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Meta-analysis of the differentially expressed colorectal cancer-related microRNA expression profiles

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Abstract. – OBJECTIVES: Unique microRNAs (miRNAs) have been identified in colorectal cancer in recent studies which can be used to accurately diagnose the presence of colorectal cancer and help predict disease recurrence. Differential expression of specific miRNAs in tissues or blood offers the prospect of their use in early detection and screening for colorectal cancer. However, the experiments under different environments would produce different results. The purpose of this study was to get a reliable result on differentially expressed miRNAs related to colorectal cancer by integrating different studies.

MATERIALS AND METHODS: A meta-analysis was performed to review three miRNA microarray datasets from three published literatures that compared the microRNAs expression profiles in colorectal cancer tissues with those in normal colorectal tissues. The R VennDiagram package was applied to identify the overlapping miRNAs with differential expression among these three studies.

RESULTS: A total of 175 differentially expressed miRNAs were reported in the three miRNA expression profiling studies that compared colorectal cancer tissues with normal tissues, of which 25 miRNAs were reported at least by two studies including 15 up-regulated miRNAs and 10 down-regulated miRNAs. Among the 25 miRNAs, 15 ones were differentially expressed between early stage colorectal cancer and normal tissues including 11 up-regulated miRNAs and 4 down-regulated miRNAs, of which hsa-miR-195 (down-regulated) and hsa-miR-20a (up-regulated) were shared by these three studies.

CONCLUSIONS: The 15 differentially expressed miRNAs, especially hsa-miR-195 and hsa-miR-20a may be used as potential biomarkers for early detection and screening of colorectal cancer.

Key Words:
Differentially expressed miRNAs, Colorectal cancer detection, Meta-analysis.

Introduction

Colorectal cancer (CRC), also known as colon cancer or bowel cancer, is a cancer from uncontrolled cell growth in the colon or rectum (parts of the large intestine), or in the appendix. CRC is one of the most frequent cancers and globally more than one million people get it yearly[1]. It is the fourth most common cause of cancer-related deaths with about 608,000 deaths per year[2]. The development of CRC from normal epithelial cells to malignant carcinomas involves a multi-step process with accumulation of both genetic and epigenetic changes, leading to a temporal activation of oncogenes and inactivation of tumor suppressor genes that confer a selective advantage to cells containing these alterations[3]. In recent years, many studies have reported the gene expression profiling related to CRC[4,5] that can help us better understand the underlying molecular mechanism of CRC and further may help to improve the treatment of CRC. More recently, microRNAs (miRNAs), a class of short 22 nucleotide (nt) non-coding RNAs that can regulate gene expression by targeting mRNAs for cleavage or translational repression[6], have been identified and implicated in cancer initiation and progression[7]. Recent researches have shed light on the biological importance of microRNAs (miRNAs) in CRC genesis, progression and response to treatments. The potential utility of miRNAs in the preclinical stage have been explored and investigated[8]. The potential use of miRNAs as biomarkers in diagnosis and prognosis has been demonstrated for several forms of cancer[9]. miRNA expression profiles may be better-suited targets for the discovery of novel cancer biomarkers compared to gene expression profiles. This is supported by a report demonstrating the ability of miRNA profiles to correctly classify human

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Table I. Three independent colorectal cancer related miRNA profiling.

Author	Samples	Cancer Stages
Nishida et al	Epithelial and stromal tissues	Clin Cancer Res 2012, march 27
Arndt et al	64 samples from 49 patients with colorectal cancer	4 normal colon, 4 Stage I, 19 Stage II, 20 Stage III and 2 Stage IV samples
Ma et al	12 pair (12 normal; 12 cancer tissues)	TNM: 1 Stage I, 6 Stage II, 5 Stage III.

cancers of unknown primary origin as well as poorly differentiated tumors[10]. With the aim of identifying new biomarkers of CRC, a growing number of studies have addressed miRNA expression in CRC. However, comparison across studies is limited by differences in profiling platform, quantity of miRNA obtainable, methodology, in some cases of sample number and a paucity of clinic pathologic data. Consequently, translation of results into clinically useful and widely applicable biomarkers is hampered. Importantly, a potentially strong contributor to the variability of data among different studies relates to the tumor resection procedures. The inadvertent collection of surrounding residual non-tumor tissue may skew experimental results, diluting quantitative estimates of particular miRNA species based on the extent and type of tissue present in the sample. Most studies do not specifically address this potential problem[11].

Meta-analysis, combining independent results from different studies, can improve the precision and accuracy of estimates. In this study, we carried out a meta-analysis combining three published literatures that compared the miRNAs expression profiles in CRC tissues with those in normal tissues and applied the R VennDiagram package to obtain overlapping miRNAs among these three studies, in an attempt to provide potential biomarkers which were more reliable and accurate for early diagnose of CRC.

Methods

Meta-analysis of gene expression profiles integrating multiple microarray studies are particularly useful to identify conserved genetic signatures of cancer[12]. We applied the meta-analysis method to analyzed three independent CRC related miRNA profiling studies that compared cancer tissues versus normal tissues (Table I).

Nishida et al collected RNA samples specific for epithelium or stroma from thirteen CRC tissues and four normal tissues using a laser microdissection (LMD) technique, and then miRNA microarray and gene expression microarray were performed. The aim of Arndt's et al[13] was to profile miRNA expression in CRC and to analyze the function of specific miRNAs in CRC cells. MirVana miRNA Bioarrays were used to determine the miRNA expression profiles in eight CRC cell line models, through 45 human CRC samples of different stages, and four matched normal colon tissue samples. In the study of Ma et al[14], total RNA was extracted and purified from 12 pairs of CRC tissue and normal colonic mucosa. After fluorescent tags being added, hybridization was carried out on miRNA microarray chip (Affymetrix company). miRNA expression profiles of three studies are listed in Table II.

As different platform of miRNA expression profile was used in different studies, different standards were used to get the miRNAs with sig-

Table II. miRNA expression profiles of three studies.

Author	Platform	Methods	Samples	miRNA probes	Country	Year
Nishida et al	Agilent	t-test	13 cases of CRC and 4 normal colorectal tissues	455 miRNAs (miRBase15.0)	Japan	2012
Arndt et al	Ambion	t-test	64 samples from 49 patients with CRC	287 human miRNA probes	USA	2009
Ma et al	Affymetrix	FDR	12 N/T	848 miRNAs	China	2011

nificant difference between CRC tissues and normal tissues (Figure 1).

Results

Nishida's Study (Japan)

A total of 127 miRNAs were up-regulated and 29 miRNAs were down-regulated in colorectal cancer tissues.

In the study of Nishida et al (Japan), differences between groups were estimated using the χ^2 test and Student's *t*-test after expression signals were calculated by log2-transformation to the normalized data. Differentially expressed miRNAs were identified depending on the fold-change value and q-value. We used the significance analysis of microarrays (SAM) method in the "samr" package of the R language (http://www.r-project.org/). All differences were considered statistically significant at the level of fold change > 1.5 and q < 0.05. A total of 127 miRNAs (Supplementary Table IA) were up-regulated and 29 miRNAs (Table III) were down-regulated after individual statistics of microarray dataset.

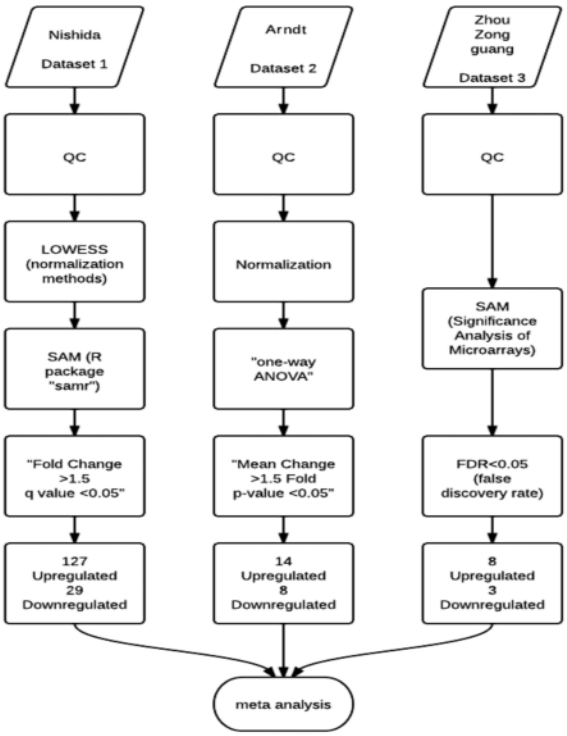

Figure 1. Meta-analysis pipeline.

Supplementary Table IA. Up-regulated microRNAs in colorectal cancer stromal tissue compared with normal stromal tissue in Nishida's study. (Nishida N, Nagahara M, Sato T, Minori K, Sudo T, Tanaka F, Shibata K, Ishii H, Sugihara K, Doki Y, Mori M. Clin Cancer Res March 27, 2012.

Number	Systematic name	Signal in cancer stromal tissue (log2)	Signal in normal stromal tissue (log2)	Fold change	q-value (%)
1	hsa-miR-214	7.3612	5.1633	4.5882	0.0000
2	hsa-miR-21	13.7414	11.7651	3.9348	0.0000
3	hsa-miR-455-3p	5.0351	3.4322	3.0376	0.0000
4	hsa-miR-663	4.4278	2.7578	3.1820	0.0000
5	hsa-miR-127-3p	3.7355	1.9303	3.4947	0.0000
6	hsa-miR-92a	7.2865	5.7584	2.8842	0.0000
7	hsa-miR-381	3.9035	2.5478	2.5593	0.0000
8	hsa-miR-93	5.5042	4.0246	2.7886	0.0000
9	hsa-miR-224	4.5767	2.2308	5.0840	0.0000
10	hsa-miR-432	2.6401	1.3709	2.4102	0.0000
11	hsa-miR-221	5.1187	3.6628	2.7433	0.0000
12	hsa-miR-125b	7.7959	5.7433	4.1486	0.0000
13	hsa-miR-17-5p	6.3813	4.9449	2.7064	0.0000
14	hsa-miR-337-5p	2.8150	1.4391	2.5952	0.0000
15	hsa-miR-502-3p	1.8047	0.5097	2.4538	0.0000
16	hsa-miR-300	3.6767	2.3557	2.4985	0.0000
17	hsa-miR-128	2.5359	1.3263	2.3127	0.0000
18	hsa-miR-532-5p	2.7469	1.5402	2.3080	0.0000
19	hsa-miR-609	3.8840	2.8980	1.9806	0.0000
20	hsa-miR-130b	4.1915	2.7904	2.6409	0.0000
21	hsa-miR-18a	3.7635	1.3688	5.2588	0.0000
22	hsa-miR-181d	3.1301	2.0477	2.1175	0.0000

Table Continued

Table I. ***(Continued).*** Up-regulated microRNAs in colorectal cancer stromal tissue compared with normal stromal tissue in Nishida's study.

Number	Systematic name	Signal in cancer stromal tissue (log2)	Signal in normal stromal tissue (log2)	Fold change	q-value (%)
23	hsa-miR-362-5p	3.2330	2.0853	2.2156	0.0000
24	hsa-miR-424	7.7230	5.9653	3.3816	0.0000
25	hsa-miR-152	3.1990	1.6066	3.0156	0.0000
26	hsa-miR-654-3p	4.3411	2.6129	3.3130	0.0000
27	hsa-miR-149	3.8869	2.7086	2.2632	0.0000
28	hsa-miR-7	4.7678	2.8344	3.8196	0.0000
29	hsa-miR-485-3p	5.3006	4.2385	2.0880	0.0000
30	hsa-miR-99b	3.9191	2.6203	2.4603	0.0000
31	hsa-miR-933	2.7300	1.7161	2.0193	0.0000
32	hsa-miR-615-3p	3.8335	2.6442	2.2804	0.0000
33	hsa-miR-640	2.9456	1.4328	2.8535	0.0000
34	hsa-miR-483-3p	6.2669	5.0075	2.3940	0.0000
35	hsa-miR-605	3.8121	2.5917	2.3301	0.0000
36	hsa-miR-135b	3.8255	0.7653	8.3408	0.0000
37	hsa-miR-296-5p	1.9389	1.1457	1.7329	0.0000
38	hsa-miR-647	3.8730	2.1903	3.2102	0.0000
39	hsa-miR-1236	3.4103	1.8925	2.8635	0.0000
40	hsa-miR-34b	4.2683	2.3956	3.6620	0.0000
41	hsa-miR-592	2.5548	0.8012	3.3721	0.4839
42	hsa-miR-25	5.9887	4.7911	2.2936	0.4839
43	hsa-let-7i	8.6952	7.6400	2.0781	0.4839
44	hsa-miR-20b	5.5041	4.2857	2.3269	0.4839
45	hsa-miR-130a	7.3585	6.0856	2.4165	0.4839
46	hsa-miR-199a-5p	7.4083	5.9666	2.7163	0.4839
47	hsa-miR-1238	4.2775	3.5734	1.6291	0.4839
48	hsa-miR-331-3p	6.6993	5.5074	2.2846	0.4839
49	hsa-miR-24	9.5984	8.5492	2.0693	0.4839
50	hsa-miR-20a	7.5321	6.3601	2.2532	0.4839
51	hsa-miR-23a	10.0837	9.0655	2.0254	0.4839
52	hsa-miR-199b-5p	6.7800	5.3520	2.6907	0.4839
53	hsa-miR-181b	4.6692	3.7467	1.8955	0.4839
54	hsa-miR-377	4.4965	3.2165	2.4283	0.4839
55	hsa-miR-18b	2.5247	0.9391	3.0013	0.4839
56	hsa-miR-539	2.0809	1.0446	2.0510	0.4839
57	hsa-miR-22	9.7884	8.6262	2.2379	0.4839
58	hsa-miR-223	8.8473	7.7969	2.0712	0.4839
59	hsa-miR-491-3p	2.5471	0.9916	2.9393	0.4839
60	hsa-miR-125a-5p	5.6168	4.5834	2.0469	0.4839
61	hsa-miR-299-5p	3.1356	1.7790	2.5607	0.4839
62	hsa-miR-483-5p	4.9531	3.9757	1.9689	0.4839
63	hsa-miR-379	2.4551	0.4574	3.9938	0.4839
64	hsa-miR-1234	5.0701	4.3136	1.6894	0.4839
65	hsa-miR-99a	3.9888	2.3726	3.0657	0.4839
66	hsa-miR-1225-3p	3.9937	3.3510	1.5613	0.4839
67	hsa-miR-495	2.3389	0.7363	3.0369	0.4839
68	hsa-miR-574-5p	7.1083	6.2371	1.8292	0.4839
69	hsa-miR-125a-3p	3.2257	2.3094	1.8873	0.4839
70	hsa-miR-326	1.9496	0.8913	2.0825	0.4839
71	hsa-miR-1224-3p	4.8757	3.6868	2.2798	0.4839
72	hsa-miR-1229	3.7613	2.5318	2.3448	0.4839
73	hsa-miR-937	4.9785	3.8112	2.2459	0.4839
74	hsa-miR-574-3p	7.9370	7.0906	1.7979	0.4839
75	hsa-miR-1237	2.8475	2.0403	1.7498	0.4839
76	hsa-miR-206	3.0294	1.7743	2.3868	0.4839
77	hsa-miR-129-3p	1.7338	0.7030	2.0432	0.4839
78	hsa-miR-885-5p	6.2987	5.1769	2.1761	0.4839
79	hsa-miR-1227	4.6286	3.0407	3.0061	0.4839

Table I. ***(Continued).*** Up-regulated microRNAs in colorectal cancer stromal tissue compared with normal stromal tissue in Nishida's study.

Number	Systematic name	Signal in cancer stromal tissue (log2)	Signal in normal stromal tissue (log2)	Fold change	q-value (%)
80	hsa-miR-631	4.3710	2.9772	2.6277	0.4839
81	hsa-miR-487b	2.2864	0.5419	3.3507	0.8249
82	hsa-miR-543	1.4302	0.1764	2.3847	0.8249
83	hsa-miR-365	6.3121	5.3710	1.9200	0.8249
84	hsa-let-7e	6.8754	5.9369	1.9166	0.8249
85	hsa-miR-151-3p	3.8653	2.8896	1.9666	0.8249
86	hsa-miR-106b	6.7077	5.7538	1.9371	0.8249
87	hsa-miR-301a	4.3942	3.3753	2.0263	0.8249
88	hsa-miR-382	2.2284	0.8661	2.5709	0.8249
89	hsa-miR-100	4.7280	3.4939	2.3523	0.8249
90	hsa-miR-452	2.3510	1.1210	2.3456	0.8249
91	hsa-miR-371-5p	2.2495	1.1375	2.1614	0.8249
92	hsa-miR-765	4.3601	3.6031	1.6900	0.8249
93	hsa-miR-362-3p	2.5044	1.5707	1.9102	0.8249
94	hsa-miR-183	1.8260	0.5658	2.3953	0.8249
95	hsa-miR-423-5p	3.8130	3.0448	1.7032	0.8249
96	hsa-miR-328	6.1455	5.1186	2.0377	0.8249
97	hsa-miR-595	4.4023	3.3593	2.0605	0.8249
98	hsa-miR-542-3p	2.8903	1.2498	3.1177	0.8249
99	hsa-miR-550	1.9877	1.2773	1.6363	0.8249
100	hsa-miR-634	2.1901	1.4710	1.6462	0.8249
101	hsa-miR-188-5p	4.1637	3.5119	1.5711	1.3079
102	hsa-miR-450a	2.6452	1.0431	3.0358	1.3079
103	hsa-miR-181c	2.7493	1.7464	2.0040	1.3079
104	hsa-miR-126	8.1419	7.2671	1.8338	1.3079
105	hsa-miR-484	3.8659	3.2581	1.5239	1.3079
106	hsa-miR-95	2.2341	1.4163	1.7627	1.3079
107	hsa-miR-19a	7.0755	6.2318	1.7946	1.3079
108	hsa-miR-181a	6.7619	5.9594	1.7441	1.3079
109	hsa-miR-376a	4.3021	3.2870	2.0211	1.3079
110	hsa-miR-425	4.4042	3.5379	1.8230	1.3079
111	hsa-miR-455-5p	1.9466	0.6533	2.4510	1.3079
112	hsa-miR-324-3p	5.1796	4.5422	1.5555	1.3079
113	hsa-miR-622	2.5718	1.6357	1.9133	1.3079
114	hsa-miR-613	1.6581	0.9437	1.6408	1.3079
115	hsa-miR-575	3.9488	3.2521	1.6208	1.3079
116	hsa-miR-222	3.2655	2.5148	1.6827	1.3079
117	hsa-miR-32	2.5684	1.6119	1.9406	1.3079
118	hsa-miR-197	7.9098	7.0983	1.7551	1.3079
119	hsa-miR-766	6.9197	6.0107	1.8777	1.3079
120	hsa-miR-876-5p	1.3810	0.6503	1.6595	1.9848
121	hsa-miR-505	2.8023	2.0524	1.6817	1.9848
122	hsa-let-7c	7.3815	6.6304	1.6831	1.9848
123	hsa-miR-98	4.5308	3.7445	1.7247	1.9848
124	hsa-miR-27a	9.1504	8.3246	1.7725	1.9848
125	hsa-miR-324-5p	4.1229	3.2487	1.8330	1.9848
126	hsa-miR-874	3.6715	3.0471	1.5416	1.9848
127	hsa-miR-133a	1.3162	0.6960	1.5371	1.9848

Arndt's Study (USA)

A total of 22 miRNAs were up-regulated and 14 miRNAs were down-regulated in colorectal cancer tissues, among which 14 were up-regulated and 8 were down-regulated in early stage colorectal cancer tissues.

In the study of Arndt et al[13] (USA), the data were analyzed using the R software package. The miRNA expression data were normalized prior to determining differential gene expression. Replicate samples and probe values were averaged and the Student's t-test was performed to find genes

Table III. Twenty-nine down-regulated miRNAs in CRC stromal tissues compared with normal tissues in Nishida's study.

Number	Systematic name	Signal in cancer stromal tissue (log2)	Signal in normal stromal tissue (log2)	Fold change	q-value (%)
1	hsa-miR-192-5p	7.9568	9.5176	0.3390	0.0000
2	hsa-miR-215	7.4765	8.9222	0.3671	0.0000
3	hsa-miR-29c-3p	7.9993	9.3317	0.3971	0.0000
4	hsa-miR-194	7.2959	8.4937	0.4359	0.0000
5	hsa-miR-638	8.6130	9.5065	0.5383	4.8814
6	hsa-miR-10b	6.9414	7.6296	0.6206	0.0000
7	hsa-miR-768-3p	7.0889	7.7048	0.6525	0.0000
8	hsa-miR-26b	8.1355	8.7371	0.6590	0.0000
9	hsa-miR-195	7.4141	7.9933	0.6693	1.9661
10	hsa-miR-338-3p	6.4844	6.9691	0.7147	0.0000
11	hsa-miR-497	6.9495	7.3849	0.7395	3.0119
12	hsa-let-7g	8.3868	8.8167	0.7423	0.0000
13	hsa-miR-375	6.1614	6.5733	0.7517	0.0000
14	hsa-miR-101	6.9437	7.3310	0.7645	4.8814
15	hsa-miR-30e	6.7244	7.0558	0.7947	0.0000
16	hsa-miR-30b	7.0081	7.3252	0.8027	1.9661
17	hsa-miR-30a-5p	6.5269	6.7850	0.8362	0.0000
18	hsa-miR-378a-3p	6.1499	6.3985	0.8417	0.0000
19	hsa-miR-139-3p	6.2961	6.5203	0.8561	3.0119
20	hsa-miR-551b	6.0496	6.2655	0.8610	0.0000
21	hsa-miR-186	6.3496	6.5568	0.8662	0.0000
22	hsa-miR-30c	6.5390	6.7460	0.8663	0.0000
23	hsa-miR-196a	6.1481	6.2924	0.9048	3.0119
24	hsa-miR-218	6.0789	6.1869	0.9279	4.8814
25	hsa-miR-361-3p	6.1635	6.2395	0.9487	3.0119
26	hsa-miR-9	5.9865	6.0449	0.9604	0.0000
27	hsa-miR-498	6.0219	6.0772	0.9624	3.0119
28	hsa-miR-139-5p	6.0374	6.0768	0.9730	4.8814
29	hsa-miR-520g	5.9742	6.0109	0.9749	4.8814

that vary significantly across sample groups. Genes were selected if the median normalized signal intensity was greater than 100 (75th percentile of median signal) for at least one group, with a mean change > 1.5-fold and a p-value < 0.05. A one-way ANOVA was used to evaluate miRNA expression level between normal tissues and cancer tissues at different stages. Both probe level and gene level data analysis was performed for all group comparisons.

A total of 22 miRNAs were up-regulated and 14 miRNAs were down-regulated in CRC (stage I to stage IV) compared with normal tissues (Table IV). Of those miRNAs, 14 miRNAs was up-regulated and 8 miRNAs was down-regulated in early stage CRC (stage I and stage II) compared with normal tissues (Table V).

Ma's Study (China)

A total of 8 miRNAs were up-regulated and 3 miRNAs were down-regulated in colorectal cancer tissues.

In the study of Ma et al[14] (China), SAM (Significance Analysis of Microarray) analysis was performed to find out the differentially expressed miRNAs between CRC tissues and normal tissues (FDR: false discovery rate < 0.05) (Table VI).

Meta-analysis

CRC vs. Normal

The R VennDiagram package15 was used to analyze the three datasets [Table III, Supplementary Table IA (Japan), Table IV (USA) and Table VI (China)] from different studies. We can get the overlap between them (Figure 2). Thus, we got miRNAs with significant expression difference between CRC tissues and normal tissues reported by two or three studies (Table VII).

Early Stage CRC vs. Normal

The R VennDiagram package15 was applied to analyze the three datasets [Table III, Supplemen-

Table IV. 37 miRNAs with significant difference between CRC (Stage I to IV) and normal tissues in Arndt's study[13] (USA).

Number	miRNA	Normal (Normalized)	CRC (Normalized)	p-value	Fold Change (Cancer/Normal)
1	hsa-miR-20a	9.20	10.30	2.00E-03	2.10
2	hsa-miR-18a	7.60	8.60	2.90E-03	2.00
3	hsa-miR-19a	7.70	8.70	2.30E-03	1.90
4	hsa-miR-17-5p	10.50	11.40	1.50E-03	1.90
5	hsa-miR-19b	11.00	11.80	3.40E-03	1.80
6	hsa-miR-203	8.40	9.70	2.20E-02	2.60
7	hsa-miR-21	13.00	14.50	6.00E-06	2.90
8	hsa-miR-34a	9.50	10.30	1.50E-02	1.70
9	hsa-miR-181b	8.50	9.20	2.20E-04	1.70
10	hsa-miR-29b	9.70	10.50	4.90E-03	1.80
11	hsa-miR-130b	7.10	7.80	1.20E-03	1.70
12	hsa-miR-95	6.10	6.80	1.10E-02	1.60
13	hsa-miR-106b	9.50	10.30	1.00E-04	1.70
14	hsa-miR-93	9.90	10.50	3.40E-03	1.60
15	hsa-miR-25	9.00	9.60	1.40E-02	1.60
16	hsa-miR-182	7.20	8.70	2.80E-03	2.80
17	hsa-miR-96	6.70	7.70	8.40E-03	2.00
18	hsa-miR-183	5.90	6.80	2.30E-03	1.80
19	hsa-miR-29a	12.10	12.90	3.60E-04	1.70
20	hsa-miR-31	6.40	8.70	2.60E-03	5.00
21	hsa-miR-106a	10.70	11.70	5.10E-04	2.00
22	hsa-miR-224	6.90	8.40	1.10E-03	2.80
23	hsa-miR-30a-5p	11.60	11.00	1.60E-05	0.70
24	hsa-miR-30a-3p	7.10	6.30	7.30E-04	0.50
25	hsa-miR-378a-5p	7.60	6.50	1.30E-05	0.40
26	hsa-miR-422b	10.90	9.60	8.30E-05	0.40
27	hsa-miR-143	14.10	12.60	1.00E-02	0.40
28	hsa-miR-145	15.00	13.20	1.20E-03	0.30
29	hsa-miR-10b	11.00	10.10	2.10E-02	0.50
30	hsa-miR-30c	10.90	10.30	4.00E-04	0.70
31	hsa-miR-125a-5p	11.20	10.20	6.40E-03	0.50
32	hsa-miR-1	9.00	7.00	1.60E-03	0.20
33	hsa-miR-133a	10.00	7.60	6.50E-04	0.20
34	hsa-miR-497	9.30	8.10	9.80E-04	0.40
35	hsa-miR-195	11.10	9.50	4.20E-05	0.30
36	hsa-miR-422a	10.00	8.80	7.30E-05	0.40
37	hsa-miR-139-5p	7.60	6.20	1.10E-05	0.40

tary Table IA (Japan), Table V (USA, early stage CRC) and Table VI (China)] from different studies. We can get the overlap between different datasets (Figure 3). Thus, we got miRNAs with significant expression difference between early stage CRC tissues and normal tissues reported by two or three studies (Table VIII).

Discussion

In our research, we collected 3 microRNA expression profiling studies that compared the microRNAs expression profiles in CRC tissues with those in normal tissues. By individually statistical analysis and meta-analysis, we got the overlapping miRNAs among the 3 datasets.

Three experiments were done on different platform (Agilent, Ambion, and Affymetrix) and the numbers of probes in different platforms

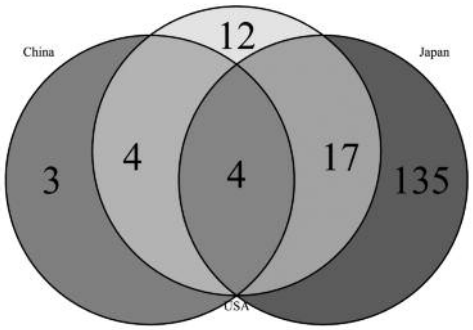

Figure 2. Meta-analysis of 3 datasets.

Table V. 22 miRNAs with significant difference between early stage CRC (Stage I and II) and normal tissues in Arndt's study[13] (USA).

Number	miRNA	Normal	Stage I/II	Fold Change (C/N)	*p*-value
1	hsa-miR-224	6.89	8.67	3.45	1.80E-02
2	hsa-miR-21	13.02	14.49	2.78	1.52E-04
3	hsa-miR-34a	9.48	10.43	1.92	1.92E-02
4	hsa-miR-106a	10.68	11.57	1.85	2.88E-02
5	hsa-miR-18a	7.51	8.38	1.83	3.32E-02
6	hsa-miR-29b	9.70	10.56	1.82	3.27E-03
7	hsa-miR-19b	10.84	11.69	1.80	2.77E-02
8	hsa-miR-106b	9.45	10.26	1.76	1.37E-02
9	hsa-miR-20a	9.28	10.06	1.72	3.75E-02
10	hsa-miR-29a	12.14	12.89	1.68	9.84E-04
11	hsa-miR-181b	8.41	9.11	1.63	9.47E-03
12	hsa-miR-19a	7.78	8.45	1.60	2.58E-02
13	hsa-miR-95	6.12	6.79	1.59	3.33E-02
14	hsa-miR-516-3p	4.87	5.52	1.57	3.20E-02
15	hsa-miR-378a-5p	7.53	6.85	0.62	1.87E-03
16	hsa-miR-422b	10.88	10.02	0.55	4.91E-03
17	hsa-miR-422a	10.05	9.19	0.55	9.57E-03
18	hsa-miR-30a-3p	7.14	6.24	0.54	1.51E-02
19	hsa-miR-139-5p	7.62	6.17	0.37	5.75E-03
20	hsa-miR-195	11.04	9.52	0.35	2.37E-02
21	hsa-miR-145	15.11	13.46	0.32	4.30E-02
22	hsa-miR-133a	10.51	7.64	0.14	3.00E-02

Table VI. 11 miRNAs with significant expression difference between CRC tissues and normal tissues in Ma's study[14].

Number	miRNAs	Ratio under FDR<5%	status
1	hsa-miR-182	2.21	up-regulated
2	hsa-miR-17-5p	1.61	up-regulated
3	hsa-miR-106a	1.54	up-regulated
4	hsa-miR-93	1.41	up-regulated
5	hsa-miR-200c	1.64	up-regulated
6	hsa-miR-92c	1.5	up-regulated
7	hsa-let-7a	1.45	up-regulated
8	hsa-miR-20a	1.37	up-regulated
9	hsa-miR-195	0.55	down-regulated
10	hsa-miR-143	0.38	down-regulated
11	hsa-miR-145	0.39	down-regulated

were different. The meta-analysis depended on their common part.

As some unpredictable noises existed in each experiment, the most significant differential expressed miRNAs sifted by the microarray data may not reflect the objective situation in clinical CRC. To take one step ahead, we sifted miRNAs from a number of experiments by meta-analysis to get a more credible result.

R packages were used in both individual analysis and meta-analysis. They are popular and effective statistic tools with high quality. Relationship between different datasets was found by the open source R packages in a short while.

A total of 25 miRNAs related to CRC (not only in early stage) were sifted through the meta-analysis and the R VennDiagram package, of which 15 were up-regulated and 10 were down-regulated (Table VII). Besides, 15 miRNAs only related to early stage CRC were sifted, of which 11 were up-regulated and 4 were down-regulated (Table VIII). These miRNAs in Table VIII is the subgroup of miRNAs in Table

Table VII. miRNAs with significant expression difference between CRC tissues and normal tissues in different experiments.

miRNA	USA	China	Japan	status in CRC
hsa-miR-106a	1	1		up-regulated
hsa-miR-106b	1		1	up-regulated
hsa-miR-10b	1		1	down-regulated
hsa-miR-125a-5p	1		1	down-regulated
hsa-miR-130b	1		1	up-regulated
hsa-miR-133a	1		1	down-regulated
hsa-miR-139-5p	1		1	down-regulated
hsa-miR-143	1	1		down-regulated
hsa-miR-145	1	1		down-regulated
hsa-miR-17-5p	**1**	**1**	**1**	up-regulated
hsa-miR-181b	1		1	up-regulated
hsa-miR-182	1	1		up-regulated
hsa-miR-183	1		1	up-regulated
hsa-miR-18a	1		1	up-regulated
hsa-miR-195	**1**	**1**	**1**	down-regulated
hsa-miR-19a	1		1	up-regulated
hsa-miR-20a	**1**	**1**	**1**	up-regulated
hsa-miR-21	1		1	up-regulated
hsa-miR-224	1		1	up-regulated
hsa-miR-25	1		1	up-regulated
hsa-miR-30a-5p	1		1	down-regulated
hsa-miR-30c	1		1	down-regulated
hsa-miR-497	1		1	down-regulated
hsa-miR-93	**1**	**1**	**1**	up-regulated
hsa-miR-95	1		1	up-regulated

1 means the miRNA was differentially expressed in the dataset.

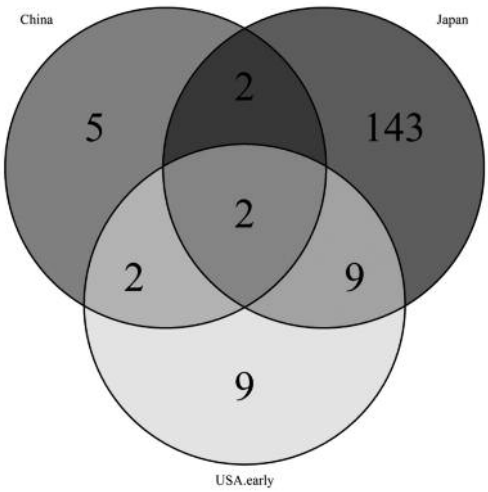

Figure 3. Meta-analysis of 3 datasets (2).

VII, which indicates that there are specific miRNAs with different expression levels in different CRC stages.

Meta-analysis of the differentially expressed colorectal cancer-related microRNA expression profiles got a satisfactory result. And the differentially expressed miRNAs reported by at least two studies can act as stabile biomarkers of CRC. To develop the diagnosis kit for detection of early stage CRC, the 15 miRNAs in Table VIII are worth to be validated in wet lab. Especially, the differentially expressed hsa-miR-195 and hsa-miR-20a, which appeared in the result of all 3 experiments, are the most credible potential biomarkers for detection of early stage CRC.

Conclusions

By meta-analysis of the differentially expressed colorectal cancer-related microRNA expression profiles, 15 miRNAs (Table VIII) from 3 different datasets were selected as potential biomarkers in early detection and screening for CRC. The miRNAs expression profile in tissue and blood has potential use in the detection, screening and surveillance of CRC. Furthermore, differentially expressed miRNAs may be targeted by gene therapy to improve the treatment of CRC.

Table VIII. miRNAs with significant expression difference between early stage CRC tissues and normal tissues in different experiments.

miRNA	USA	China	Japan	status in CRC
hsa-miR-106b	1	1		up-regulated
hsa-miR-133a	1	1		down-regulated
hsa-miR-139-5p	1	1		down-regulated
hsa-miR-181b	1	1		up-regulated
hsa-miR-18a	1	1		up-regulated
hsa-miR-195	**1**	**1**	**1**	**down-regulated**
hsa-miR-19a	1	1		up-regulated
hsa-miR-20a	**1**	**1**	**1**	**up-regulated**
hsa-miR-21	1	1		up-regulated
hsa-miR-224	1	1		up-regulated
hsa-miR-95	1	1		up-regulated
hsa-miR-17-5p		1	1	up-regulated
hsa-miR-93		1	1	up-regulated
Has-miR-145	1		1	down-regulated
Has-miR-106a	1		1	up-regulated

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Conflict of Interest

The Authors declare that there are no conflicts of interest.

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