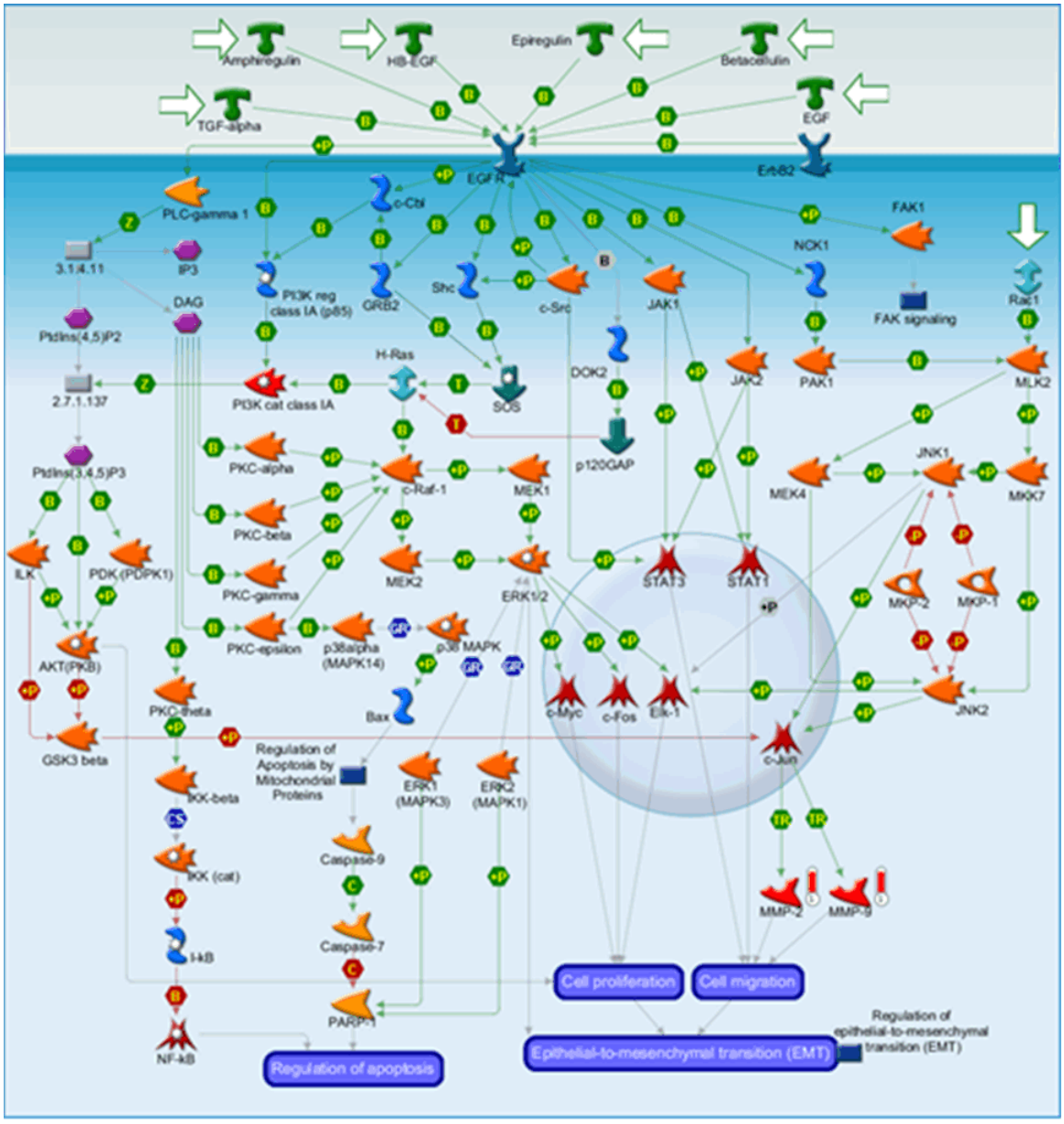

Figure S5. The fourth scored map (map with the lowest *p*-value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files is linked to and visualized on the maps as thermometer like figures.

(5). Map: Immune response_IL 18 signaling.

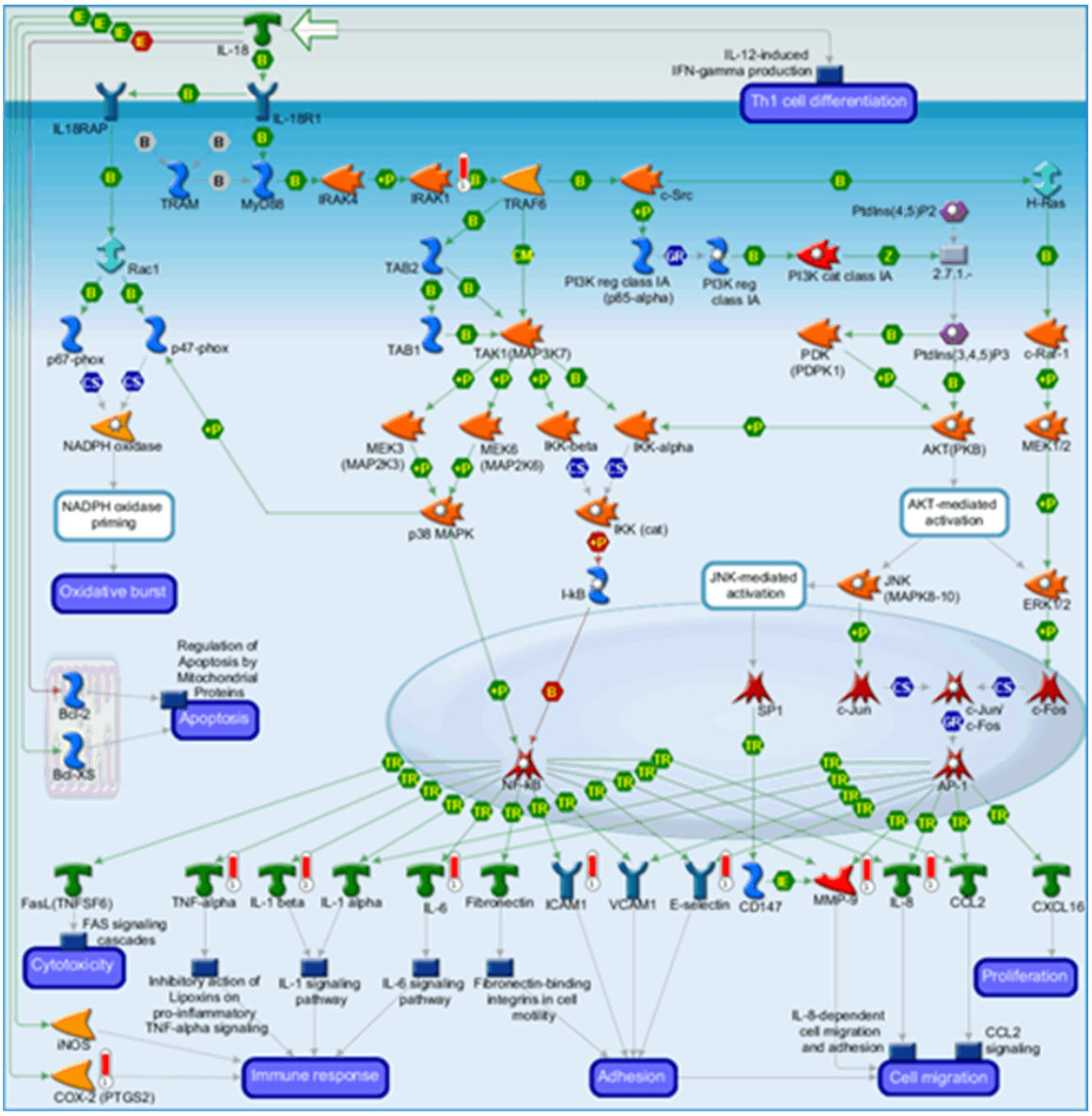

Figure S6. The fifth scored map (map with the lowest *p*-value) based on the enrichment distribution sorted by 'Statistically significant Maps' set. Experimental data from all files is linked to and visualized on the maps as thermometer like figures.

Process networks

The content of these cellular and molecular processes is defined and annotated by Thomson Reuters scientists. Each process represents a pre-set network of protein interactions characteristic for the process.

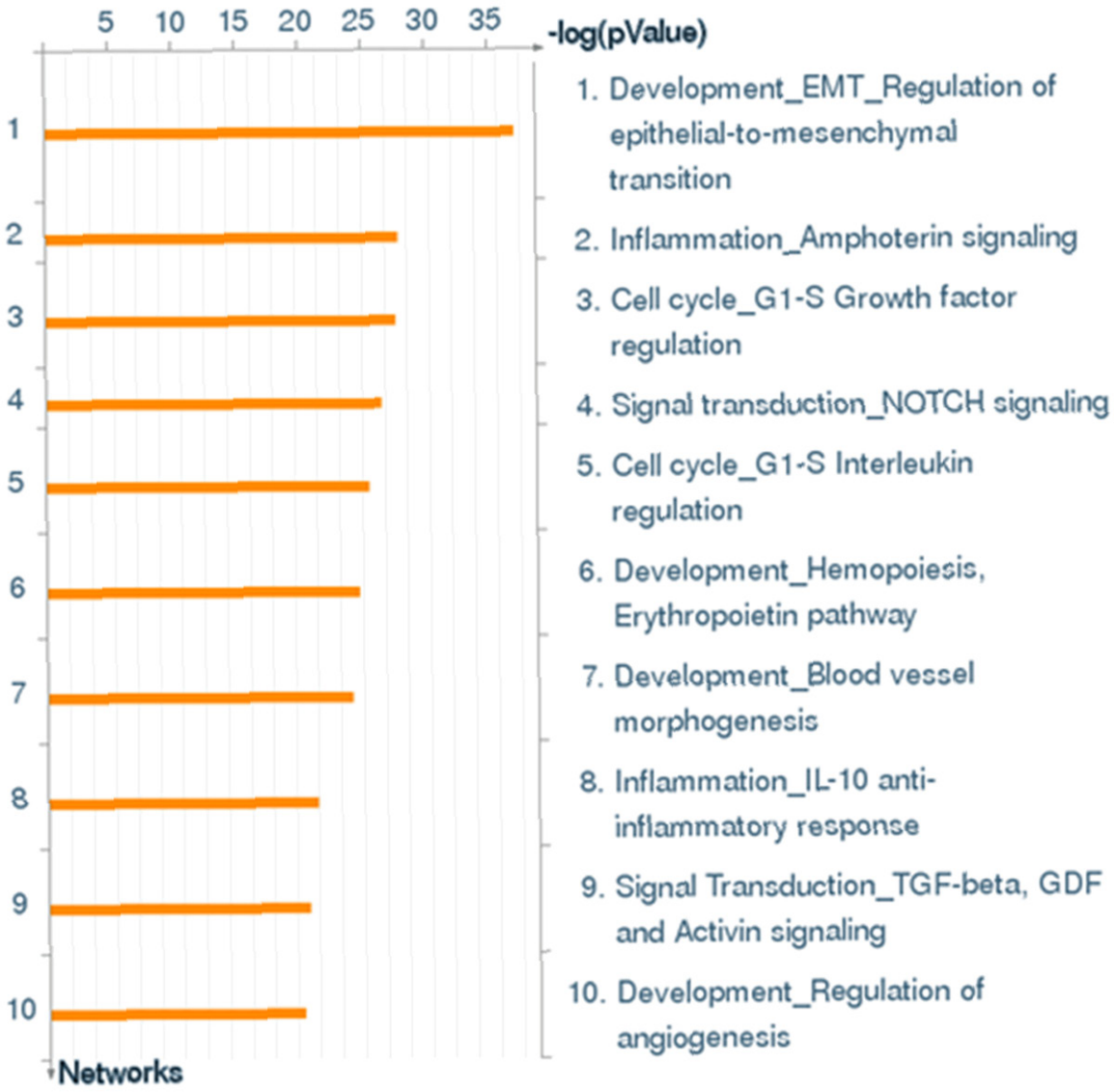

Figure S7. Process Networks. Sorting is done for the 'Statistically significant Networks'.

Diseases (by biomarkers)

Disease folders are organized into a hierarchical tree. Gene content may very greatly between such complex diseases as cancers and some Mendelian diseases. Also, coverage of different diseases in literature is skewed. These two factors may affect *p*-value prioritization for diseases.

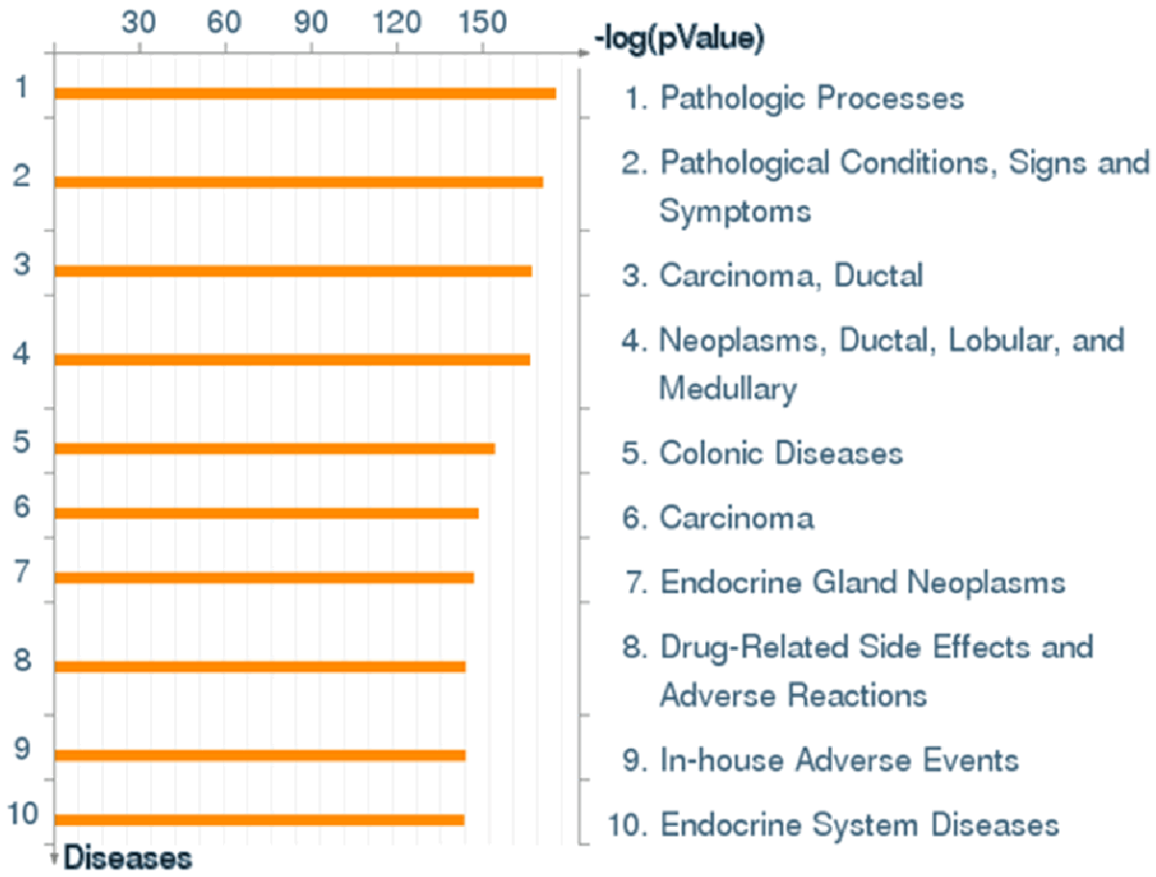

Figure S8. Diseases (by Biomarkers). Sorting is done for the 'Statistically significant Diseases'.