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The expression and significance of microRNA in different stages of colorectal cancer

Binbin Du, MS[a], Dewang Wu, MS[a,b], Xiongfei Yang, MD[a], Tao Wang, MS[a], Xinlong Shi, MS[a], Yaochun Lv, MS[a,b], Zhuolong Zhou, MD[c], Qing Liu, MD[c], Weisheng Zhang, MS[a,*]

Abstract

Background: The aim of this study is to compare microRNA expression patterns in different stages of colorectal cancer (CRC) and to discuss the significance of the application of microRNAs in the clinical treatment of CRC.

Methods: The study used gene chip technology to analyze genetic sequences in CRC tissues and surrounding normal tissues at different cancer stages. The bioinformatics profiles of the target genes of the different microRNAs were analyzed to clarify the target gene-related pathways and their functions in the disease.

Results: A total of 368 target genes with differential expression, including 275 upregulated and 93 downregulated genes, were screened from CRC patients in different stages of the disease. These microRNAs participated widely in the occurrence and development processes of CRC. The microRNA expression profiles obviously differed in tissues at different CRC stages.

Conclusion: microRNA regulation of CRC samples can be used as a tool to control the occurrence and development of tumor cells.

Abbreviations: AGCCC = Affymetrix Gene Chip Command Console, C = cancer tissues, CRC = colorectal cancer, CS = cancer-surrounding tissues, GO = gene ontology, KOBAS = KEGG Orthology-Based Annotation System, LC = late cancer tissues, LCS = late cancer-surrounding tissues, MPS3 = mucopoly-saccharidosis type III, PC = primary cancer tissues, PCS = primary cancer-surrounding tissues, SAM = significance analysis of microarray, TAC = Transcriptome Analysis Console, TGF = transforming growth factor.

Keywords: colorectal cancer, microRNA, target genes, tumor

1. Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors of the digestive system, and the morbidity and mortality of the disease are increasing worldwide.[1] Due to the extreme difficulty involved in the diagnosis of CRC in its early stages and the shortage of simple and noninvasive detection tools, CRC is diagnosed in its advanced stages for most patients. Thus, the goal of our study is to develop a detection method with high sensitivity and specificity for the early diagnosis of CRC. MicroRNAs are single stranded, short (usually 18–22 nt), evolutionarily conserved, endogenous, and noncoding RNA molecules.[2] At present, more than 2500 microRNAs have been found in the human genome. These microRNAs are involved in gene expression regulation for approximately one-third of the human genome; additionally, microRNAs participate in the cell growth, proliferation, differentiation and apoptosis processes[3] and affect insulin secretion and various skeletal muscle-, brain- and heart-related processes.[3,4] MicroRNAs can serve as noninvasive tumor markers for the early diagnosis and prognostic evaluation of CRC.[5] Since microRNAs had the potential for use as regulators of the biological activities described above, gene chip technology was used to analyze colorectal cancer (C) and cancer-surrounding tissues (CS) from patients in 9 different stages. The microRNA expression profiles were obtained, and the identified microRNAs from both groups were screened to ultimately predict their target genes via bioinformatics analysis. KOBAS (KEGG Orthology-Based Annotation System) was employed to evaluate the differentially expressed microRNAs, analyze the target gene-related pathways and their functions in the disease, identify the significantly enriched GO (gene ontology) terms, and analyze the metabolism and signaling pathways. The results provide an innovative method for the early diagnosis and prognostic evaluation of CRC.

2. Materials and methods

2.1. Patients and tissue materials

A total of 35 cases of colorectal cancer patients from the Department of Anorectal Surgery of Gansu Provincial Hospital were collected from April to June in 2016. Nine patients were

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BD and WZ proposed the study. BD and DW wrote the first draft.

BD and DW collected and analyzed the data. All authors contributed to the design and interpretation of the study and to further drafts. WZ is the guarantor.

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The authors have no conflicts of interest to disclose.

[a] Department of Colorectal Surgery, Gansu Provincial Hospital, Lanzhou, [b] Department of Surgery, Ningxia Medical University, Yinchuan, [c] School of Medical Instrument and Food Engineering, University of Shanghai for Science and Technology, Shanghai, China.

[*] Correspondence: Weisheng Zhang, Department of Colorectal Surgery, Gansu Provincial Hospital, Donggang West Road, Lanzhou 730000, China (e-mail: zh9189@sina.com).

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selected. Both cancer tissues and cancer-surrounding tissues were collected. The normal surrounding tissues were located 5 cm from the edge of the patients' cancer tissues, and each piece weighed approximately 300 mg. First, the specimens were placed in prepared frozen storage tubes and immediately immersed in a liquid nitrogen tank. For long-term preservation, the samples were stored at −80°C. The patients approved the use of the samples for this experiment. A microarray was used to detect the microRNA expression profiles of CRC. Five patients were male, and the other patients were female. There were 5 cases in the primary stage of the disease (3 males and 2 females) and 4 cases in the late cancer stages (2 males and 2 females). All of the patients were diagnosed and staged based on the references.[6] The criteria for patient selection were as follows: the postoperative pathological diagnosis was clear adenocarcinoma; the preoperative imaging examination revealed no distant metastasis or other multiple cancers; the postoperative pathological stage of the tumor (TNM) staging was I, IIa, IIb and III, including I, IIa, and IIb for the primary stages and III for the advanced stages; the patients had no serious diseases of the heart, liver, brain, lung, or kidney or other diseases; and no patients received radiotherapy, chemotherapy or molecular targeted therapy prior to surgery. All patients were approved by the hospital's ethics committee.

2.2. Methods

Total RNA extraction and quality inspection of all samples were performed by CapitalBio Technology Corporation. The samples were analyzed by the Affymetrix microarray company for quality inspection.

2.2.1. Comparison of the similarities between samples from different stages and gene chip detection. Gene chip detection of the CRC samples was performed by the CapitalBio Technology Corporation. Box-whisker plots were used to analyze and compare the normalized expression values between different stages, and the AGCCC software (Affymetrix Gene Chip Command Console Software) was used to transform each sample's fluorescence by scanning images from this experiment. The RMA algorithm was used to preprocess the data,[7] and a correlation analysis of the preprocessed data was performed (Fig. 1A) to evaluate the similarity between the samples and to determine whether samples from different stages were grouped as expected.

2.2.2. Detection of differentially expressed miRNAs in different disease stages. An Affymetrix miRNA 4.0 Array was used to detect the microRNA expression profiles of the samples in different stages and to characterize the up- and downregulated target genes. Then, the Affymetrix Expression Console and Affymetrix Transcriptome Analysis Console (TAC) were used to analyze the differentially expressed genes. A minimum of 3 experiments was performed. The SAM (significance analysis of microarray) R package was used to analyze the expression profiles of the differentially expressed genes. The screening criteria for the differentially expressed genes were as follows: a Q-value no higher than 5% and a fold change larger than 2 or less than 0.5.

2.2.3. KOBAS (KEGG Orthology-Based Annotation System). KOBAS was used to predict the target gene-related pathways and their functions in the disease process, to identify the significantly

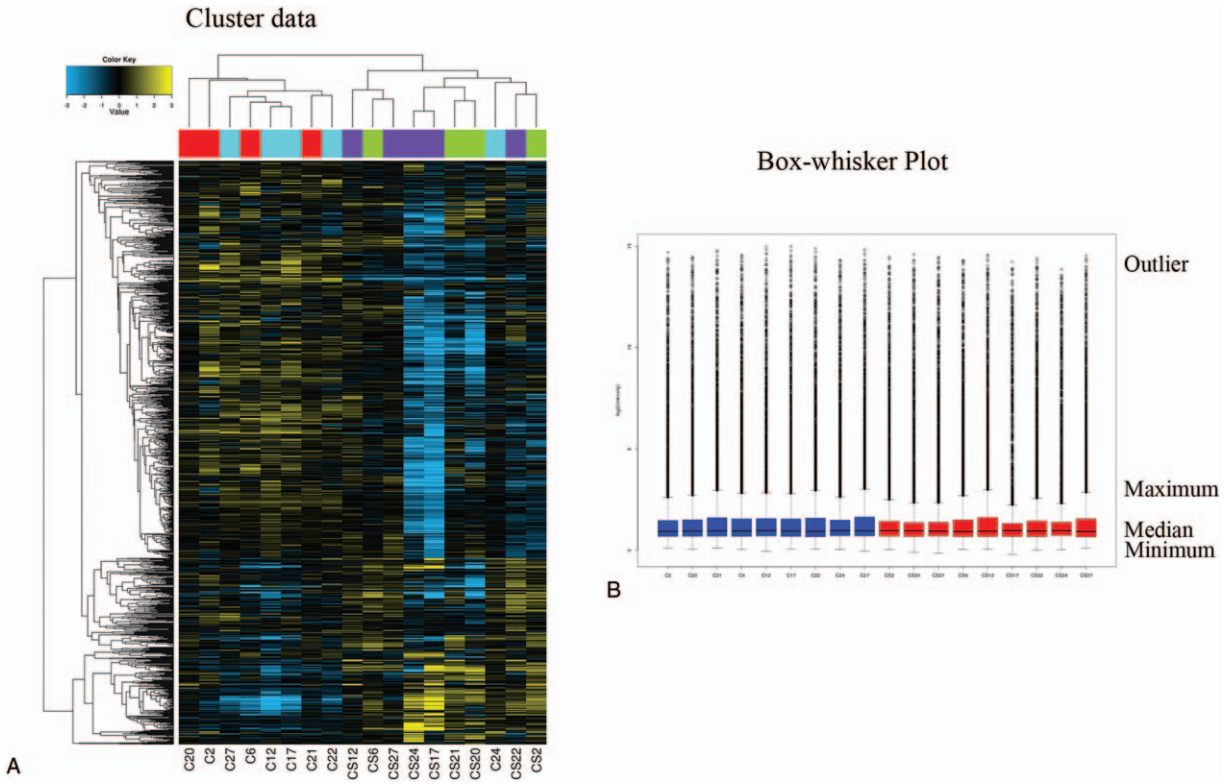

Figure 1. (A) Cluster data for all samples. In the diagram, each column represents a sample, and each row represents the degree of miRNA expression. (B) Box-whisker plots for all samples. In the image, the blue represents the cancer tissues, whereas red represents the cancer-surrounding tissues. C = cancer tissues, CS = cancer-surrounding tissues.

Table 1

The results of quality inspection for all samples.

Numbers	A260/280	Total, μg	Numbers	A260/280	Total, μg
C2	1.98	68.1	CS2	1.93	43.6
C6	1.95	105.2	CS6	1.90	22.9
C12	1.96	68.0	CS12	1.92	26.5
C17	1.98	127.6	CS17	1.91	25.2
C20	1.98	94.8	CS20	1.97	81.3
C21	1.96	96.2	CS21	1.96	43.7
C22	1.96	184.5	CS22	1.94	155.5
C24	1.98	78.4	CS24	1.92	10.5
C27	1.95	178.6	CS27	1.97	171.3

C = cancer tissues, CS = cancer-surrounding tissues.

enriched GO terms and pathways, and to analyze the metabolic and signaling pathways of these target genes.

3. Results

3.1. Quality inspection of all samples at different stages

The RNA purity was evaluated in all samples as follows: the A260/280 was at least 1.70; the amount of total RNA larger than or equal to 1 mg; and all samples were in accordance with the requirements for miRNA expression profiling (Table 1).

3.2. Similarity comparisons between samples at different stages

The box-whisker plot (Fig. 1B) shows that the total gene expression levels in the different stages and different samples were essentially the same after normalization and that all of the samples had good consistency. According to the correlation of the expression patterns of the samples, a sample number more than 3 indicated that the samples would be clustered (Fig. 1A). Based on the top of the tree diagram and the expected color block, the actual clustering behaviors of the cancer tissues and cancer-surrounding tissues were better in all stages. The sample differences were small and were in accordance with the expected grouping.

3.3. Detection of differentially expressed genes in different stages and regulation of target genes by microRNAs

A total of 368 microRNAs with different expression profiles were screened out from the CRC samples at different stages. First, upon comparing the primary cancer-surrounding tissues (PCS) with the late cancer-surrounding tissues (LCS) (Fig. 2A), neither upregulated nor downregulated miRNAs were identified. Second, upon comparing the cancer tissues (C) with the cancer-surrounding tissues (CS) (Fig. 2B), 275 upregulated and 89 downregulated miRNAs were detected (Tables 2 and 3). Third, upon comparing the primary cancer tissues (PC) with the late cancer tissues (LC) (Fig. 2C), 4 downregulated and no upregulated miRNAs were detected (Table 4). In these experiments, a total of 3972 genes were regulated by the identified microRNAs. The regulated targeted miRNAs included 275 significantly upregulated miRNAs and 93 downregulated miRNAs, accounting for 6.92% and 2.34% of the total, respectively.

3.4. GO enrichment and pathway analysis results

KOBAS analysis was conducted for the target genes and their potentially related diseases and pathways.[8] The analysis predicted target genes associated with MPS3 (mucopolysaccharidosis type III), prostate cancer and CRC (Fig. 3A) in the comparison of the PC and LC tissues. The pathway analysis (Fig. 3B) indicated that these target genes were related to the Wnt,

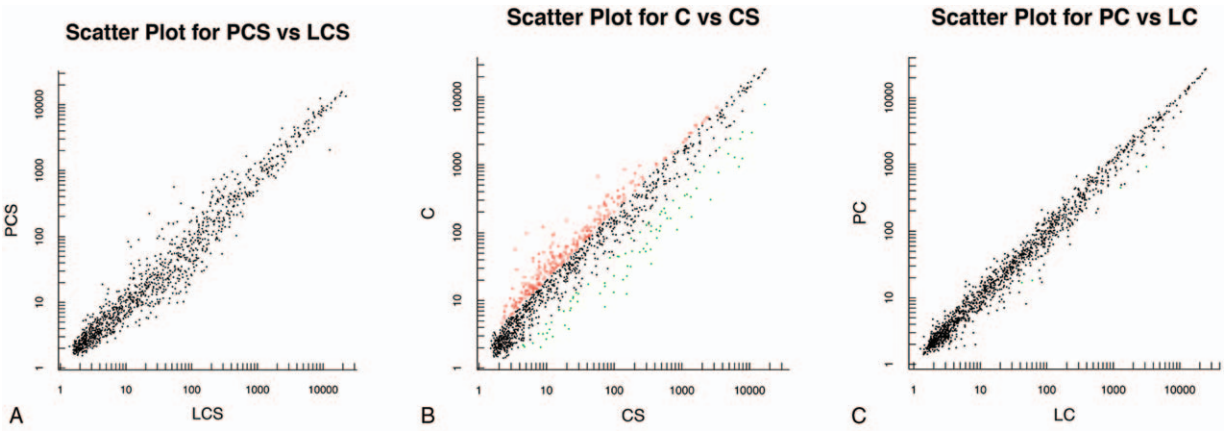

Figure 2. (A) The scatter plot of signal values compared PCS with the LCS. (B) The scatter plot of signal values compared C with the CS. (C) The scatter plot of signal values compared PC with the LC. C = cancer tissues, CS = cancer-surrounding tissues, LC = later cancer tissues, LCS = later cancer-surrounding tissues, PC = primary cancer tissues, PCS = primary cancer-surrounding tissues.

Table 2

Compared to C and CS, the number of differentially upregulated genes.

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20517821	0	19.1819	hsa-miR-3613–3p
20534320	0	16.4355	HBII-85-26
20519463	0	13.6522	hsa-miR-4668–5p
20506797	0	11.7342	hsa-miR-663b
20500164	0	7.8179	hsa-miR-31–5p
20538241	0	7.6276	U71d
20538242	0	7.552	U71d
20509228	0	7.2783	hsa-miR-1910–5p
20500170	0	6.7106	hsa-miR-92a-1–5p
20519702	0	6.368	hsa-miR-4800–3p
20504368	0	6.0544	hsa-miR-622
20536590	0	5.9979	hsa-mir-3687
20519663	0	5.6187	hsa-miR-4436b-5p
20538292	0	5.609	mgU6-47
20534329	0	5.3879	HBII-85–6
20534139	0	5.3492	ENSG00000263864
20538191	0	5.3492	U48
20534145	0	5.3492	ENSG00000264202
20534171	0	5.3492	ENSG00000265732
20534143	0	5.3492	ENSG00000264086
20538103	0	4.7566	SNORA38B
20532834	0	4.7566	ENSG00000201042
20533921	0	4.7433	ENSG00000252277
20500142	0	4.6639	hsa-miR-21–3p
20517899	0	4.513	hsa-miR-3648
20538228	0	4.4346	U70D
20500132	0	4.3166	hsa-miR-18a-5p
20500174	0	4.2856	hsa-miR-93–3p
20505760	0	4.1342	hsa-miR-708–5p
20529783	0	4.092	hsa-miR-8073
20517948	0	4.0302	hsa-miR-3687
20524034	0	3.9522	hsa-miR-6124
20532673	0	3.9396	ACA43
20517730	0	3.8531	hsa-miR-4271
20525644	0.148844963	3.8352	hsa-miR-6840–3p
20521783	0	3.7352	hsa-miR-5571–5p
20509071	0	3.686	hsa-miR-1825
20538202	0	3.6793	U53
20532668	0	3.6686	ACA3
20500416	0	3.6347	hsa-miR-208a-5p
20536552	0	3.6176	hsa-mir-3648
20500450	0.265875736	3.6167	hsa-miR-182–5p
20518785	0.265875736	3.6073	hsa-miR-4417
20521810	0.608000124	3.6063	hsa-miR-664b-5p
20538189	0	3.5067	U46
20503882	0	3.5008	hsa-miR-503–5p
20538289	0	3.4962	mgU2–25-61
20534245	0	3.4962	HBII-382
20538235	0	3.4639	U70
20534195	0	3.4639	ENSG00000268237
20534219	0	3.4442	HBII-115
20512260	0	3.4328	hsa-miR-2276–3p
20500133	0	3.4315	hsa-miR-18a-3p
20532722	0	3.406	ACA9
20534168	0	3.3893	ENSG00000265706
20532683	0	3.378	ACA48
20529143	0	3.3332	hsa-miR-7851–3p
20538205	0	3.3071	U56
20506795	0.148844963	3.2884	hsa-miR-1202
20529774	0.878060202	3.2754	hsa-miR-8064
20500489	0	3.2655	hsa-miR-224–5p
20520576	0.878060202	3.2381	hsa-miR-5195–3p
20534228	0.265875736	3.2376	HBII-180C

(*continued*)

Table 2

(continued).

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20538188	0.148844963	3.2112	U46
20538252	0.443690718	3.2015	U78
20533073	0.443690718	3.2015	ENSG00000212378
20504572	0.608000124	3.183	hsa-miR-1301–3p
20500490	0	3.1829	hsa-miR-224–3p
20525497	0.265875736	3.1786	hsa-miR-6768–5p
20501312	0.344848726	3.1765	hsa-miR-345–5p
20503100	0.677950781	3.1747	hsa-miR-483–5p
20538146	0	3.1643	U19
20532674	0.148844963	3.1609	ACA43
20517902	0.344848726	3.1299	hsa-miR-3651
20533922	0	3.0881	ENSG00000252277
20519564	0	3.0657	hsa-miR-4725–5p
20506790	0.148844963	3.0433	hsa-miR-1238–3p
20500145	0.443690718	3.0405	hsa-miR-23a-5p
20502126	0	3.0396	hsa-miR-424–3p
20532689	0.503319673	3.0356	ACA52
20518879	0	3.0084	hsa-miR-4485
20536555	0	2.984	hsa-mir-3651
20538106	0	2.984	SNORA84
20532581	0.608000124	2.9686	14qII-14
20529773	0.148844963	2.9413	hsa-miR-8063
20510799	0.265875736	2.9362	hsa-miR-1972
20501158	0	2.9345	hsa-miR-106b-3p
20525395	0.782690368	2.9234	hsa-miR-6723–5p
20537986	0	2.917	hsa-mir-6516
20538171	0	2.904	U37
20538224	0.148844963	2.8977	U68
20525621	0	2.8956	hsa-miR-6830–5p
20519554	0	2.8617	hsa-miR-4721
20538322	0.148844963	2.8601	U17a
20503811	0.265875736	2.8443	hsa-miR-181d-5p
20525470	0.148844963	2.8385	hsa-miR-6754–3p
20500446	0.503319673	2.8371	hsa-miR-181b-5p
20519427	0	2.8366	hsa-miR-4647
20525711	0.148844963	2.8341	hsa-miR-6875–5p
20522180	0.344848726	2.8324	hsa-miR-5739
20500150	0.677950781	2.829	hsa-miR-25–5p
20515637	0.608000124	2.8156	hsa-miR-3195
20517690	0	2.8139	hsa-miR-4310
20538206	0.344848726	2.806	U56
20536949	0	2.7968	hsa-mir-4716
20501229	0	2.7947	hsa-miR-371a-5p
20532720	0.344848726	2.7809	ACA7
20532719	0.344848726	2.7809	ACA7B
20532968	0.344848726	2.7809	ENSG00000206913
20520577	0.344848726	2.7774	hsa-miR-5196–5p
20506901	0.148844963	2.7693	hsa-miR-1307–3p
20501162	1.063502942	2.7639	hsa-miR-200a-5p
20504552	0.344848726	2.7535	hsa-miR-671–5p
20525692	0	2.7525	hsa-miR-6865–3p
20525659	0	2.7445	hsa-miR-6849–5p
20519425	0	2.7379	hsa-miR-4646–5p
20500762	0.608000124	2.7324	hsa-miR-191–3p
20519681	0.344848726	2.7154	hsa-miR-4788
20503875	0.148844963	2.7056	hsa-miR-500a-5p
20500396	0	2.6986	hsa-miR-198
20538274	0	2.6961	U94
20518880	0.344848726	2.6898	hsa-miR-4486
20518439	0	2.6735	hsa-miR-3916
20538305	0.503319673	2.67	E2
20504312	0.148844963	2.6692	hsa-miR-584–5p
20538223	0.148844963	2.6596	U68

(*continued*)

Table 2

(continued).

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20518440	0.265875736	2.6457	hsa-miR-3917
20532737	1.301466457	2.6403	ENSG00000199411
20517717	0.344848726	2.6399	hsa-miR-4327
20517750	0	2.6393	hsa-miR-4290
20538187	0.677950781	2.6341	U46
20532829	0.677950781	2.6341	ENSG00000201009
20534321	0	2.6107	HBII-85-26
20518826	0.93358016	2.6087	hsa-miR-4449
20538111	0	2.5994	SNORD121B
20501177	0.148844963	2.5868	hsa-miR-99b-3p
20532618	0	2.5851	ACA11
20529132	0.503319673	2.583	hsa-miR-1273h-5p
20515585	0	2.5691	hsa-miR-3162–5p
20520574	0	2.568	hsa-miR-5194
20517817	0	2.557	hsa-miR-3610
20525017	0.344848726	2.5436	hsa-miR-6508–5p
20515650	0.344848726	2.5387	hsa-miR-1273d
20503877	0	2.5329	hsa-miR-501–5p
20519498	0.344848726	2.5096	hsa-miR-4690–5p
20519488	0.148844963	2.4919	hsa-miR-4684–3p
20538165	0.148844963	2.4861	U34
20519439	0.265875736	2.4845	hsa-miR-4655–5p
20502130	0.344848726	2.4833	hsa-miR-425–3p
20517936	0.148844963	2.4813	hsa-miR-3679–5p
20517910	0.148844963	2.4675	hsa-miR-1273e
20538200	0	2.4484	U51
20519409	0.782690368	2.4482	hsa-miR-4634
20500131	0.265875736	2.4387	hsa-miR-17–3p
20518945	0.608000124	2.4328	hsa-miR-4538
20532718	0	2.4328	ACA6
20538180	0	2.4286	U43
20515533	0	2.4282	hsa-miR-3131
20534200	0	2.4064	ENSG00000268874
20532693	0	2.4064	ACA56
20519417	0.503319673	2.4047	hsa-miR-4640–5p
20500791	0	2.3924	hsa-miR-188–5p
20538253	0.503319673	2.3894	U78
20502235	0.148844963	2.387	hsa-miR-18b-5p
20519588	0.503319673	2.3797	hsa-miR-4738–3p
20504433	0.93358016	2.3797	hsa-miR-421
20525635	1.390770124	2.3688	hsa-miR-6836–5p
20525627	0	2.3682	hsa-miR-6833–5p
20532640	0	2.367	ACA27
20523016	1.063502942	2.3604	hsa-miR-6084
20526180	0.93358016	2.3556	hsa-miR-7111–5p
20525505	0.265875736	2.3544	hsa-miR-6772–5p
20538181	0	2.3539	U43
20538323	0	2.3492	U17a
20500418	0	2.3468	hsa-miR-129–5p
20525555	0.722275328	2.3466	hsa-miR-6797–5p
20538175	0.148844963	2.3259	U3
20517679	0.344848726	2.3235	hsa-miR-4299
20519518	0	2.3226	hsa-miR-4701–3p
20538112	0.148844963	2.3215	SNORD121B
20504292	0	2.3187	hsa-miR-570–5p
20518847	0	2.3187	hsa-miR-548ai
20503106	0.344848726	2.3067	hsa-miR-486–3p
20519472	0.265875736	2.3039	hsa-miR-4672
20500130	0	2.3009	hsa-miR-17–5p
20500469	0.608000124	2.2984	hsa-miR-212–3p
20504273	0.608000124	2.2941	hsa-miR-92b-5p
20518937	0	2.286	hsa-miR-4535
20517903	0.503319673	2.286	hsa-miR-3652

(*continued*)

Table 2

(continued).

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20538140	0.503319673	2.2824	U17b
20524053	0.503319673	2.2802	hsa-miR-6132
20525684	0.93358016	2.2792	hsa-miR-6861–5p
20500465	2.183514642	2.2757	hsa-miR-210–3p
20501181	0.148844963	2.2651	hsa-miR-130b-3p
20518800	0.265875736	2.2629	hsa-miR-4428
20538192	0.878060202	2.2612	U49A
20538195	0.878060202	2.2612	U49B
20518721	0.503319673	2.248	hsa-miR-642b-3p
20500783	2.00732039	2.247	hsa-miR-150–3p
20500156	0.782690368	2.246	hsa-miR-27a-5p
20500194	0	2.2447	hsa-miR-106a-5p
20538207	0.148844963	2.2446	U57
20504576	1.152454016	2.2408	hsa-miR-1185–2–3p
20501772	1.390770124	2.2363	hsa-miR-196b-3p
20536948	0	2.2331	hsa-mir-4716
20538124	0.677950781	2.2311	U105B
20534226	0.608000124	2.2309	HBII-180A
20525587	1.152454016	2.23	hsa-miR-6813–5p
20515624	0	2.2212	hsa-miR-3188
20525601	0.878060202	2.2195	hsa-miR-6820–5p
20525619	0.722275328	2.2194	hsa-miR-6829–5p
20519405	0	2.2152	hsa-miR-4632–5p
20525453	0.443690718	2.2118	hsa-miR-6746–5p
20532617	0.608000124	2.2094	ACA10
20532999	0.608000124	2.2094	ENSG00000207187
20500777	0.93358016	2.2064	hsa-miR-138–1–3p
20525691	0.782690368	2.2056	hsa-miR-6865–5p
20525541	0.148844963	2.204	hsa-miR-6790–5p
20538166	0.148844963	2.2002	U35A
20529795	0	2.1967	hsa-miR-8085
20538303	0.782690368	2.1948	snR38C
20519496	2.089783282	2.1888	hsa-miR-4688
20538328	0.265875736	2.1885	U3–4
20538326	0.265875736	2.1885	U3–2
20538327	0.265875736	2.1885	U3–3
20538325	0.265875736	2.1885	U3–2B
20525531	0.608000124	2.1837	hsa-miR-6785–5p
20538176	0.503319673	2.1825	U41
20538144	0.608000124	2.182	U18C
20501276	1.77003502	2.1797	hsa-miR-330–3p
20518940	1.529683708	2.1694	hsa-miR-1587
20538238	0.148844963	2.1621	U71b
20525603	0	2.151	hsa-miR-6821–5p
20506002	0.503319673	2.1377	hsa-miR-933
20525749	0.148844963	2.137	hsa-miR-6894–5p
20525561	0	2.1366	hsa-miR-6800–5p
20538247	0.608000124	2.1348	U75
20532648	0.93358016	2.1256	ACA3–2
20525539	0	2.1175	hsa-miR-6789–5p
20538138	0	2.1156	U15B
20501209	2.00732039	2.1151	hsa-miR-365a-5p
20503789	0.677950781	2.1047	hsa-miR-491–5p
20538132	0.148844963	2.1032	U13
20525444	0.878060202	2.0975	hsa-miR-6741–5p
20525735	0.344848726	2.0949	hsa-miR-6887–5p
20519508	0.265875736	2.0926	hsa-miR-4695–3p
20519636	0	2.0903	hsa-miR-4763–3p
20500141	3.535890221	2.0872	hsa-miR-21–5p
20519592	0	2.0824	hsa-miR-4741
20523000	0.148844963	2.0822	hsa-miR-6068
20506830	0.265875736	2.0805	hsa-miR-1304–3p
20538272	0	2.0785	U92

(*continued*)

Table 2

(continued).

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20537087	0	2.0766	hsa-mir-5095
20504585	1.675061226	2.0742	hsa-miR-1185–1–3p
20511549	2.00732039	2.0692	hsa-miR-2110
20520198	0.148844963	2.0671	hsa-miR-5001–5p
20536702	0.503319673	2.0599	hsa-mir-4449
20534337	0.608000124	2.0552	HBII-99
20501298	1.152454016	2.054	hsa-miR-339–5p
20538167	0.503319673	2.0452	U35B
20519580	0	2.0414	hsa-miR-4734
20538222	0.148844963	2.0305	U67
20518818	0	2.0289	hsa-miR-4443
20515617	0	2.0289	hsa-miR-3185
20519689	0	2.0227	hsa-miR-4793–3p
20505795	0.782690368	2.0177	hsa-miR-665
20529138	1.529683708	2.0177	hsa-miR-7846–3p
20518903	1.301466457	2.0167	hsa-miR-2392
20532647	0.93358016	2.0146	ACA3–2
20500116	0.148844963	2.0107	hsa-let-7b-3p
20517736	0.148844963	2.0095	hsa-miR-4281
20524250	0.148844963	2.0079	hsa-miR-6165
20501299	1.77003502	2.0057	hsa-miR-339–3p
20518625	0.608000124	2.0009	hsa-miR-3937
20538248	0.782690368	2.0006	U75

C = cancer tissues, CS = cancer-surrounding tissues.

Table 3

Compared to C and CS, the number of differentially downregulated genes.

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20501243	3.706777575	0.4953	hsa-miR-378a-3p
20537464	3.643870306	0.4926	hsa-mir-6722
20501280	3.588226789	0.4893	hsa-miR-342–3p
20533836	1.301466457	0.489	ENSG00000251940
20534201	1.301466457	0.489	ENSG00000268890
20529566	3.588226789	0.473	hsa-miR-7975
20500146	1.063502942	0.4706	hsa-miR-23a-3p
20534505	0.677950781	0.4682	hsa-mir-139
20518881	4.692086816	0.4673	hsa-miR-4487
20534637	0.200170812	0.4652	hsa-mir-320a
20500115	0.200170812	0.46	hsa-let-7b-5p
20500720	4.084117525	0.4563	hsa-miR-23b-5p
20506835	3.965301167	0.4553	hsa-miR-1244
20500128	3.535890221	0.4432	hsa-miR-16–5p
20504218	3.854489164	0.4391	hsa-miR-487b-3p
20503884	3.588226789	0.4346	hsa-miR-504–5p
20500161	3.706777575	0.4319	hsa-miR-29a-3p
20536308	0.728994168	0.4314	hsa-mir-320e
20501293	4.084117525	0.4309	hsa-miR-331–3p
20518446	2.469463258	0.425	hsa-miR-3921
20533759	1.063502942	0.4245	ENSG00000239155
20518801	0.200170812	0.4227	hsa-miR-4429
20504569	4.692086816	0.4199	hsa-miR-1271–5p
20501286	0	0.415	hsa-miR-151a-5p
20500769	3.854489164	0.4099	hsa-miR-126–3p
20521785	3.588226789	0.409	hsa-miR-5100
20515627	3.429833865	0.3998	hsa-miR-320e
20500144	0.200170812	0.397	hsa-miR-22–3p
20517745	3.588226789	0.397	hsa-miR-4286
20500424	0	0.3868	hsa-miR-30d-5p
20533275	0.878060202	0.3864	ENSG00000238414
20500721	0.200170812	0.3852	hsa-miR-23b-3p
20504561	0.878060202	0.378	hsa-miR-151b

(*continued*)

Table 3

(continued).

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20501296	1.675061226	0.3702	hsa-miR-338–5p
20533758	0.878060202	0.3595	ENSG00000239154
20500159	1.063502942	0.3534	hsa-miR-28–3p
20500158	0.200170812	0.3379	hsa-miR-28–5p
20501182	4.084117525	0.3371	hsa-miR-30e-5p
20501282	0.677950781	0.3352	hsa-miR-337–3p
20529785	0.200170812	0.3348	hsa-miR-8075
20501279	4.154003465	0.3332	hsa-miR-342–5p
20500152	0.608000124	0.3298	hsa-miR-26a-5p
20533693	0.200170812	0.3213	ENSG00000239055
20500189	3.643870306	0.3173	hsa-miR-29b-2–5p
20528493	0.377217199	0.3154	hsa-miR-7641
20509070	0	0.3054	hsa-miR-320d
20518936	0.878060202	0.3	hsa-miR-378i
20500119	0	0.299	hsa-let-7d-5p
20500758	1.301466457	0.2911	hsa-miR-152–3p
20500755	1.390770124	0.2759	hsa-miR-145–5p
20518834	0	0.2717	hsa-miR-4454
20501309	1.390770124	0.269	hsa-miR-133b
20500782	0.377217199	0.2657	hsa-miR-150–5p
20500715	0	0.2641	hsa-let-7i-5p
20500400	3.550136888	0.2595	hsa-miR-199a-3p
20500458	3.550136888	0.2595	hsa-miR-199b-3p
20517675	0	0.2487	hsa-miR-378c
20500765	0	0.2473	hsa-miR-125a-5p
20500163	0.200170812	0.2473	hsa-miR-30a-3p
20500751	3.6712409	0.2469	hsa-miR-143–5p
20504378	0	0.2361	hsa-miR-628–3p
20500723	0.377217199	0.2359	hsa-miR-27b-3p
20504584	1.152454016	0.2313	hsa-miR-378d
20518783	0.377217199	0.23	hsa-miR-378e
20500718	0	0.2283	hsa-miR-15b-5p
20500399	0	0.2257	hsa-miR-199a-5p
20518794	2.469463258	0.2214	hsa-miR-378g
20500121	0	0.219	hsa-let-7e-5p
20500438	0.503319673	0.2162	hsa-miR-10a-5p
20502122	0	0.2141	hsa-miR-422a
20500752	0	0.2118	hsa-miR-143–3p
20533260	0.200170812	0.2045	ENSG00000238388
20500162	0.265875736	0.2036	hsa-miR-30a-5p
20518788	0	0.1969	hsa-miR-378f
20500713	0	0.1819	hsa-let-7g-5p
20533259	0	0.1808	ENSG00000238388
20500739	1.110891676	0.1766	hsa-miR-133a-3p
20500123	0	0.1649	hsa-let-7f-5p
20500117	0	0.1576	hsa-let-7c-5p
20500432	0	0.1495	hsa-miR-139–5p
20500735	0	0.1491	hsa-miR-130a-3p
20500112	0	0.1337	hsa-let-7a-5p
20500440	0	0.1334	hsa-miR-10b-5p
20500183	0	0.1303	hsa-miR-100–5p
20500798	0	0.1218	hsa-miR-195–5p
20500730	0	0.1145	hsa-miR-125b-5p
20533757	0	0.1098	ENSG00000239154
20500181	0	0.0985	hsa-miR-99a-5p
20503809	0	0.0816	hsa-miR-497–5p

C = cancer tissues, CS = cancer-surrounding tissues.

Table 4

Compared to PC and LC, the number of differentially downregulated genes.

Gene ID	*Q*-value (%)	Fold change	Transcript ID (array design)
20500766	0	0.3971	hsa-miR-125a-3p
20501176	0	0.345	hsa-miR-99b-5p
20501177	0	0.3015	hsa-miR-99b-3p
20500472	0	0.2919	hsa-miR-214–3p

LC = late cancer tissues, PC = primary cancer tissues.

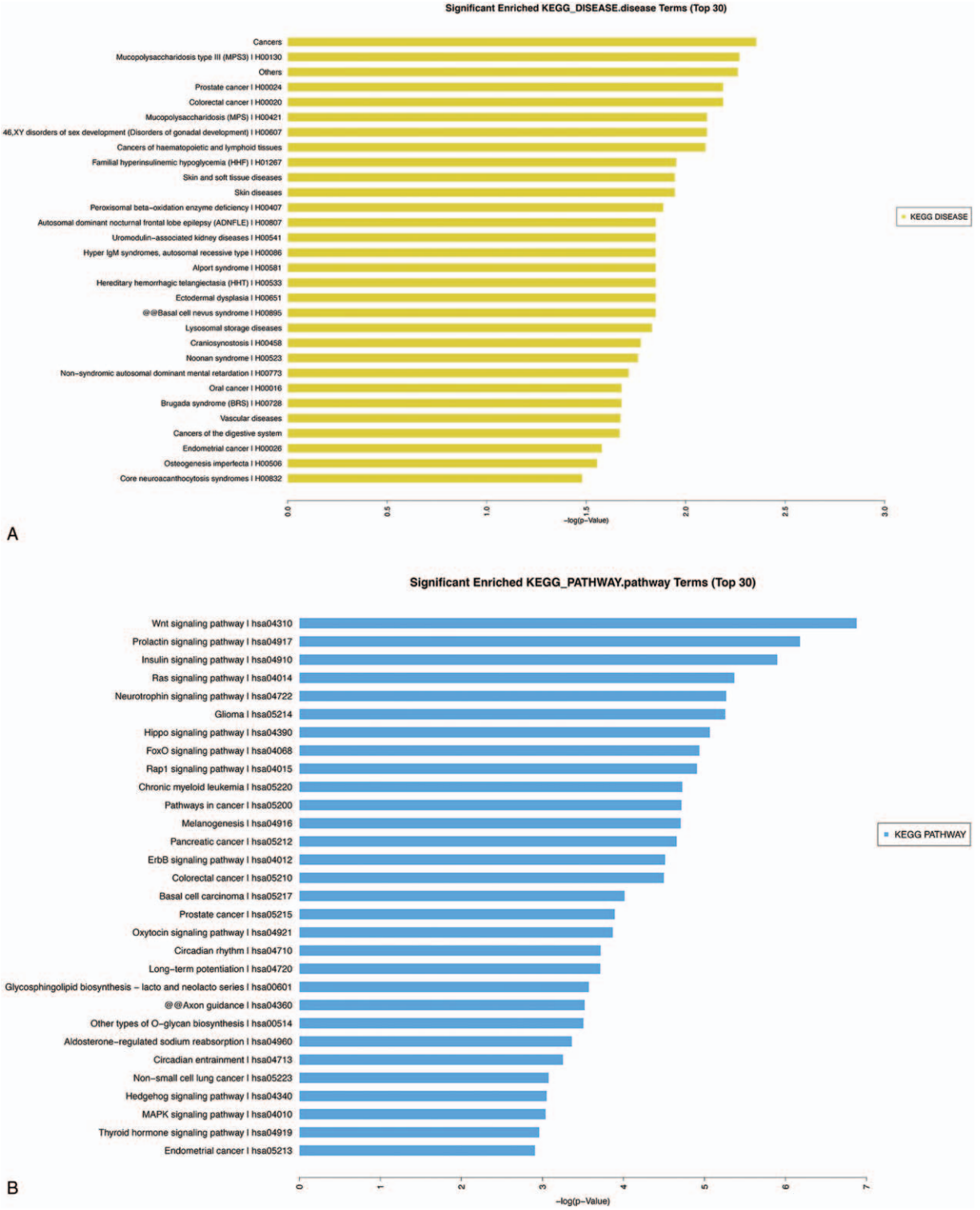

Figure 3. (A) Significantly enriched KEGG-DISEASE terms (top 30). Following comparisons of the PC and LC samples, the target gene annotation results were analyzed in the KEGG-DISEASE database. (B) Significantly enriched KEGG-PATHWAY terms (top 30). Following comparisons of the PC and LC samples, the target gene annotation results were analyzed in the KEGG-PATHWAY database. LC= later cancer tissues, PC=primary cancer tissues.

prolactin, insulin, and Ras signaling pathways. Upon comparing C with CS, the target genes were predicted in the tumor, skin, and soft tissue disease categories (Fig. 4A). The pathway analysis (Fig. 4B) showed that the target genes were mainly related to the thyroid hormone signaling pathway, proteoglycans in cancer, Wnt signaling pathway, choline metabolism in cancer, cAMP signaling pathway, T cell receptor signaling pathway, AMPK signaling pathway, and TNF signaling pathway. After conducting the significance analysis of GO terms for the target genes (http://www.geneongoloty.org/), we obtained the corresponding

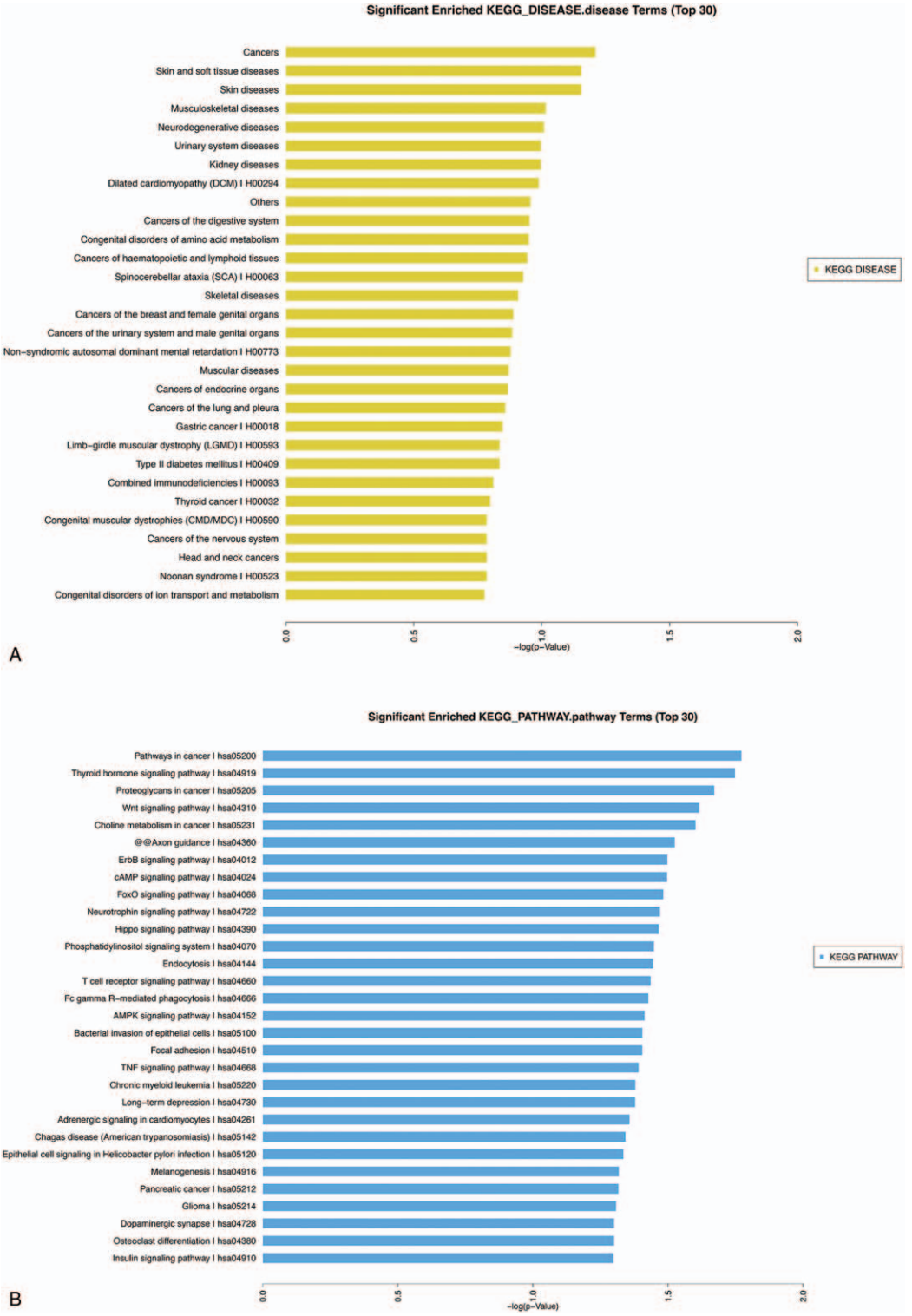

Figure 4. (A) Significantly enriched KEGG-DISEASE terms (top 30). Following comparisons of the C and CS samples, the target gene annotation results were analyzed with the KEGG-DISEASE database. (B) Significantly enriched KEGG-PATHWAY terms (top 30). Following comparisons of the C and CS samples, the target gene annotation results were analyzed with the KEGG-PATHWAY database. C = cancer tissues, CS = cancer-surrounding tissues.

significant GO terms and their genes. Figure 5 indicates that in the biological process category, the targeted genes were mainly related to metabolic processes, anatomical structure development, positive regulation of biological processes, and positive regulation of cellular processes. In the cellular component category, the target genes were mainly related to intracellular components, cytoplasm, organelles and membrane-bounded organelles. In the molecular function category, the target genes were mainly related to the aspects of binding, protein binding, ion binding, anion binding, small molecule binding, enzyme binding,

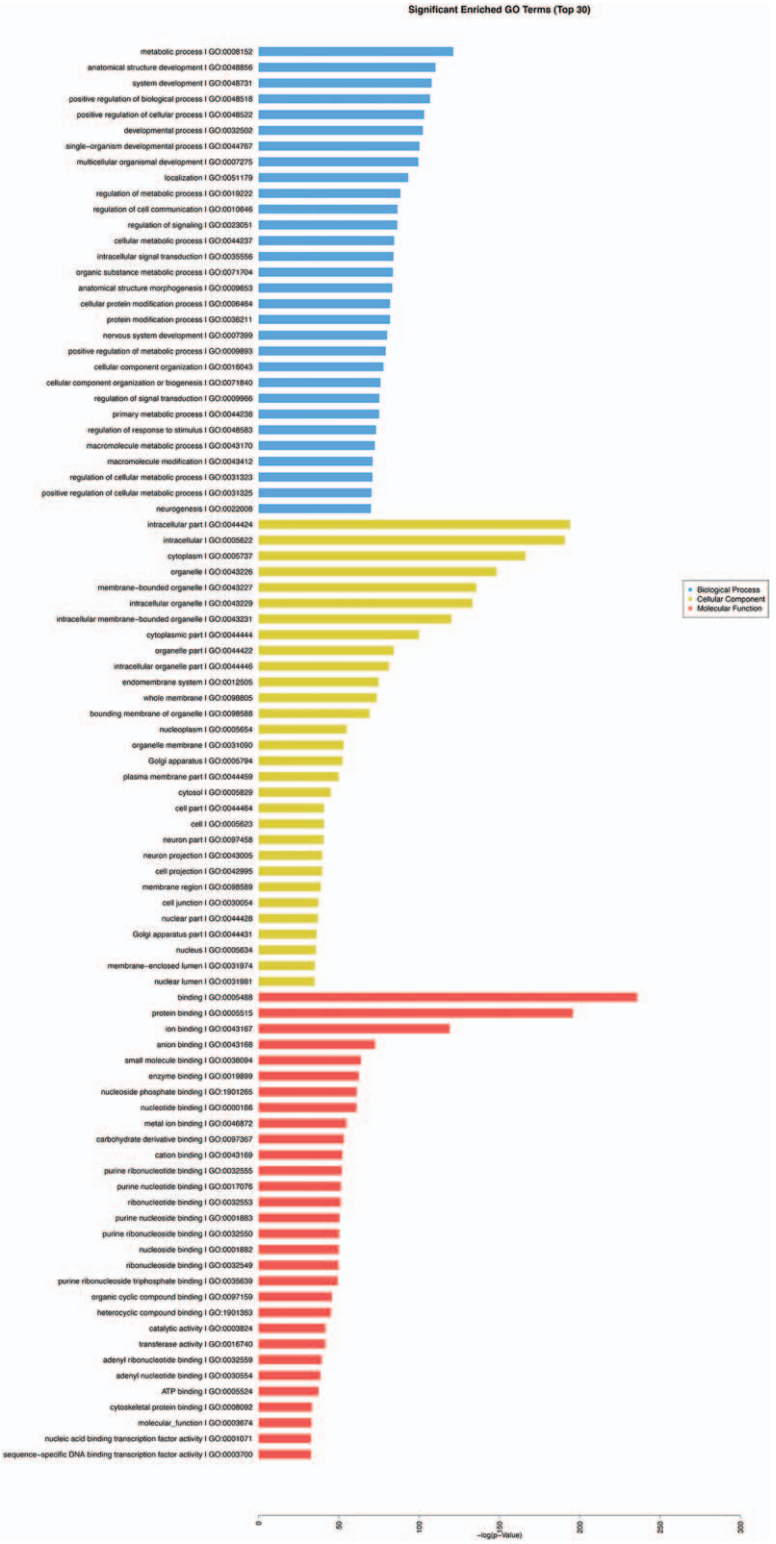

Figure 5. Significantly enriched GO terms (top 30). The GO (gene ontology) annotation results following comparisons of the C and CS samples. C = cancer tissues, CS = cancer-surrounding tissues, GO = gene ontology.

and nucleoside phosphate binding. Figure 6 shows that the target genes with biological process terms were mainly related to the positive regulation of biological processes, positive regulation of cellular processes, regulation of cell communication, and multicellular organismal development. In the cellular components category, the target genes were mainly related to intracellular components, cytoplasm, intracellular organelles, neurons, cytoplasmic structures, and membrane-bounded organelles. In terms of molecular functions, the targeted genes were mainly related to the aspects of protein binding, enzyme

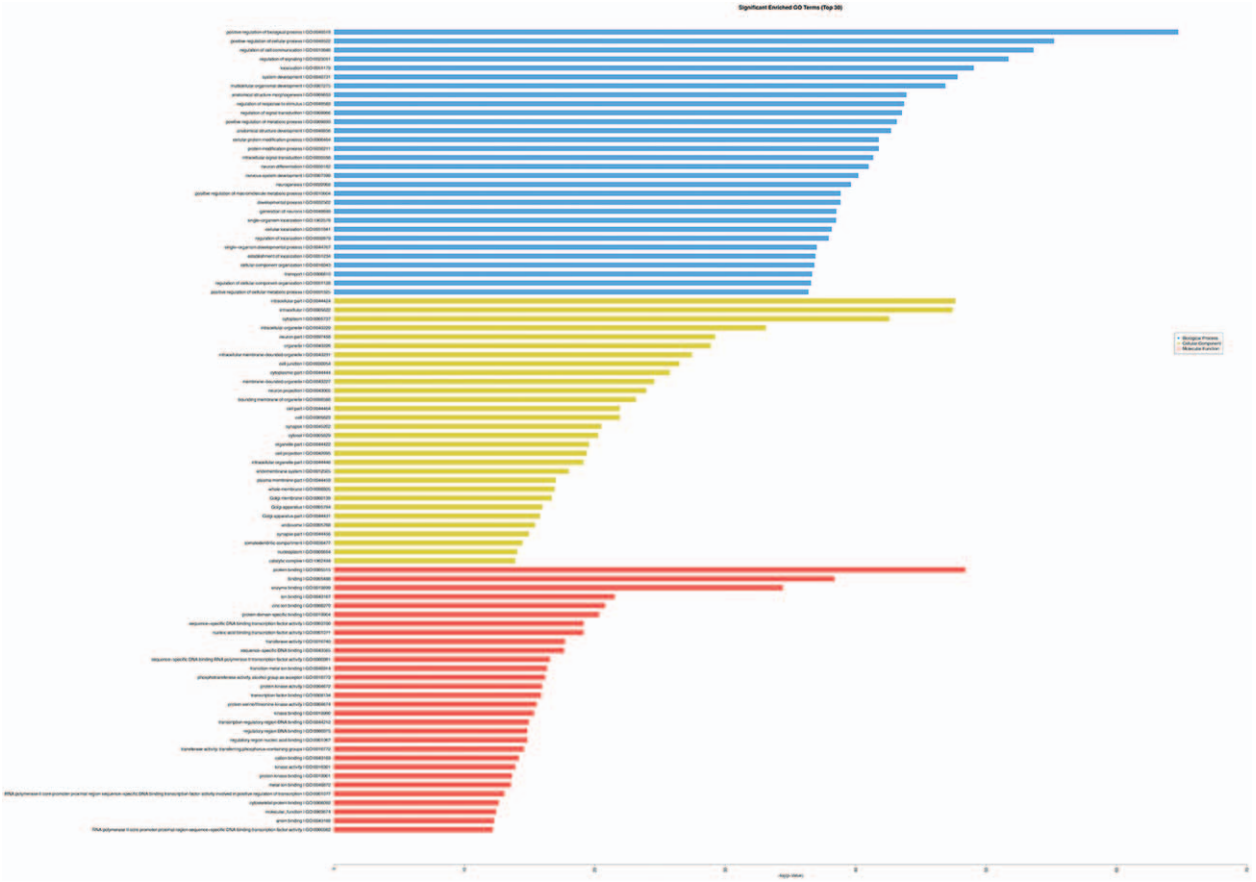

Figure 6. Significantly enriched GO terms (top 30). Following comparisons of the PC and LC samples, the target gene annotation results from the GO (gene ontology) analysis are shown. GO = gene ontology, LC = later cancer tissues, PC = primary cancer tissues.

binding, ion binding, zinc ion binding and protein domain-specific binding.

4. Discussion

The occurrence of colorectal cancer is commonly due to multistep, multifactor, and polygenic effects and involves changes in multiple oncogenes and tumor suppressor genes.[9] In this study, we evaluated 9 pairs of C and CS tissues in different stages using gene chip technology and obtained a number of target genes that were differentially expressed due to regulation by microRNAs. Previous studies showed that abnormal microRNA expression profiles were related to the occurrence and development of many tumors, including colorectal cancer.[10,11] In this study, 275 upregulated and 93 downregulated miRNAs were screened out by comparing C with CS, no upregulated miRNAs and 4 downregulated miRNAs were identified by comparing PC with LC, and neither upregulated nor downregulated miRNAs were identified by comparing PCS with LCS. In the above 3 groups of comparisons, a total of 3972 miRNAs were regulated by microRNAs. Among the differentially regulated genes, 275 and 93 genes were up- and downregulated by the miRNAs, respectively. The microRNA expression profiles were not only different between C and CS but were also between the different CRC stages. Some researchers have suggested that the microRNA expression profile in CRC is significantly different from the profile in the surrounding tissues.[12,13] Qiu et al[14] also confirmed that miRNA-21 expression was related to the TNM stage and suggested that the later TNM stages were associated with higher miRNA-21 expression levels. Furthermore, some studies[15,16] showed that specific microRNAs were associated with the TNM stage of the tumor and suggested that microRNAs might be used for prediction of the tumor prognosis. In different stages of CRC, the microRNA families were associated with CRC-related genes and pathways, participated in tumor-related signaling pathways, and regulated the expression profiles of target genes and biological processes, such as cell proliferation and apoptosis, among others. Other studies showed that[17,18] microRNA alterations in cellular pathways affected the susceptibility and progression of diseases, inhibited or induced the expression of messenger RNAs, and ultimately affected the occurrence of oncogenes and tumor suppressor genes.

The KEGG-Disease analysis revealed that target genes in different stages of cancer were mainly related to tumor occurrence and development, and CRC was no exception. The abnormal regulation of microRNAs in different stages of CRC tissues often leads to the occurrence of many diseases, including tumor invasion and metastasis.[19,20] MicroRNAs regulate the expression of multiple target genes and participate in the regulation of normal physiological processes and tumor cell occurrence, development, and invasion.[21] For example, microRNAs regulate the invasion and metastasis of CRC cells via the PI3K/AKT pathway,[22] the transforming growth factor (TGF-β) signaling pathway,[23] and regulation of matrix metalloproteinases.[24] Similarly, the KEGG pathway analysis of tissues from different CRC stages showed that microRNAs regulated tumor-related cell signaling pathways, such

as the Wnt, prolactin, insulin, Ras, thyroid hormone, proteoglycan, and choline signaling pathways. In addition, some of these signaling pathways are involved in the development of colorectal cancer, such as the Wnt and Ras signaling pathways.[25,26] Based on the previous research, the identification of some tumor-related signaling pathways, including the p53, Wnt, and TGF-β signaling pathways, which were mapped to some pathways in CRC, such as cell cycle and survival,[27] showed that microRNAs and their target genes were directly involved in the biological process of CRC and that CRC cell carcinogenesis was indirectly inhibited by the cell signaling pathways in different stages of CRC. Therefore, intervening in these signaling pathways may provide a new method for individualized treatment for and diagnosis of CRC.

These results suggested that microRNAs and their regulated target genes could affect the occurrence and development of CRC by regulating biological processes and that these microRNAs could play important roles in the assessment of the CRC prognosis. For example, over-expression of miRNA-21 in colon cancer was not associated with malignancy but was closely related to survival and treatment outcome.[28] Abnormal microRNA expression also affects the biological processes and molecular functions of other tumors, such as gastric cancer, thyroid cancer and cancer of the nervous system. Thus, microRNAs could become new therapeutic tools for the treatment of malignant tumors by inhibiting or enhancing the expression of target genes, thereby affecting the occurrence and development of malignant tumors.

In summary, the microRNA expression profiles significantly differed between different stages of CRC. The target mRNAs regulated by the affected microRNAs were mainly involved in biological processes, cellular components and molecular functions. MicroRNAs are involved in the entire CRC development process and have particularly important clinical value for the early diagnosis and prognostic evaluation of CRC.

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