Pathol. Oncol. Res. (2014) 20:245–252
DOI 10.1007/s12253-013-9688-z

RESEARCH

Research on the Typical miRNA and Target Genes in Squamous Cell Carcinoma and Adenocarcinoma of Esophagus Cancer with DNA Microarray

Hong Bin Wang · Zhi Biao Jiang · Min Li

Received: 22 April 2013 / Accepted: 22 August 2013 / Published online: 12 February 2014

Abstract To identify the typically expressed miRNAs in squamous cell carcinoma (SCC) and adenocarcinoma (ADC) of esophagus cancer and their target genes, and explore the related functions and pathways, providing potential biomarkers for esophageal carcinoma diagnosis and treatment. Gene expression profile GSE13937 was downloaded from Gene Expression Omnibus database which includes 152 samples, paired non-cancerous and cancerous, 44 SCC cases and 32 ADC cases; the differentially expressed miRNAs were identified with limma packages in R language after the data were normalized. Selected differentially expressed miRNAs were further analyzed using bioinformatics methods. Firstly, verified targets of miRNAs in two miRNA databases: miRecods and miRTarBase were integrated to select the targets genes of differentially expressed miRNAs. Next, String software was used to construct the target genes interaction network. Finally, function and pathway enrichment analysis of genes in the interaction network was carried out with Gestalt software. Up-regulated hsa-miR-21 and down-regulated hsa-miR-203 were identified by comparing normal and cancer tissue samples, and the targets genes regulated by these two miRNAs were most significantly related to cell cycle function and pathway, especially in the phase of G1/S. The two differentially expressed miRNA: hsa-miR-21 and hsa-miR-203 provide evidence for early diagnosis and treatment of esophageal carcinoma. The functions and pathways of target genes shows that deep understanding of cell cycle G1/S will help to illustrate the relationship between cell cycle regulation and pathogenesis of esophageal cancer.

Keywords Esophageal cancer · Differentially expressed miRNA · Target genes interaction network · Function and pathway enrichment analysis

Introduction

Esophageal cancer (EC) ranks eighth of occurrence and sixth as the leading cause of cancer mortality [1]. EC is most common in China with the highest mortality [2]. Squamous cell carcinoma (SCC) and esophageal adenocarcinoma (ADC) account for more than 90 % in EC [3]. The formation of EC is a combined cumulative process of multi factors, and the clinical treatments at present are mainly surgery, radiotherapy and chemotherapy, with low survival rate [4]. The establishment of early prevention and detection by finding specific genetic biomarker is the hotspot in the research field of tumor and even EC.

MiRNAs are well-conserved, small (20–24 nucleotides), non-coding RNA molecules, regulating mRNAs translation, and controlling gene expression by targeting mRNAs [5]. As the biological characteristics of miRNAs, such as formation and functions, have been gradually known, it is reported that miRNAs have very close relationship with the formation of tumor [6]. MiRNA, normally positioned in cancer-associated genomic regions: genomic instability region, expresses abnormally in almost all tumors, involves in basic signal transduction

H. B. Wang
Department of Cardiac-Thoracic Surgery, Sixth People's Hospital in Southern Branch, Affiliated to Shanghai Jiao Tong University, Fengxian District Central Hospital, Shanghai 201499, China

Z. B. Jiang
Department of Cardiac-Thoracic Surgery, Shanghai Fengxian Hospital, Affiliated to Southern Medical University, Shanghai 201499, China

M. Li (✉)
Department of Minimally Invasive Gynecological Ward, Sixth People's Hospital in Southern Branch, affiliated to Shanghai Jiao Tong University, Fengxian District Central Hospital, No. 6600 Nanfeng Road, Fengxian District, Shanghai 201499, China
e-mail: limin568856@hotmail.com

Table 1 The differentially expressed miRNAs

SCC			ADC		
miRNA_ID	FDR	logFC	miRNA_ID	FDR	logFC
hsa-miR-521	0.0349	−3.21888	hsa-mir-101	0.03110064	−2.7524059
hsa-miR-302c	0.0407	−3.01481	hsa-miR-205	0.00008318	−2.7126889
hsa-mir-498	0.0331	−2.90049	hsa-miR-203	0.00020111	−2.4498658
hsa-mir-509	0.0434	−2.44217	hsa-miR-194*	0.04947571	−2.4378198
hsa-mir-450a	0.0338	−2.12801	hsa-miR-136*	0.01494514	−2.1281385
hsa-mir-331	0.00371	−1.861	hsa-miR-202	0.0000631	−1.8122706
hsa-miR-202	0.000119	−1.64359	hsa-miR-450a	0.00535475	−1.1080344
hsa-miR-203	0.0035	−1.56716	hsa-miR-496	0.00799396	−1.0811464
hsa-miR-375	6.22E-07	−1.40221	hsa-miR-424*	0.03857966	−1.0164284
hsa-miR-133a	0.00114	−1.2097	hsa-miR-21	0.00006686	1.1190711
hsa-miR-1	0.000842	−1.11102	hsa-miR-192	0.02401597	1.1554549
hsa-miR-223	2.51E-06	1.252839	hsa-miR-335	0.00554656	1.6218158
hsa-miR-21	1.29E-10	1.406785	hsa-miR-183*	0.03908016	2.0359841
hsa-miR-452	0.0286	1.498027	hsa-miR-208a	0.04544495	3.0559119
hsa-miR-154*	0.0324	1.932731	hsa-miR-511	0.00915115	4.9617077
hsa-mir-512	0.00931	3.044924	hsa-miR-518b	0.00124996	5.4469899
hsa-miR-381	0.00188	4.329163	hsa-miR-381	0.00132844	8.2431998
hsa-miR-519a	0.0177	4.373794			
hsa-miR-518c*	2.23E-05	5.722046			

SCC squamous cell carcinoma, *ADC* adenocarcinoma of esophagus cancer, *FDR* false discovery rate, *FC* fold chance

pathways, and regulates the expression of many tumor-related genes by regulating the translation of mRNA [7].

Recent studies have pointed out that miRNA is closely related with the formation and development of EC [8, 9], including SCC and ADC. Hsa-miR-21* is found to be up-regulated in SCC tissues [10] and may play a role in abnormal proliferation of SCC tissues by negatively regulating the expression of tumor suppressor gene PDCD4 [11]. Hsa-miR-203 is one of the down-regulated miRNAs in SCC tissues [12], and miR-203 mediates the post-transcriptional suppression of SOCS3 [13]. The miRNA expression profiles of EC are significantly different from that of adjacent normal tissue. However, high-throughput screenings of miRNA expression in EC remains rare. In this study, we aimed to screen the differentially expressed miRNAs by comparing miRNA microarray expression data of non-cancerous, SCC and ADC samples. In addition, their underlying target genes and the molecular mechanism were also predicted.

Materials and Methods

Affymetrix Chip Data

GSE13937 was downloaded from a public functional genomics data repository Gene Expression Omnibus (GEO) database [2]. Total 152 non-cancerous and cancerous paired samples were available, including 44 SCC cases, and 32 ADC cases (18 cases were also diagnosed with Barrett's esophagus). The annotation information of chip was also downloaded from GPL8835 OSU-CCC Human and Mouse MicroRNA Microarray (Version 3.0).

Data Processing and Differentially Expressed miRNAs Analysis

Original CEL expression profiling data were processed by Affy package in R language [14, 15], and then was standardized using median method. Significance of differentially expressed miRNA was tested by R package-limma [16], and adjusted for multiple testing with the Benjamin and Hochberg (BH) [17] in multtest package. Only the miRNAs with False Discovery Rate (FDR) < 0.05 and |logFC| > 1 were selected as differentially expressed miRNAs.

Target Gene Screening of Differentially Expressed miRNAs

Every single miRNA has multiple target genes. In order to detect target genes with high certainty factor (HCF), we integrated the verified targets of miRNAs in two miRNA databases: miRecods and miRTarBase to select the target genes of differentially expressed miRNAs. MiRecords is a database of target gene interaction of animal miRNA [18], including target genes that have been validated experimentally, and have now gathered 548 miRNAs of 9 species with their verified target genes.

Table 2 The target genes of differentially expressed miRNAs

a).miRNA-203

Tarbase	miRecord
ABCE1	—
ABL1	ABL1
BCL2L2	—
CDK6	—
EDNRA	—
EYA4	—
GDAP1	—
PPM1D	—
SOCS3	SOCS3
TP63	p63

b).miRNA-21

Tarbase	miRecord	Tarbase	miRecord	Tarbase	miRecord
TIMP3	TIMP3	ANKRD46	—	SPATS2L	—
MTAP	MTAP	BASP1	—	SPRY2	—
MARCKS	MARCKS	BCL2	—	TGFBI	—
RASGRP1	RASGRP1	CCR1	—	TGIF1	—
HNRNPK	HNRNPK	CDK2AP1	—	WIBG	—
PTEN	PTEN	DERL1	—	WFS1	—
CDC25A	CDC25A	E2F2	—	RTN4	—
BMPR2	BMPR2	EGFR	—	—	ACTA2
TP53BP2	TP53BP2	EIF2S1	—	—	BMPRII
SOX5	SOX5	EIF4A2	—	—	CDK6
APAF1	APAF1	ERBB2	—	—	CDKN1A
PPIF	PPIF	FMOD	—	—	CFL2
E2F1	E2F1	ICAM1	—	—	FAM3C
TOPORS	TOPORS	IL1B	—	—	FAS
LRRFIP1	LRRFIP1	ISCU	—	—	Glcci1
DAXX	DAXX	MEF2C	—	—	HIPK3
BTG2	BTG2	MSH2	—	—	IL-6R
TGFBR3	TGFBR3	MSH6	—	—	NFIB
JMY	JMY	MYC	—	—	PRRG4
RECK	RECK	NCAPG	—	—	pten
TGFBR2	TGFBR2	NCOA3	—	—	RASA1
SERPINB5	SERPINB5	PCBP1	—	—	RP2
JAG1	JAG1	PDHA2	—	—	SESN1
PDCD4	PDCD4	PLAT	—	—	SGK3
TIAM1	—	PLOD3	—	—	SLC16A10
TM9SF3	—	PTX3	—	—	SOCS5
TNFAIP3	—	REST	—	—	TGFB1
TP63	—	RHOB	—	—	TP73L
TPM1	—	RPS7	—	—	TPM1

MiRTarBase is also a database that collects comprehensively the verified target genes, and up to Nov, 2012 [19], it has collected 14 species with 773 miRNA and the corresponding 2,632 target genes. We chose the common target genes of differentially expressed miRNA in these two databases as the target genes of HCF.

Construction of the Target Genes Interaction Network

To study genes and their expression product-protein, it is necessary to study the interactive protein of them [20]. The online String (Search Tool for the Retrieval of Interacting Genes) Software [21] was used to construct the target genes

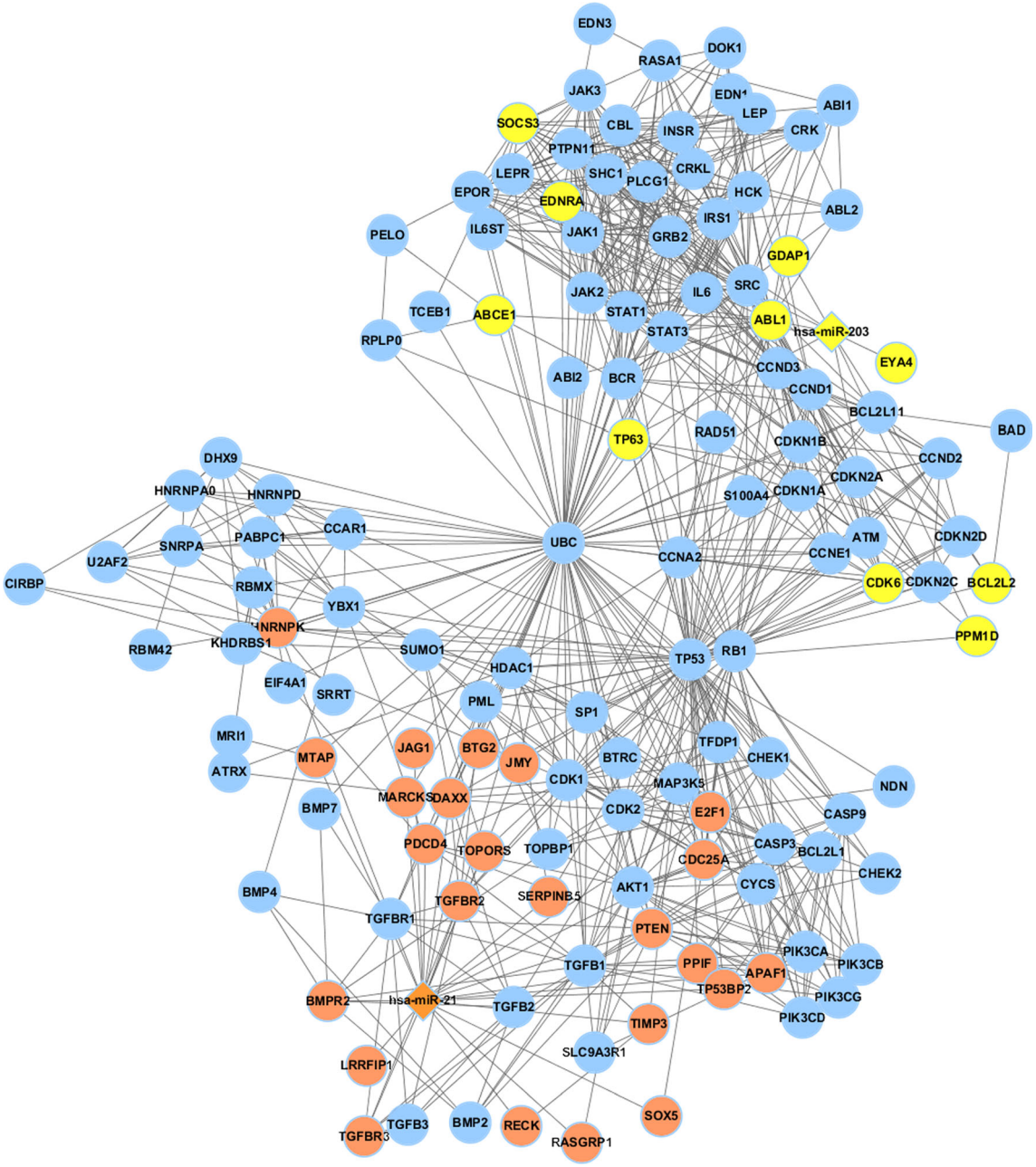

Fig. 1 Interaction network of target genes. The lozenge nodes are for the two miRNA, the orange nodes and the yellow nodes represent common target genes of hsa-miR-21 and hsa-miR-203 respectively in Tarbase and miRecord database, and the blue nodes are the predicted interacting objects

interaction network after the interactions of all target genes were collected. Interaction scores were calculated according to experimental data and text mining by String.

Function and Pathway Enrichment Analysis of Target Genes

To gain further insight into the functions and pathways of target genes in the target genes interaction network, Gestalt (Gene Set Analysis Toolkit) software was used to identify the significant functions and pathways. A group of similar or related genes were used as a whole in the analysis. Hypergeometric distribution analysis in Gestalt was used to identify the related genes in the interaction network (FDR<0.05). Biological function and characteristics changes were assessed by overall significance of gene expression changes in gene sets. Because the data dimension in this strategy is greatly reduced, and the analysis processes are very close to biological problem, enrichment analysis is increasingly used in microarray data analysis [22]. Gestalt is a rich analysis suite used in the analyses of biological problems, which contains all the information stored in different public resources,

such as NCBI (National Center for Biotechnology Information), Ensemble, KEGG (Kyoto Encyclopedia of Genes and Genomes) and GO (Gene Ontology) of 8 species including human, rat and mice [23, 24].

Results

Screening for Differentially Expressed miRNAs

Based on normalized expression data after data processing, 19 and 17 differentially expressed miRNAs (FDR<0.05 and |logFC|>1) in SCC and ADC were screened respectively (as shown in Table 1), of which up-regulated hsa-miR-21 and down-regulated hsa-miR-203 were the two common differentially expressed miRNAs.

Screening for Target Genes of Differentially Expressed miRNAs

The numbers of target genes with HCF of up-regulated hsa-miR-21 and down-regulated hsa-miR-203 were 87 and 10 respectively (as shown in Table 2).

Target Genes Interaction Network Construction

The network is shown in Fig. 1. Total 24 common target genes were screened from Tarbase and miRecord databases of has-miR-21, such as PTEN, TGFBR2; and total 10 common target genes were selected of has-miR-203, such as ABL1, p63.

Function and Pathway Enrichment Analysis of Target Genes

We found 12 and 11 significantly enriched functions of target genes of has-miR-21 and has-miR203 respectively (as shown in Table 3), of which cell cycle regulation function was the most significant. The pathway of has-miR-203 target genes cannot be enriched because the gene number (10) was low, thus the genes in the interaction network were analyzed as a whole in the pathway enrichment. Judged by the enriched pathways (Table 4), cell cycle pathway was the most significant pathway of the interactive genes (Fig. 2). Genes regulated by the two miRNAs were mainly distributed in the phase of G1/S in cell cycle.

Discussion

By DNA microarray, differentially expressed miRNAs and their target genes were screened in this study, such as up-regulated has-miR-21/PTEN, E2F1, and down-regulated has-miR-203/ABL1, p63.

Table 3 Enriched gene functions in the network

Term	Count	FDR
a).hsa-miR-21		
GO:0051726~regulation of cell cycle	21	3.66E-13
GO:0010941~regulation of cell death	28	1.83E-12
GO:0042981~regulation of apoptosis	27	1.35E-11
GO:0043067~regulation of programmed cell death	27	1.70E-11
GO:0010942~positive regulation of cell death	20	6.80E-10
GO:0042127~regulation of cell proliferation	24	6.16E-09
GO:0006468~protein amino acid phosphorylation	21	1.42E-07
GO:0010604~positive regulation of macromolecule metabolic process	22	1.76E-06
GO:0007049~cell cycle	21	2.04E-06
GO:0006793~phosphorus metabolic process	23	2.85E-06
GO:0006796~phosphate metabolic process	23	2.85E-06
GO:0016310~phosphorylation	21	3.46E-06
b).hsa-miR0-203		
GO:0007049~cell cycle	19	2.27E-16
GO:0007242~intracellular signaling cascade	32	1.20E-14
GO:0042325~regulation of phosphorylation	20	3.47E-11
GO:0042127~regulation of cell proliferation	24	3.73E-11
GO:0051174~regulation of phosphorus metabolic process	20	7.17E-11
GO:0019220~regulation of phosphate metabolic process	20	7.17E-11
GO:0010033~response to organic substance	20	7.82E-08
GO:0042981~regulation of apoptosis	19	4.00E-06
GO:0043067~regulation of programmed cell death	19	4.68E-06
GO:0010941~regulation of cell death	19	4.96E-06
GO:0010604~positive regulation of macromolecule metabolic process	19	1.10E-05

Count gene numbers, *FDR* false discovery rate

Multiple studies have shown that expression levels of many miRNAs in tumor tissues are up-regulated or down-regulated to varying degree [5]. Major biological function of miRNA is the post-transcriptional regulation, which may has potential impact on every genetic pathway. The degradation and post-transcriptional translation of target genes are inhibited by the

Table 4 Enriched genes pathways in the network

Term	Count	FDR
hsa04110:Cell cycle	14	2.43E-08
hsa04115:p53 signaling pathway	11	2.07E-07
hsa04350:TGF-beta signaling pathway	11	2.52E-06
hsa04210:Apoptosis	11	2.52E-06
hsa05214:Glioma	9	5.38E-05
hsa05014:Amyotrophic lateral sclerosis (ALS)	8	3.02E-04
hsa04914:Progesterone-mediated oocyte maturation	9	6.34E-04

Count gene numbers, *FDR* false discovery rate

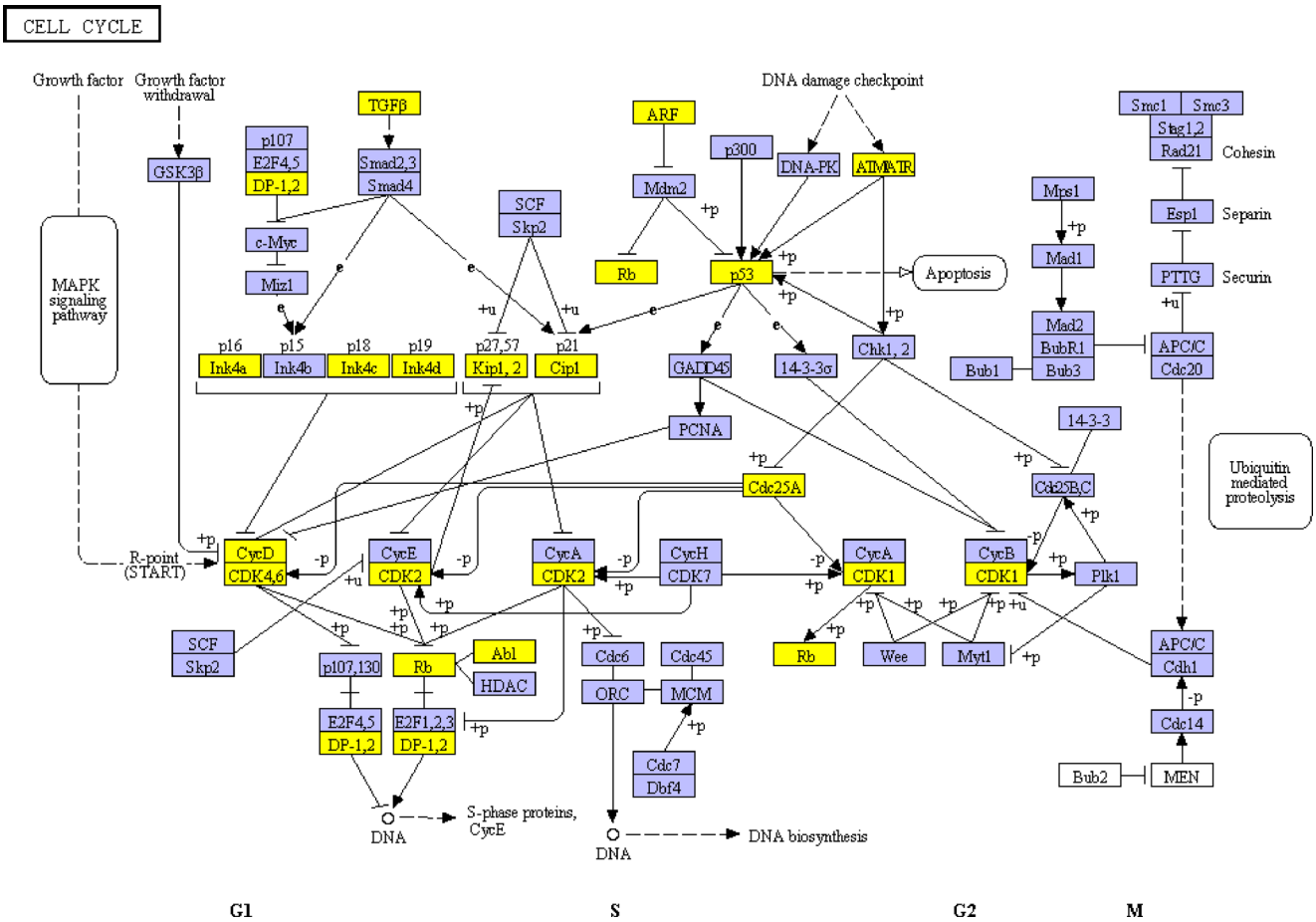

Fig. 2 Cell cycle pathway. The yellow nodes target genes, and the rest are other genes participated in cell cycle pathway

completely or partially complementary of miRNA to 3′-untranslated regions of target mRNA, thus the expression of target genes are regulated [25].

MiR-21 expression is elevated, while miR-203 is reduced in SCC and ADC patients {Mathé, 2009 #156} [12]. The expression of miR-21 is significantly up-regulated in whole spectrum of preneoplastic/neoplastic lesions [26]. Zhang et al. [27]. report that miR-21 post-transcriptionally down-regulates the expression of tumor suppressor PTEN, and regulates cell proliferation and invasion in colorectal cancer [28]. Over-expressed PTEN significantly suppresses growth and induces apoptosis in EC cell line Eca-109 and TE-1 via changes in cell-cycle progression [29]. PTEN is the upstream regulator of Akt kinase pathway that is involved in oncogenesis [30]. MiR-21 is direct transcriptional target of E2F1 [31] that limit E2F-induced proliferation and apoptosis. Genetic analysis and computational modeling shows that E2F1 ensures the endocycle [32].

MiRNA-203 is a tumor-suppressive miRNA for hepatocellular carcinoma epigenetically silenced and activating multiple targets during hepatocarcinogenesis [33], and leads to G1 phase cell cycle arrest in laryngeal carcinoma cells [34]. María J. Bueno et al. report that miR-203 can inhibit tumor cell proliferation in an ABL1-dependent manner, and ABL1 is a direct target of miR-203 [35]. ABL1 has been implicated in multiple processes including cell division, adhesion and cellular stress response [36]. AM et al. [37]. reported that miR-203 is a key molecule controlling the p63-dependent proliferative potential of epithelial precursor cells both during keratinocyte differentiation and in epithelial development. It is found that p63 regulates cell proliferation and cell cycle progression-associated genes in stromal cells of giant cell tumor of bone [38].

There are many studies focus on exploring the relationship between miRNAs and EC, but few are about target genes of typical miRNAs. We found in this study that 25 target genes of has-miR-21 and 10 target genes of has-miR-203 in SCC and ADC, which are close related to cell cycle pathway, and they are mainly distributed in the phase of G1/S in cell cycle with largest quantity, little genes distributed in the latter two phases: G2/M, medium/late stage (spindle assembles). Cell cycle regulation is a complex biological process that involves the biological effects of a lot of proto-oncogenes and tumor suppressor genes [39]. Seeking for new drugs treatments that can protect normal tissue and induce the differentiation and apoptosis of cancerous tissue at the same time, to activate the cell

cycle regulation mechanism and assure normal cell growth, proliferation, differentiation, will be new direction in tumor cure [40].

Conclusion

Up-regulated hsa-miR21 and down-regulated hsa-miR203 provide evidence for esophageal carcinoma early diagnosis and treatment, functions and pathways study of these two genes showed G1/S phase of cell cycle may be related with pathogenesis of EC.

References

1. Enzinger PC, Mayer RJ (2003) Esophageal cancer. N Engl J Med 349(23):2241–2252
2. Mathé EA, Nguyen GH, Bowman ED, Zhao Y, Budhu A, Schetter AJ, Braun R, Reimers M, Kumamoto K, Hughes D (2009) MicroRNA expression in squamous cell carcinoma and adenocarcinoma of the esophagus: associations with survival. Clin Cancer Res 15(19):6192–6200
3. Enzinger PC, Mayer RJ (2008) Oesophageal cancer. In: Baert A (ed) Encyclopedia of diagnostic imaging. Springer, Berlin, pp 1401–1401. doi:10.1007/978-3-540-35280-8_1772
4. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D (2011) Global cancer statistics. CA Cancer J Clin 61(2):69–90
5. Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116(2):281–297. doi:10.1016/S0092-8674(04)00045-5
6. Zhao Y, Srivastava D (2007) A developmental view of microRNA function. Trends Biochem Sci 32(4):189
7. Zhang B, Pan X, Cobb GP, Anderson TA (2007) microRNAs as oncogenes and tumor suppressors. Dev Biol 302(1):1–12
8. Feber A, Xi L, Luketich JD, Pennathur A, Landreneau RJ, Wu M, Swanson SJ, Godfrey TE, Litle VR (2008) MicroRNA expression profiles of esophageal cancer. J Thorac Cardiovasc Surg 135(2):255–260
9. Ye Y, Wang KK, Gu J, Yang H, Lin J, Ajani JA, Wu X (2008) Genetic variations in microRNA-related genes are novel susceptibility loci for esophageal cancer risk. Cancer Prev Res 1(6):460–469
10. Yang M, Liu R, Sheng J, Liao J, Wang Y, Pan E, Guo W, Pu Y, Yin L (2013) Differential expression profiles of microRNAs as potential biomarkers for the early diagnosis of esophageal squamous cell carcinoma. Oncol Rep 29(1):169–176
11. Hiyoshi Y, Kamohara H, Karashima R, Sato N, Imamura Y, Nagai Y, Yoshida N, Toyama E, Hayashi N, Watanabe M (2009) MicroRNA-21 regulates the proliferation and invasion in esophageal squamous cell carcinoma. Clin Cancer Res 15(6):1915–1922
12. Kano M, Seki N, Kikkawa N, Fujimura L, Hoshino I, Akutsu Y, Chiyomaru T, Enokida H, Nakagawa M, Matsubara H (2010) miR–145, miR–133a and miR–133b: tumor–suppressive miRNAs target FSCN1 in esophageal squamous cell carcinoma. Int J Cancer 127(12):2804–2814
13. Sonkoly E, Wei T, Loriè EP, Suzuki H, Kato M, Törmä H, Ståhle M, Pivarcsi A (2009) Protein kinase C-dependent upregulation of miR-203 induces the differentiation of human keratinocytes. J Investig Dermatol 130(1):124–134
14. Troyanskaya O, Cantor M, Sherlock G, Brown P, Hastie T, Tibshirani R, Botstein D, Altman RB (2001) Missing value estimation methods for DNA microarrays. Bioinformatics 17(6):520–525
15. Fujita A, Sato J, Rodrigues L, Ferreira C, Sogayar M (2006) Evaluating different methods of microarray data normalization. BMC Bioinforma 7(1):469
16. Gentleman R, Carey V, Huber W, Irizarry R, Dudoit S (2005) Bioinformatics and computational biology solutions using R and Bioconductor, vol 746718470. Springer, New York
17. Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B (Methodological) 289–300
18. Xiao F, Zuo Z, Cai G, Kang S, Gao X, Li T (2009) miRecords: an integrated resource for microRNA–target interactions. Nucleic Acids Res 37(suppl 1):D105–D110
19. Hsu S-D, Lin F-M, Wu W-Y, Liang C, Huang W-C, Chan W-L, Tsai W-T, Chen G-Z, Lee C-J, Chiu C-M (2011) miRTarBase: a database curates experimentally validated microRNA–target interactions. Nucleic Acids Res 39(suppl 1):D163–D169
20. Li S, Armstrong CM, Bertin N, Ge H, Milstein S, Boxem M, Vidalain P-O, Han J-DJ, Chesneau A, Hao T (2004) A map of the interactome network of the metazoan C. elegans. Sci Signal 303(5657):540
21. Szklarczyk D, Franceschini A, Kuhn M, Simonovic M, Roth A, Minguez P, Doerks T, Stark M, Muller J, Bork P (2011) The STRING database in 2011: functional interaction networks of proteins, globally integrated and scored. Nucleic Acids Res 39(suppl 1):D561–D568
22. Nam D, Kim S-Y (2008) Gene-set approach for expression pattern analysis. Brief Bioinform 9(3):189–197
23. Zhang B, Kirov S, Snoddy J (2005) WebGestalt: an integrated system for exploring gene sets in various biological contexts. Nucleic Acids Res 33(suppl 2):W741–W748
24. Duncan D, Prodduturi N, Zhang B (2010) WebGestalt2: an updated and expanded version of the Web-based Gene Set Analysis Toolkit. BMC Bioinforma 11(Suppl 4):P10
25. Ec L (2002) MicroRNAs are complementary to 3′UTR sequence motifs that mediate negative post-transcriptional regulation. Nat Genet 30(4):363–364
26. Fassan M, Realdon S, Pizzi M, Balistreri M, Battaglia G, Zaninotto G, Ancona E, Rugge M (2012) Programmed cell death 4 nuclear loss and miR–21 or activated Akt overexpression in esophageal squamous cell carcinogenesis. Dis Esophagus 25(3):263–268
27. Zhang J-g, Wang J-j, Zhao F, Liu Q, Jiang K, Yang G-h (2010) MicroRNA-21 (miR-21) represses tumor suppressor PTEN and promotes growth and invasion in non-small cell lung cancer (NSCLC). Clin Chim Acta 11–12:846–852. doi:10.1016/j.cca.2010.02.074
28. Xiong B, Cheng Y, Ma L, Zhang C (2013) MiR-21 regulates biological behavior through the PTEN/PI-3 K/Akt signaling pathway in human colorectal cancer cells. Int J Oncol 42(1):219–228
29. Y-a Z, Zhang T, Zhao J-B, X-p W, Jiang T, Gu Z-P, X-n W, X-f L (2010) The adenovirus-mediated transfer of PTEN inhibits the growth of esophageal cancer cells in vitro and in vivo. Biosci Biotechnol Biochem 74(4):736–740
30. Yuan T, Cantley L (2008) PI3K pathway alterations in cancer: variations on a theme. Oncogene 27(41):5497–5510
31. Ofir M, Ben-Shachar B, Hacohen D, Ginsberg D (2012) Abstract A17: MiR-15, miR-16 and miR-21 are direct transcriptional targets of E2F1 that limit E2F-induced proliferation and apoptosis. Cancer Res 72(2 Supplement):A17–A17
32. Schuldt A (2011) Cell cycle: E2F1 ensures the endocycle. Nat Rev Mol Cell Biol 12(12):768–769
33. Furuta M, K-i K, Tanaka S, Arii S, Imoto I, Inazawa J (2010) miR-124 and miR-203 are epigenetically silenced tumor-suppressive microRNAs in hepatocellular carcinoma. Carcinogenesis 31(5):766–776
34. Bian K, Fan J, Zhang X, Yang X-W, Zhu H-Y, Wang L, Sun J-Y, Meng Y-L, Cui P-C, Cheng S-Y (2012) MicroRNA-203 leads to G1 phase cell cycle arrest in laryngeal carcinoma cells by directly targeting survivin. FEBS Lett 586(6):804–809
35. Bueno MJ, Pérez de Castro I, Gómez de Cedrón M, Santos J, Calin GA, Cigudosa Juan C, Croce CM, Fernández-Piqueras J, Malumbres

M (2008) Genetic and epigenetic silencing of MicroRNA-203 enhances ABL1 and BCR-ABL1 oncogene expression. Cancer Cell 13(6):496–506. doi:10.1016/j.ccr.2008.04.018
36. Cunningham J, Vierkant R, Sellers T, Phelan C, Rider D, Liebow M, Schildkraut J, Berchuck A, Couch F, Wang X (2009) Cell cycle genes and ovarian cancer susceptibility: a tagSNP analysis. Br J Cancer 101(8):1461–1468
37. Lena A, Shalom-Feuerstein R, di Val Cervo PR, Aberdam D, Knight R, Melino G, Candi E (2008) miR-203 represses 'stemness' by repressing ΔNp63. Cell Death Differ 15(7):1187–1195
38. Lau CPY, Ng PKS, Li MS, Tsui SKW, Huang L, Kumta SM (2013) p63 regulates cell proliferation and cell cycle progression-associated genes in stromal cells of giant cell tumor of the bone. Int J Oncol 42(2):437
39. Mishina T, Dosaka-Akita H, Hommura F, Nishi M, Kojima T, Ogura S, Shimizu M, Katoh H, Kawakami Y (2000) Cyclin E expression, a potential prognostic marker for non-small cell lung cancers. Clin Cancer Res 6(1):11–16
40. Blagosklonny MV, Pardee AB (2001) Exploiting cancer cell cycling for selective protection of normal cells. Cancer Res 61(11):4301–4305