Identifying cancer-related microRNAs based on subpathways

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Abstract: MicroRNAs (miRNAs) are a class of small endogenous non-coding genes that play important roles in post-transcriptional regulation as well as other important biological processes. Accumulating evidence indicated that miRNAs were extensively involved in the pathology of cancer. However, determining which miRNAs are related to a specific cancer is problematic because one miRNA may target multiple genes and one gene may be targeted by multiple miRNAs. The authors proposed a new approach, named miR_SubPath, to identify cancer-associated miRNAs by three steps. The targeted genes were determined based on differentially expressed genes in significant dysfunctional subpathways. Then the candidate miRNAs were determined according to miRNA–genes associations. Finally, these candidate miRNAs were ranked based on their relations with some seed miRNAs in a functional similarity network. Results on real-world datasets showed that the proposed miR_SubPath method was more robust and could identify more cancer-related miRNAs than a prior approach, miR_Path, miR_Clust and Zhang's method.

1 Introduction

MicroRNAs (miRNAs) are a class of small ($\sim$22 nt) non-coding RNAs that may degrade their target genes or suppress their expression at the post-transcriptional stage [1, 2]. A single miRNA can usually regulate many genes, and different miRNAs could also target the same gene [3]. MiRNAs are involving in many critical processes, such as cell development, proliferation, differentiation, apoptosis, signal transduction and viral infection [4–6]. Therefore, they have critical influence in the initiation and progression of human cancers [7]. More and more miRNAs have been identified to associate to cancers. For example, miR-21 played a pivotal role in gastric cancer pathogenesis and progression [8], and altered expression of miR-21, miR-31, miR-143 and miR-145 were found to be related to colorectal cancer [9]. MiR-205 could suppress cell growth and invasion in breast cancer [10]. The up-regulation of miR-155 and down-regulation of let-7a were used to predict survival in patients with lung cancer [11].

The human miRNA disease database (HMDD) and miR2Disease database have collected hundreds of miRNAs related to various diseases [12, 13]. However, there are still many cancer-related miRNAs not being identified, so many approaches have been proposed to tackle this problem. For example, Kuo *et al.* developed a statistical approach, imputed miRNA regulation based on weighted ranked expression and putative miRNA targets, to detect differentially expressed miRNAs between normal and cancerous samples [14]. Li *et al.* [15] proposed to identify cancer related miRNAs through the functional consistency between the target genes and cancer. Zhang *et al.* [16] proposed to predict cancer-related miRNAs by investigating matched gene and miRNA expression data as well as miRNA-gene regulations. Other approaches, such as RWRMDA and RLSMDA, identify cancer miRNA through the Random Walk strategy in the miRNA–miRNA functional similarity network [17, 18]. Recently, Zhao *et al.* proposed two novel approaches, miR_Clust and miR_Path, to predict cancer-associated miRNAs based solely on differentially expressed genes (DEGs) [19]. For each miRNA, they first clustered all its possible target genes into different groups according to the Pearson's correlation coefficients of their expressions. MiR_Clust ranked each miRNA according to the discriminant power of its clusters for separating the cancers form the controls. MiR_Path

ranked each miRNA based on the enrichment score of each cluster on the dysfunctional pathways.

In recent years, more and more works showed that cancers were caused by the dysfunction in some small networks of the pathways, named subpathway, instead of the entire pathway [20–22]. Then a lot of methods had been proposed to detect cancer-related genes based on subpathways, such as DEgraph [23], the clipper approach [24], PATHWAYS [25], and sub-SPIA [26]. In this paper, we proposed a new approach, named miR_SubPath, to identify cancer-associated miRNAs based on genes significantly differentially expressed in some subpathways. Results on real-world datasets showed that the proposed miR_SubPath method was more robust and could identify more cancer-related miRNAs than prior approaches, miR_Path, miR_Clust and Zhang's method.

2 Materials and methods

2.1 Gene expression datasets

We used eight cancer datasets downloaded from NCBI's Gene Expression Omnibus (GEO) [27]: GSE7670 and GSE10072 for lung cancer, GSE9348 and GSE20916 for colon cancer, GSE13911 and GSE19826 for gastric cancer, and GSE37290 and GSE69428 for ovarian cancer. The number of samples and the platform of these datasets are presented in Table 1.

2.2 miRNA networks

In this paper, we used the relations in three miRNA networks to identify cancer related miRNAs: miRNA–gene associations, miRNA–miRNA functional similarity network and disease-miRNA networks. The relationship between miRNA and their targeting genes was the same as the one used by Zhao *et al.* [16]. First, they downloaded all candidate miRNAs from miRBase (version 16) [28]. Only those miRNA–gene relations affirmed by at least two tools, including PicTar [29], miRanda (version 3.0) [30], microT (version 5.0) [31], or TargetScan (release 6.2) [32], were retained. Second, they added these experimentally determined relations in TarBase (version 6.0) [33] to the candidate miRNAs. As miRNAs with similar functions are more likely associated with similar diseases, we downloaded the miRNA–miRNA functional similarity network including 271 miRNAs (http://cmbi.bjmu.edu.cn/misim).

Table 1 Eight gene expression datasets for four different types of cancers

Cancer	GEO accession number	Number of samples (case/control)	platform
colon	GSE9348	82 (70/12)	GPL570
	GSE20916	145 (101/44)	GPL570
lung	GSE7670	66 (36/30)	GPL96
	GSE10072	107 (58/59)	GPL96
gastric	GSE13911	69 (38/31)	GPL570
	GSE19826	27 (12/15)	GPL570
ovarian	GSE37290	20 (10/10)	GPL570
	GSE69428	20 (10/10)	GPL570
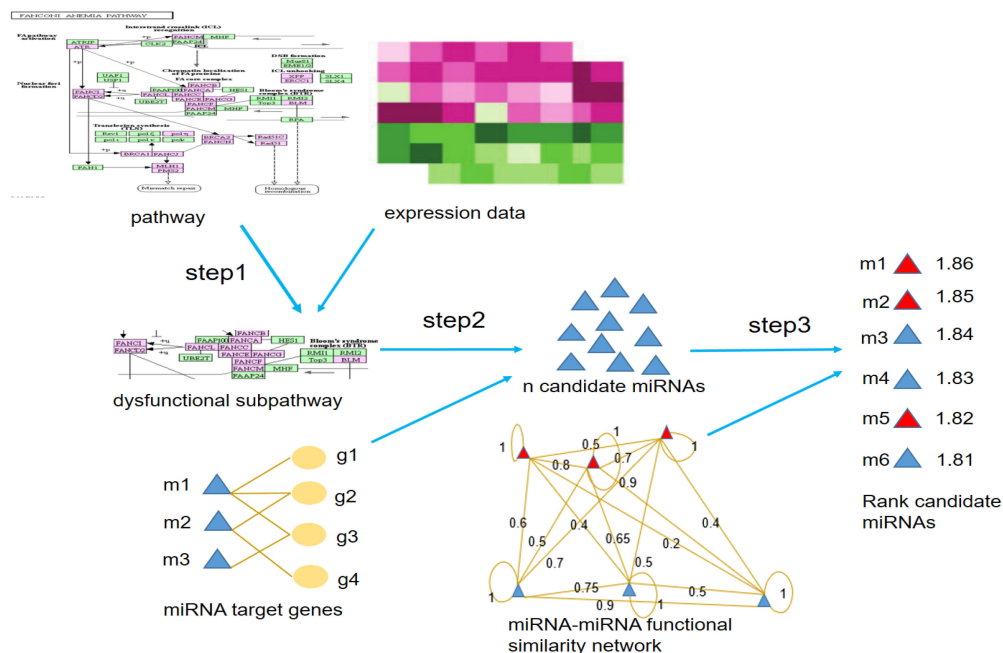
Fig. 1 *Main workflow to identify cancer-related miRNAs based on subpathways and functional similarity network*

In this network, miRNAs in the same family or cluster were assigned a higher score than those outside. Concerning the disease–miRNA association, we combined HMDD (10,368 curated and experimentally supported miRNA–disease associations between 572 miRNAs and 378 diseases) and miR2Diseas (containing 1939 miRNA–disease associations between 299 miRNAs and 94 diseases). These miRNAs related with a specific disease were used as seed to identify other potential cancer-related miRNAs in the functional similarity network.

2.3 Methods

2.3.1 Identifying cancer-related candidate genes:
The Kyoto Encyclopaedia of Genes and Genomes (KEGG; http://www.kegg.jp/kegg/xml/) is an important pathway database that includes both metabolic and signalling pathways. We downloaded 137 signalling pathways from the KEGG database, and the gene network for signalling pathway was reconstructed by graphite package [34]. The DEGs were mapped onto each pathway network using the Limma package for R [35]. We applied sub-SPIA method to detect cancer-related subpathways with p-values = 0.01 corrected by false discovery rate.

2.3.2 Rank cancer-associated candidate miRNA:
The miRNAs targeting genes in the identified significant subpathways were considered as potential candidate cancer-related miRNAs. We ranked them based on their relations with seed miRNAs in the functional similarity network. Given a seed set S with N verified cancer-related miRNAs, miRNA r was scored as

$$score(r) = \frac{\sum_{i=1}^{N} sim(r, s_i)}{N} \times \log_2^N$$

The item $\log_2^N$ was adopted to emphasise that the more cancer-related miRNAs associated with r the more likely it is associated with the cancer.

Fig. 1 shows the three steps to identify cancer-related miRNAs in this paper:

Step 1: Identify significant cancer-related subpathways by sub_SPIA method based on DEGs.
Step 2: Identify potential cancer-related miRNAs based on genes in the subpathways and the miRNA-gene relations.
Step 3: Rank candidate miRNAs based on their similarity score with seed miRNAs in the functional similarity network.

As the potential targeted genes are determined based on significant dysfunctional subpathways, we call the proposed method as miR_SubPath.

3 Result

We applied the proposed miR_SubPath to the eight cancer datasets. For each dataset, N seeds were randomly generated to calculate the scores of all candidate miRNAs. The final score for each candidate miRNA was the average value of 1000 random tests. For fair comparison, we compared our results with miR_Path, miR-Clust and Zhang's method based on the results of the top 100 miRNAs. The results for the miR_Path method were obtained by the code and data files provided at http://comp-sysbio.org/miR_Path/ [19]. Zhang's pipeline identified cancer miRNAs that regulated the top 30% genes differentially expressed between normal and control samples were regarded as cancer miRNAs.

3.1 Performance and evaluation

We implemented miR_SubPath by setting the seed number to $N = 10$, 20 and 30, respectively. As the results of miR_SubPath on the eight datasets for $N > 10$ have no obvious difference, we only present the precision, recall, F1, and mean of the two approaches for the eight cancer datasets for $N = 10$ in Table 2. The precision scores of miR_SubPath are all higher than the other three methods on the eight datasets, while the recall scores of miR_SubPath are all higher than them apart from datasets GSE10072 and GSE9348. Concerning the average F1 score, miR_SubPath performs about 14, 16 and 19% higher than that of miR_Path, miR_Clust and Zhang's method on the eight datasets. Furthermore, the F1 scores of miR_SubPath in two independent datasets of a cancer are very close while that of other three methods differ a lot. These observations demonstrate that miR_SubPath performs better than other three methods when using only a few known cancer-related miRNAs as seeds, resulting in the identification of an increased number of potential miRNAs.

3.2 Identification of novel cancer-related miRNAs

Although the precision, recall, F1, and mean of miR_SubPath had no obvious difference for $N > 10$, the ranks of the miRNAs might be different given different seeds. In this section, we used all of the cancer-related miRNAs in the HMDD and miR2Disease databases as seeds to identify potential cancer-related miRNAs. Among the top 100 miRNAs detected from each of the eight datasets, we found a number of novel miRNAs not recorded in HMDD or miR2Disease that were reported to be associated with a specific cancer in previous publications. Table 3 presents the rank of these potential miRNAs, and the supporting literatures are listed in Table 4. Interestingly, the ranks of most miRNAs from the two independent datasets for a specific cancer are very close. The overlap of the miRNAs in the two independent of a cancer is over

88. All these results indicate the proposed miR_SubPath method is very robust to rank candidate miRNAs.

A total of 12 of the 31 miRNAs were found to be related to colon cancer. For example, decreased expression of mir-218 (ranked 29 in GSE9348 and 37 in GSE20916) was associated with poor prognosis in patients with colorectal cancer [36]. It could inhibit cell-cycle progression, the invasion and migration of colon cancer cells by targeting the PI3K/Akt/mTOR signalling pathway [37]. Mir-9 (ranked 43 in GSE9348 and 44 in GSE20916) suppressed cell migration and invasion via down-regulation of the TM4SF1 transmembrane protein in colorectal cancer [38]. Its up-regulation promoted cell motility to induce metastasis of colorectal cancer [39].

A total of 12 of the 28 miRNAs were found to be associated with lung cancer. Mir-373 (ranked 74 in GSE7670 and 74 GSE10072) could affect human lung cancer cell growth and the expression of E-cadherin [40]. Mir-133a (ranked 71 in GSE7670 and 71 in GSE10072) could suppress multiple oncogenic membrane receptors and cell invasion in non-small cell lung carcinomas [41]. A clinical study also observed the down-regulation of mir-133a in non-small cell lung cancer [42]. It could regulate some novel molecular networks in lung squamous cell carcinomas [43].

A total of 22 of the 36 miRNAs were found to be associated with gastric cancer. For example, let-7b (ranked 30 in GSE13911 and 27 in GSE19826) could inhibit cell proliferation, migration, and invasion of cancer cells by targeting CTHRC1 [44, 45]. Silencing of let-7b could activate AKT signalling to promote gastric carcinogenesis [46]. Mir-203 (ranked 80 in GSE13911 and 80 in GSE19826) could suppress growth of gastric cancer by targeting PIBF1/Akt signalling [47], and inhibit tumour invasion and metastasis in gastric cancer by regulating the serine/threonine kinase ATM [48]. It could promote the proliferation and invasion of gastric cancer cells by targeting the calcium/calmodulin-dependent serine protein kinase CASK [49].

Table 2 Performance of different approaches over eight cancer dataset

| Index | Method | Colon cancer | | Lung cancer | | Gastric cancer | | Ovarian cancer | | Mean |
		GSE9348	GSE20916	GSE7670	GSE10072	GSE13911	GSE19826	GSE37290	GSE69428	
precision	miR_SubPath miR_Path	0.66	0.69	0.71	0.72	0.57	0.56	0.63	0.62	0.64
		0.51	0.49	0.39	0.48	0.39	0.40	0.38	0.40	0.43
	miR_Clust	0.47	0.46	0.38	0.46	0.37	0.36	0.37	0.39	0.41
	zhang	0.43	0.44	0.33	0.42	0.36	0.37	0.35	0.35	0.38
recall	miR_SubPath miR_Path	0.61	0.75	0.66	0.67	0.61	0.62	0.60	0.61	0.64
		0.94	0.48	0.66	0.88	0.57	0.57	0.31	0.42	0.60
	miR_Clust	0.92	0.41	0.69	0.90	0.61	0.62	0.34	0.45	0.61
	zhang	0.84	0.47	0.38	0.79	0.53	0.55	0.43	0.43	0.55
F1	miR_SubPath miR_Path	0.63	0.71	0.68	0.69	0.58	0.58	0.61	0.61	0.63
		0.66	0.48	0.49	0.62	0.46	0.47	0.34	0.40	0.49
	miR_Clust	0.62	0.43	0.49	0.60	0.46	0.45	0.35	0.41	0.47
	zhang	0.57	0.45	0.35	0.55	0.42	0.44	0.39	0.39	0.44

Table 3 Rank of the predicted cancer-related miRNAs supported by at least one publication

| No. | Rank (colon cancer) | | | Rank (lung cancer) | | |
	miRNA	GSE9348	GSE20919	miRNA	GSE7670	GSE10072
1	Mir-16	61	55	Mir-92a	49	50
2	Mir-101	40	38	Mir-194	64	66
3	Mir-9	43	44	Mir-30c	25	24
4	Mir-29c	93	85	Mir-153	97	95
5	Mir-7	72	68	Mir-302b	59	60
6	Mir-218	29	37	Mir-373	74	74
7	Mir-30b	46	52	Mir-367	58	58
8	Mir-204	66	67	Mir-24	47	48
9	Mir-125b	64	62	Mir-135a	81	81
10	Mir-92a	58	51	Mir-133a	71	71
11	Mir-214	33	31	Mir-181a	84	83
12	Mir-302a	62	64	Mir-23b	85	85

No.	Rank (gastric cancer)			Rank (ovarian cancer)		
	miRNA	GSE13911	GSE19826	miRNA	GSE37290	GSE69428
1	Mir-19	10	10	Mir-132	25	28
2	Let-7c	7	7	Mir-194	34	37
3	Let-7b	30	27	Mir-29b	42	41
4	Let-7f	6	6	Mir-205	46	45
5	Let-7i	19	20	Mir-153	53	53
6	Mir-218	29	28	Mir-7	60	60
7	Mir-92a	63	60	Mir-196a	62	62
8	Mir-7	59	55	Mir-1	64	64
9	Mir-23b	85	83	Mir-92a	66	66
10	Mir-30a	79	74	Mir-98	69	69
11	Mir-24	62	61	Mir-106a	71	70
12	Mir-101	48	46	Mir-373	72	71
13	Mir-194	50	49	Mir-372	75	75
14	Mir-196a	49	48	Mir-181b	78	77
15	Mir-125b	48	65	Mir-203	80	82
16	Mir-1	73	69	Mir-181a	82	83
17	Mir-133a	67	66	Mir-135a	89	89
18	Mir-367	47	45	Mir-15a	91	91
19	Mir-215	56	54	Mir-130b	98	95
20	Mir-15a	97	95	—	—	—
21	Mir-181a	86	84	—	—	—
22	Mir-203	80	80	—	—	—

Table 4 PubMed IDs for the identified novel miRNA in PubMed database for cancer

Cancer	MiRNA/PubMed ID	MiRNA/PubMed ID	MiRNA/PubMed ID
colon cancer	Mir-204/27095441	Mir -302a/26191138	Mir-9/26983891/25940709
	Mir-29c/26187445/25193986	Mir-106/25623762/25435873	Mir-125b/26693202/26038573
	Mir-30b/24593661/24293274	Mir-214/27811858/27537384	Mir-7/27919977/26648422
	Mir-92a/27565378/27131314	Mir-101/27435782/26071354	Mir-21827779719/27462788
lung cancer	Mir-302b/27160836	Mir-194/27035759/26909612	Mir-135a/27525941/26235874
	Mir-367/22835608	Mir-153/26339455/25475731	Mir-133a/27282282/25903369
	Mir-92a/26432332/23820254	Mir-373/25591738/25063738	Mir-181a/27802900/26323677
	Mir-23b/27268921/24966325	Mir-24/25725584/23794259	Mir-30c/25119247/25249344
gastric cancer	Mir-19/26762410	Mir-367/25489984	Let-7c/26701848/25549793
	Let-7i/25549793/23107361	Let-7f/25549793/21533124	Mir-92a/26790436/26499948
	Mir-15a/26894855/25743273	Mir-7/24573489/22614005	Mir-30a/27876712/27212164
	Let-7b/27497248/26564501	Mir-23b/26835790/26041881	Mir-24/26758252/26045155
	Mir-194/27874950/25412959	Mir-1/27349337/2587449	Mir-125b/26504803/25240408
	Mir-181a/26793992/26589846	Mir-203/27542403/27142767	Mir-218/27696291/27642088
	Mir-215/26716895/24981590	Mir-133a/26629938/25815687	Mir-196a/27420607/25374225
ovarian cancer	Mir-194/27486333	Mir-153/25954928	Mir-1/27354590
	Mir-92a/25448599	Mir-98/21109987	Mir-372/28456593
	Mir-98/21109987	Mir-372/28456593	Mir-181b/24735543
	Mir-132/27812929/27186275	Mir-29b/26512921/25738313	Mir-205/28145479/26275944
	Mir-196a/27890373/26097603	Mir-181a/27249598/24394555	Mir-135a/24607788/24016480
	Mir-130b/27048832/26573160	Mir-203/27655286/27347348	Mir-106a/27510094/27393101

A total of 19 of the 37 miRNAs were found to be associated with ovarian cancer. For example, mir-194 (ranked 34 in GSE37290 and 37 in GSE69428) could promote the growth, migration and invasion of ovarian carcinoma cells by targeting the protein tyrosine phosphatase PTPN12 [50]. Mir-181b (ranked 78 in GSE37290 and 77 in GSE69428) could promote cell growth and invasion in ovarian cancer by targeting the serine/threonine-protein kinase LATS2 [51]. Mir-130b (ranked 98 in GSE37290 and 95 in GSE69428) was a tumour suppressor by regulating the transcription factor RUNX3 in epithelial ovarian cancer [52].

4 Discussion

As important regulators of gene expression, the aberrant function of miRNAs may drive the initiation and development of numerous cancers. Identifying potential cancer-miRNAs is critical to our understanding of the pathogenesis of cancer and for designing new targeted therapies. However, only a limited number of miRNAs is known to be directly related to cancer, which hinders the development of miRNA-based therapeutic strategies. The performance of previous methods based on miRNA–gene interactions was affected by two factors: background noise present in the data and the lack of context. Integrating other information, such as pathway data, known miRNA-diseases relations, and miRNA–miRNA similarities, may help to improve the identification validity.

In this paper, we developed a new method, named miR_SubPath, to identify cancer-related miRNAs. Compared with miR_Path and miR_Clust, the proposed miR_SubPath determines the candidate miRNAs based on genes in the significantly differentially expressed subpathways, while the former two check if the genes in a highly correlated cluster targeted by a miRNA could classify the controls and the cases or are significantly different in some pathways. If only a few of the targeted genes of a miRNA are significantly differentially expressed, then both miR_Path and miR_Clust may lose to consider them. However, our proposed miR-SubPath will consider an miRNA even if only one of its target gene is significantly differentially expressed in a subpathway. This is one of main reasons that the proposed miR-SubPath could identify more cancer-related miRNAs. Moreover, the targeted genes of the candidate miRNAs determined by miR-SubPath generally functions in a co-ordinately way while those targeted genes of miRNAs from miR_Path and miR_Clust may not.

The scoring method of miR_SubPath is also different from that of miR_Path and miR_Clust. They rank an miRNA based one the classification performance of one of its targeted cluster or a combined score of the cluster considered both the enrichment and significance of the cluster. The proposed miR_SubPath rank a candidate miRNA based its similarity with known seed miRNAs in functional similarity network. This scoring method improves both the accuracy and robustness of the miR_SubPath.

Results from eight datasets, consisting of four cancers types, showed that the F1 score for miR_SubPath was dramatically higher than that of miR_Path, miR_Clust and Zhang's method. This demonstrated that the proposed miR_SubPath method could identify an increased number of cancer-related miRNAs. Furthermore, both the *F*-score and the ranks of the new novel miRNAs among the top 100 miRNAs from the two independent datasets were very close. This indirectly demonstrated that the proposed miR_SubPath was relatively robust. Concerning the implementation of the proposed MiR-SubPath, sub-SPIA implemented by our group using R can be freely downloaded from https://github.com/eshinesimida/subpathway-analysis. Interested readers for the ranking process could ask us for the R code with email. Finally, it is important to point out that the number of cancer-related miRNAs identified using this method was relatively small, as there were only 271 miRNAs included in the miRNA–miRNA functional similarity network. Identification of cancer-related miRNA outside of this dataset is the goal of our future research.

5 Acknowledgments

6 References

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