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Original Article

Hsa_circ_0101996 combined with hsa_circ_0101119 in peripheral whole blood can serve as the potential biomarkers for human cervical squamous cell carcinoma

Yi-Ming Wang, Lu-Mi Huang, Dai-Rong Li, Jiang-He Shao, Shuang-Long Xiong, Chun-Mei Wang, Song-Mei Lu

Department of Oncology, Chongqing Cancer Institute & Hospital & Cancer Center, Chongqing, China

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Abstract: Background: Previous study suggests changes in circRNAs in tumor tissues from cervical squamous cell carcinoma (CSCC) patients. However, little is known about the diagnostic value of circRNAs in CSCC. To assess the potential application of circRNAs as diagnostic tools in CSCC, the circulating circRNAs in peripheral whole blood were carried out. Methods: Five up-regulated circRNAs in peripheral whole blood from 87 patients with CSCC and 55 healthy controls were first identified by real-time quantitative polymerase chain reaction (RT-qPCR). The diagnostic value was evaluated using receiver operating characteristics (ROC) curves and the area under the ROC curves (AUC). Results: Compared with healthy controls, hsa_circ_0101996, hsa_circ_0104649, hsa_circ_0104443 and hsa_circ_0101119 expression were significantly up-regulated in peripheral whole blood from CSCC patients. ROC analysis showed that hsa_circ_0101996 and hsa_circ_0101119 could distinguish CSCC patients from healthy controls with high AUC (0.906 and 0.887, respectively). Intriguingly, the combination of hsa_circ_0101996 and hsa_circ_0101119 markedly improved AUC (0.964). Conclusion: All of the findings suggest that hsa_circ_0101996 combined with hsa_circ_0101119 can serve as potential biomarkers for CSCC detection.

Keywords: Circular RNA, biomarker, diagnosis, whole blood, cervical squamous cell carcinoma

Introduction

Cervical cancer is the most frequently occurring gynecologic malignancies in worldwide [1, 2]. In China, the incidence of cervical cancer is approximately 98,900 newly diagnosed cases and corresponding to about 30,500 death incidents in 2015 [3]. Cervical squamous cell carcinoma (CSCC) is the most common subtype and accounts for up to 80% of cervical cancer [4]. One important reason for high mortality of CSCC is the inadequate understanding of the biomarkers for early diagnosis. Conventional serum tumour markers, such as squamous cell carcinoma antigen (SCC-Ag), carcinoembryonic antigen (CEA) and cancer antigen-125 (CA-125), have been used for early detection and monitoring of CSCC [5], however, these biomarkers lack sufficient sensitivity and specificity. In recent years, non-coding RNAs, including microRNA (miR) and long non-coding RNA (lncRNA), are regarded as a class of promising blood-based biomarkers for early and non-invasive detection of CSCC [6-8]. Unfortunately, the use of non-cellular serum or plasma RNA for miR or lncRNA biomarker has shown some limitations in routine clinical practice [9], including the low yield of RNA from human serum and plasma and vulnerable to degradation. Therefore, exploring novel and specific biomarkers for the non-invasive detection of CSCC should be emphasized.

Circular RNAs (circRNAs) are also known as a class of non-coding RNA and are characterized by high stability, covalently closed continuous loop, without 5' to 3' polarity and polyadenylated tail, which give them distinct ability to counteract RNA exonucleolytic digestion [10]. With the development of microarray and high-throughput sequencing, more than 20,000 circRNAs have been identified in eukaryocytes

Table 1. Divergent primers were used to RT-qPCR

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
hsa_circ_0101996	TTCGGCATTGGGTTGAACATC	GATAGCCAGGTGTTTGCTTCTT
hsa_circ_0104649	GTTCATCCTTTGACTGTGCTGG	TCCGATCAAGAACCTGGGCT
hsa_circ_0104651	TGGTTTCATCTCACTCCTTGGT	TGATCTTTAGGTCCTCCTCCTCA
hsa_circ_0104443	TTCCGATGGGCAATTCTCCG	TTGGATCCTGTCCCATCAGC
hsa_circ_0101119	ACTACAGGGAAATGGCGAAGG	ACTACAGGGAAATGGCGAAGG
GAPDH	GCACCGTCAAGCTGAGAAC	TGGTGAAGACGCCAGTGGA

Table 2. Clinical parameters of CSCC patients and healthy subjects

Variable	CSCC	Healthy	*p*-Value[a]
Number	87	55	
Mean Age (Range, SD[b])	49.2 (32-58, 12.6)	47.5 (31-55, 10.8)	0.734
SCC-Ag (ng/ml)			
> 1.5	53 (60.9%)		
≤ 1.5	34 (39.1%)		
FIGO stage			
IIB	49 (56.3%)		
IIIB	38 (43.7%)		
Differentiation			
Low	20 (23.0%)		
Moderate	36 (41.4%)		
High	31 (35.6%)		
Lymph node metastasis			
No	62 (71.3%)		
Yes	25 (28.7%)		

[a]Pearson χ^2 test for categorical variables; two-tailed t tests assuming equal group variances for continuous variables; [b]Standard deviation.

[11]. CircRNAs perform a wide variety of biological functions in eukaryotic cells by acting competing endogenous RNAs (ceRNAs) or miRNA sponges, interacting with RNA binding proteins, modulating the stability of mRNAs, regulating gene transcription and translating proteins [12]. Intriguingly, differentially expressed circRNAs have been confirmed in tumor tissues [13, 14], and clinical diagnosis value of circRNAs begins to emerge in malignancies, including lung adenocarcinoma, breast cancer, gastric cancer and acute myeloid leukemia [14-18]. However, the clinical diagnostic significance of circRNAs has not been completely annotated in CSCC.

In the present study, based on previous circRNA microarray expression profile GSE102686 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102686) in CSCC tumor tissues, we selected five significant up-regulation of circRNAs (hsa_circ_0101996, hsa_circ_0104649, hsa_circ_0104651, hsa_circ_0104443 and hsa_circ_0101119) to validate peripheral whole blood-based clinical diagnostic significance in CSCC.

Material and methods

Patients and specimens

87 patients with CSCC with International Federation of Gynecology and Obstetrics (FIGO) stage IIB to IIIB and 55 healthy controls were enrolled at Chongqing Cancer Institute & Hospital & Cancer Center (Chongqing, China) from January 2014 to December 2016. Written informed consent was obtained from all of the participants prior to blood samples collection. The study was approved by the Ethics Committee of the Chongqing Cancer Institute & Hospital & Cancer Center (Chongqing, China). All subjects recruited in this study were not subjected to preoperative radiotherapy or chemotherapy and diagnosed with histopathological evaluation. 10 ml of blood sample from pre-operative CSCC patients were collected with ethylenediaminetetraacetic acid (EDTA)-containing tubes (Becton, Dickinson and Company) and stored at -80°C in an ultra-low temperature refrigerator.

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR)

RNA was extracted using TRIzol (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Moloney Murine Leukemia Virus reverse transcriptase (Promega Corporation, Madison, WI, USA) and oligo dT 15 primers (Thermo Fisher Scientific, Inc.) were utilized to synthesize cDNA. Divergent primers were designed to

Table 3. The annotation of circRNAs

circRNA	*p*-Value	Log_2 FC	Regulation	Gene symbol	Spliced length	Position
hsa_circ_0103384	4.51E-08	-3.14	Down	None	135	chr15: 37425511-37425646
hsa_circ_0103677	5.20E-07	-2.86	Down	SPATA5L1	690	chr15: 45706768-45710880
hsa_circ_0101308	2.39E-05	-4.29	Down	None	569	chr14: 102810157-102810726
hsa_circ_0102050	9.63E-05	-2.03	Down	ATL1	396	chr14: 51081090-51089966
hsa_circ_0101996	1.07E-04	2.89	Up	SOS2	965	chr14: 50619787-50626804
hsa_circ_0104649	1.50E-04	2.01	Up	CHRNA5	537	chr15: 78885433-78885970
hsa_circ_0104651	7.47E-04	2.46	Up	ADAMTS7	2041	chr15: 79079034-79083565
hsa_circ_0104443	9.17E-04	2.03	Up	MYO9A	2253	chr15: 72252241-72338975
hsa_circ_0001459	1.32E-03	-2.07	Down	NEIL3	421	chr4: 178274461-178274882
hsa_circ_0101120	1.77E-03	-2.56	Down	DOCK9	825	chr13: 99449368-99460929
hsa_circ_0101119	2.89E-03	2.26	Up	DOCK9	431	chr13: 99449368-99452732
hsa_circ_0102950	4.48E-03	-2.33	Down	UNC79	1663	chr14: 93933043-93954101
hsa_circ_0104152	7.11E-03	-2.14	Down	HERC1	496	chr15: 63978573-63984726
hsa_circ_0104238	9.65E-03	-2.34	Down	MTFMT	473	chr15: 65298450-65316132

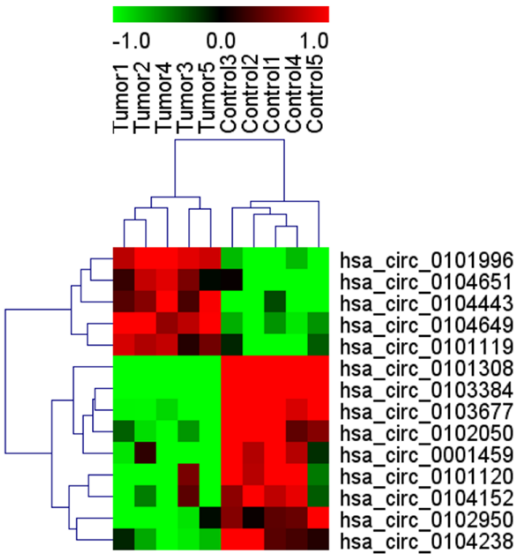

Figure 1. Microarray and hierarchical cluster analysis were performed in five pairs tumor tissues and adjacent non tumorous tissues. Each row represents an individual circRNA, and each column represents a sample. The color legend at the top indicates circRNA expression levels, red indicating high expression and green indicating low expression levels.

ensure amplification of the head-to-tail splicing of circRNA using ABI7300 System (Applied Biosystems, Foster City, CA, USA) with the SYBR Select Master Mix (Applied Biosystems), as described previously [19]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels were utilized to normalize the expression of the circRNAs. The PCR primers were used in this study as shown in **Table 1**.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 19.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism Version 5.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical descriptions were used to describe the clinical pathological features, and the Student's t test was used to analyze the measurement data. Pearson χ^2 tests were used to evaluate differences in the clinical characteristics between the two groups. Receiver operating characteristic (ROC) curves and the area under the ROC curve (AUC) were used to assess the ability of using peripheral whole blood circRNAs as diagnostic tools for CSCC. The binary logistic regression analysis was used to combine the expression level of hsa_circ_0101996 and hsa_circ_0101119 as a combined diagnostic marker for CSCC. The maximum value of the Youden index was used as a criterion for selecting the optimum cut-off point. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics of CSCC patients and healthy subjects

Demographic features and pathological classification of 87 patients with CSCC and 55 healthy controls were summarized in **Table 2**. The average age had no obvious difference between CSCC patients and healthy controls. Among CSCC patients, 49 (56.3%) patients

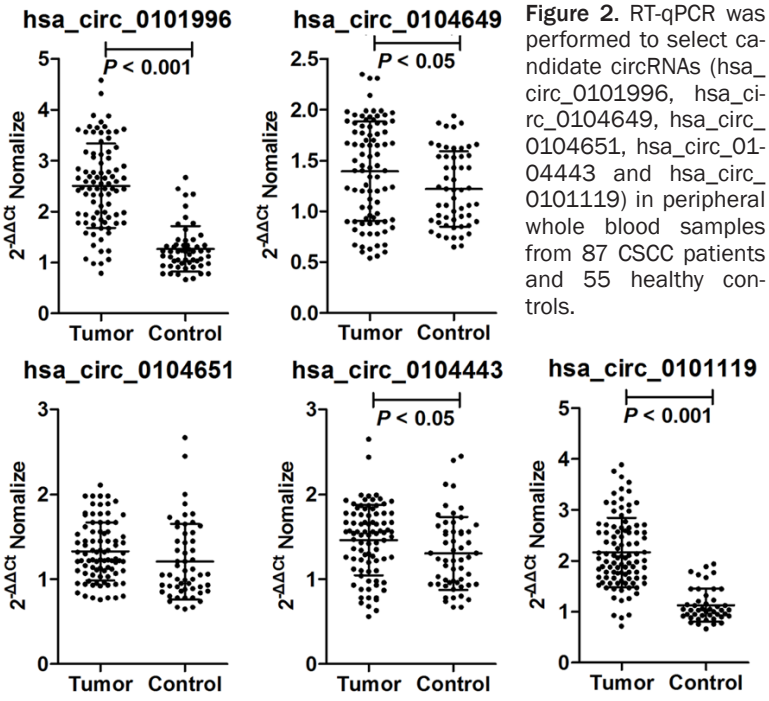

Figure 2. RT-qPCR was performed to select candidate circRNAs (hsa_circ_0101996, hsa_circ_0104649, hsa_circ_0104651, hsa_circ_0104443 and hsa_circ_0101119) in peripheral whole blood samples from 87 CSCC patients and 55 healthy controls.

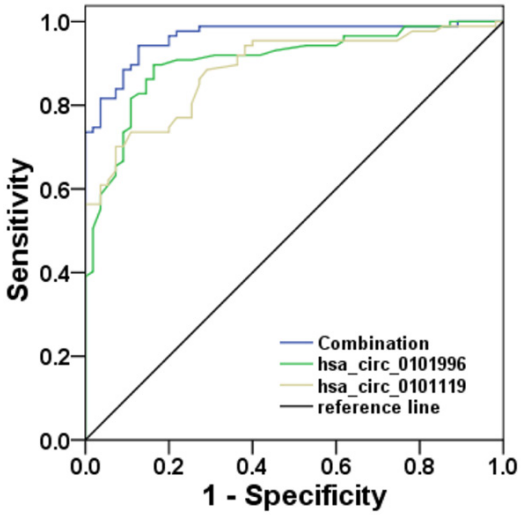

Figure 3. Evaluation of hsa_circ_0101996 and hsa_circ_0101119 for CSCC detection. Receiver operating characteristics (ROC) curves were drawn with the data of peripheral whole blood hsa_circ_0101996 and hsa_circ_0101119 levels from 87 CSCC patients and 55 healthy controls. Using binary logistic regression analysis, the ROC curves were drawn with the data of peripheral whole blood hsa_circ_0101996 combined with hsa_circ_0101119. The combination of hsa_circ_0101996 and hsa_circ_0101119 showed a higher positive diagnostic rate of CSCC patients from healthy controls than hsa_circ_0101996 or hsa_circ_0101119 diagnosis alone.

were diagnosed with clinical FIGO stage (IIB), the others 38 (43.7%) with clinical stage IIIB; 20 (23.0%) patients with low differentiation, 36 (41.4%) patients with moderate differentiation and 31 (35.6%) patients with high differentiation. Furthermore, 25 (28.7%) patients had lymph node metastasis.

CircRNA aberrant expression in CSCC tumor tissues

Recent study has identified that a large number of circRNAs are up-regulated in squamous cell carcinoma [19-21]. In our study, the microarray data were obtained from the GEO database (Accession No. GSE102686; https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE102686) and found that 5 were upregulated and 9 were downregulated in tumor tissues compared with the paired adjacent normal tissues based on the Log_2 (fold change) ≥ 2, $P < 0.01$. Additionally, the expression levels and annotation of these circRNAs were summarized in **Table 3**, gene symbols, spliced length and position according to circBase (http://www.circbase.org). Among the differentially expressed circRNAs in tumor tissues, up to 50.0% came from chromosome 15 (chr15) and approximately 28.6% from chr14, which were predominantly transcribed from chr15 and chr15. Therefore, we believe the distribution status of circRNAs in chromosomes may be related to the progression of CSCC. Hierarchical clustering analysis demonstrated differential circRNAs expression between the tumor tissues from CSCC patients and healthy controls, these findings suggest that the circRNA expression profiles in tumor tissues were different from those in adjacent normal tissues (**Figure 1**).

Verification of five up-regulated circRNAs in peripheral whole blood by RT-qPCR

To validate whether the differentially expressed circRNAs in tumor tissues can serve as the potential biomarkers for CSCC detection, RT-qPCR was performed to select candidate circRNAs using an independent set of peripheral

Table 4. Clinical diagnostic significance of hsa_circ_0101996 and hsa_circ_0101119 in CSCC patients

	AUC	*p*-Value	95% CI		Sensitivity	Specificity	Youden index	Cut-off
			Lower	Upper				
hsa_circ_0101996	0.906	< 0.001	0.856	0.956	0.897	0.836	0.733	1.545
hsa_circ_0101119	0.887	< 0.001	0.834	0.940	0.701	0.927	0.628	1.815
Combination	0.964	< 0.001	0.936	0.992	0.943	0.873	0.815	3.436

whole blood samples from 87 CSCC patients and 55 healthy controls. Consistent with the microarray data, the expression levels of hsa_circ_0101996 ($P < 0.001$), hsa_circ_0104649 ($P < 0.05$), hsa_circ_0104443 ($P < 0.05$) and hsa_circ_0101119 ($P < 0.001$) in peripheral whole blood from CSCC patients were significantly up-regulated compared with those of the healthy controls. However, hsa_circ_0104651 had no obvious difference between CSCC patients and healthy controls (**Figure 2**). Therefore, we focused on hsa_circ_0101996 and hsa_circ_0101119 in our study.

Evaluation of hsa_circ_0101996 and hsa_circ_0101119 in peripheral whole blood as novel tumor markers for CSCC

To investigate the diagnostic value of hsa_circ_0101996 and hsa_circ_0101119 as potential tumor markers of CSCC, receiver operating characteristics (ROC) curves and the area under the ROC curves (AUC) were performed on data from all participants, including 87 CSCC patients and 55 healthy controls. The ROC curves showed strong distinguishing label between the CSCC patients and healthy controls, with an AUC of 0.906 (95% CI: 0.856-0.956; $P < 0.001$; **Figure 3** and **Table 4**) for hsa_circ_0101996 and 0.887 (95% CI: 0.834-0.940; $P < 0.001$; **Figure 3** and **Table 4**) for hsa_circ_0101119. When hsa_circ_0101996 and hsa_circ_0101119 were combined, the AUC increased to 0.964 (95% CI: 0.936-0.992; $P < 0.001$; **Figure 3** and **Table 4**). Therefore, hsa_circ_0101996 combined with hsa_circ_0101119 may be more efficient for CSCC detection than hsa_circ_0101996 or hsa_circ_0101119 diagnosis alone.

Discussion

Currently, circRNAs have caused great concern and have been developing rapidly in clinical diagnosis, which attributes to their strongly expressed levels, 10-fold or greater than their linear isomers, highly conserved sequences and stability [22]. Recent studies have indicated that circRNAs can be used as diagnostic or predictive biomarkers for rheumatoid arthritis [23], breast cancer [14], non-small cell lung cancer [16, 24] and acute myeloid leukemia [18]. However, the role of circRNAs in CSCC is poorly known. In the present study, hsa_circ_0101996, hsa_circ_0104649, hsa_circ_0104443 and hsa_circ_0101119 in peripheral whole blood from CSCC patients were significantly increased as compared to those of the healthy subjects. We also found that hsa_circ_0101996 and hsa_circ_0101119 might be used as novel, non-invasive biomarkers for CSCC in routine clinical practice.

Hsa_circ_0101996 is derived from SOS2 (SOS Ras/Rho guanine nucleotide exchange factor 2), which is widely expressed in various tissues and mediates multiple signaling pathways promoting Ras activation [25, 26]. Ras-mediated signaling controls a wide variety of biological functions, including carcinogenesis [27]. Ras functions as an oncogene and has a central role in cancer progression towards a metastatic phenotype in cervical cancer, through the activation of the PI3-K/Akt/mTOR and mitogen-activated protein kinase (MAPK) signaling pathway [28, 29]. Hsa_circ_0101119 aligns with the gene DOCK9 (dedicator of cytokinesis 9), also referenced as Zizimin1, which specifically activates Cdc42 that, which specifically activates Cdc42 that plays pivotal roles in cell-cycle progression [30, 31]. Cdc42 mediates the activation of JNK through MAPK signaling participation in the development of tumors [31, 32]. All of these findings provide a hypothesis that SOS2-originated hsa_circ_0101996 and DOCK9-originated hsa_circ_0101119 may be involved in the progression of CSCC by the activation of MAPK signaling.

Some studies have announced that serum or plasma-based biomarkers, miRs or lncRNAs, may provide new genetic diagnostic methods for early detection of cervical cancer [33, 34]. The yield of RNA from human serum or plasma was ranging from 2.5 ng/ml to 120 ng/ml, which limits the stabilization and impartiality of biomarker discovery [9, 35]. Moreover, additional separated steps of serum or plasma from blood can accelerate RNA degradation, and RNA in peripheral whole blood is overwhelmingly more in amount than serum or plasma [36]. Therefore, some researchers suggest that whole blood is more suitable for practical detection of non coding RNA [9, 37]. In our study, RT-qPCR assay identified that the levels of hsa_circ_0101996, hsa_circ_0104649, hsa_circ_0104443 and hsa_circ_0101119 in whole blood had similar trend with their expression in tumor tissues using circRNA microarray assay. Among these circRNAs, hsa_circ_0101996 and hsa_circ_0101119 were significantly increased more than 4-fold in whole blood from CSCC patients compared with healthy controls. In addition, hsa_circ_0101996, hsa_circ_0101119 or hsa_circ_0101996 combined with hsa_circ_0101119 showed a high ROC AUC value in CSCC screening, indicating theirs high potential as diagnostic biomarker.

However, there are some limitations in our study. First, the number of samples was limited, which could have biased in our results. Second, the circRNAs expression profile in serum or plasma had not been performed to eliminate the possibility of serum- or plasma-based inspection. Third, the levels of hsa_circ_0101119 or hsa_circ_0101996 in peripheral whole blood had not been carried out before and after surgical operation, which is beneficial to study circRNA origination, tumors-released circRNAs into circulation and circRNAs released into blood circulation by all cells of the body. Fourth, the relationship between circRNAs and prognostic value did not involve. Fifth, the underlying molecular mechanisms associated with the present circRNAs, should be experimentally identified and characterized in the progression of CSCC.

Taken together, whole blood circRNAs expression might be specifically altered in patients with CSCC, and we discovered that hsa_circ_0101996 and hsa_circ_0101119 were significantly up-regulated in peripheral whole blood from CSCC patients. Hsa_circ_0101996 combined with hsa_circ_0101119 might serve as diagnostic biomarkers for CSCC in routine clinical practice.

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Disclosure of conflict of interest

None.

Address correspondence to: Dr. Lu-Mi Huang, Department of Oncology, Chongqing Cancer Institute & Hospital & Cancer Center, 181 Hanyu Road, Chongqing 400030, China. Tel: +86 023-65075652; E-mail: huang_lumi09@aliyun.com

References

[1] Sauvaget C, Nishino Y, Konno R, Tase T, Morimoto T and Hisamichi S. Challenges in breast and cervical cancer control in Japan. Lancet Oncol 2016; 17: e305-e312.

[2] Altobelli E. Improving cervical cancer screening in Baltic, central, and eastern European countries. Lancet Oncol 2016; 17: 1349-1350.

[3] Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ and He J. Cancer statistics in China, 2015. CA Cancer J Clin 2016; 66: 115-132.

[4] Lorin L, Bertaut A, Hudry D, Beltjens F, Roignot P, Bone-Lepinoy MC, Douvier S and Arveux P. About invasive cervical cancer: a French population based study between 1998 and 2010. Eur J Obstet Gynecol Reprod Biol 2015; 191: 1-6.

[5] Ikeda S, Yoshimura K, Onda T, Kasamatsu T, Kato T, Ishikawa M, Sasajima Y and Tsuda H. Combination of squamous cell carcinoma-antigen, carcinoembryonic antigen, and carbohydrate antigen 19-9 predicts positive pelvic lymph nodes and parametrial involvement in early stage squamous cell carcinoma of the uterine cervix. J Obstet Gynaecol Res 2012; 38: 1260-1265.

[6] Zhang J, Yao T, Lin Z and Gao Y. Aberrant methylation of MEG3 functions as a potential plasma-based biomarker for cervical cancer. Sci Rep 2017; 7: 6271.

[7] Wei H, Wen-Ming C, Jun-Bo J. Plasma miR-145 as a novel biomarker for the diagnosis and radiosensitivity prediction of human cervical cancer. J Int Med Res 2017; 45: 1054-1060.
[8] You W, Wang Y and Zheng J. Plasma miR-127 and miR-218 might serve as potential biomarkers for cervical cancer. Reprod Sci 2015; 22: 1037-1041.
[9] Patnaik SK, Yendamuri S, Kannisto E, Kucharczuk JC, Singhal S and Vachani A. MicroRNA expression profiles of whole blood in lung adenocarcinoma. PLoS One 2012; 7: e46045.
[10] Hentze MW and Preiss T. Circular RNAs: splicing's enigma variations. EMBO J 2013; 32: 923-925.
[11] Glazar P, Papavasileiou P and Rajewsky N. circBase: a database for circular RNAs. RNA 2014; 20: 1666-1670.
[12] Meng S, Zhou H, Feng Z, Xu Z, Tang Y, Li P and Wu M. CircRNA: functions and properties of a novel potential biomarker for cancer. Mol Cancer 2017; 16: 94.
[13] Chen S, Li T, Zhao Q, Xiao B and Guo J. Using circular RNA hsa_circ_0000190 as a new biomarker in the diagnosis of gastric cancer. Clin Chim Acta 2017; 466: 167-171.
[14] Lu L, Sun J, Shi P, Kong W, Xu K, He B, Zhang S and Wang J. Identification of circular RNAs as a promising new class of diagnostic biomarkers for human breast cancer. Oncotarget 2017; 8: 44096-44107.
[15] Nair AA, Niu N, Tang X, Thompson KJ, Wang L, Kocher JP, Subramanian S and Kalari KR. Circular RNAs and their associations with breast cancer subtypes. Oncotarget 2016; 7: 80967-80979.
[16] Zhu X, Wang X, Wei S, Chen Y, Chen Y, Fan X, Han S and Wu G. hsa_circ_0013958: a circular RNA and potential novel biomarker for lung adenocarcinoma. FEBS J 2017; 284: 2170-2182.
[17] Tian M, Chen R, Li T and Xiao B. Reduced expression of circRNA hsa_circ_0003159 in gastric cancer and its clinical significance. J Clin Lab Anal 2017; [Epub ahead of print].
[18] Li W, Zhong C, Jiao J, Li P, Cui B, Ji C and Ma D. Characterization of hsa_circ_0004277 as a new biomarker for acute myeloid leukemia via circular RNA profile and bioinformatics analysis. Int J Mol Sci 2017; 18.
[19] Xia W, Qiu M, Chen R, Wang S, Leng X, Wang J, Xu Y, Hu J, Dong G, Xu PL and Yin R. Circular RNA has_circ_0067934 is upregulated in esophageal squamous cell carcinoma and promoted proliferation. Sci Rep 2016; 6: 35576.
[20] Cao S, Wei D, Li X, Zhou J, Li W, Qian Y, Wang Z, Li G, Pan X and Lei D. Novel circular RNA expression profiles reflect progression of patients with hypopharyngeal squamous cell carcinoma. Oncotarget 2017; 8: 45367-45379.
[21] Sand M, Bechara FG, Gambichler T, Sand D, Bromba M, Hahn SA, Stockfleth E and Hessam S. Circular RNA expression in cutaneous squamous cell carcinoma. J Dermatol Sci 2016; 83: 210-218.
[22] Jeck WR, Sorrentino JA, Wang K, Slevin MK, Burd CE, Liu J, Marzluff WF and Sharpless NE. Circular RNAs are abundant, conserved, and associated with ALU repeats. RNA 2013; 19: 141-157.
[23] Ouyang Q, Wu J, Jiang Z, Zhao J, Wang R, Lou A, Zhu D, Shi GP and Yang M. Microarray expression profile of circular RNAs in peripheral blood mononuclear cells from rheumatoid arthritis patients. Cell Physiol Biochem 2017; 42: 651-659.
[24] Yao JT, Zhao SH, Liu QP, Lv MQ, Zhou DX, Liao ZJ and Nan KJ. Over-expression of CircRNA_100876 in non-small cell lung cancer and its prognostic value. Pathol Res Pract 2017; 213: 453-456.
[25] Baltanas FC, Perez-Andres M, Ginel-Picardo A, Diaz D, Jimeno D, Liceras-Boillos P, Kortum RL, Samelson LE, Orfao A and Santos E. Functional redundancy of Sos1 and Sos2 for lymphopoiesis and organismal homeostasis and survival. Mol Cell Biol 2013; 33: 4562-4578.
[26] Rojas JM, Oliva JL and Santos E. Mammalian son of sevenless Guanine nucleotide exchange factors: old concepts and new perspectives. Genes Cancer 2011; 2: 298-305.
[27] Steelman LS, Franklin RA, Abrams SL, Chappell W, Kempf CR, Basecke J, Stivala F, Donia M, Fagone P, Nicoletti F, Libra M, Ruvolo P, Ruvolo V, Evangelisti C, Martelli AM and McCubrey JA. Roles of the Ras/Raf/MEK/ERK pathway in leukemia therapy. Leukemia 2011; 25: 1080-1094.
[28] Mora N, Rosales R and Rosales C. R-Ras promotes metastasis of cervical cancer epithelial cells. Cancer Immunol Immunother 2007; 56: 535-544.
[29] Chakrabarti O, Veeraraghavalu K, Tergaonkar V, Liu Y, Androphy EJ, Stanley MA and Krishna S. Human papillomavirus type 16 E6 amino acid 83 variants enhance E6-mediated MAPK signaling and differentially regulate tumorigenesis by notch signaling and oncogenic Ras. J Virol 2004; 78: 5934-5945.
[30] Kwofie MA and Skowronski J. Specific recognition of Rac2 and Cdc42 by DOCK2 and DOCK9 guanine nucleotide exchange factors. J Biol Chem 2008; 283: 3088-3096.
[31] Xiao B, Chen D, Luo S, Hao W, Jing F, Liu T, Wang S, Geng Y, Li L, Xu W, Zhang Y, Liao X, Zuo D, Wu Y, Li M and Ma Q. Extracellular translationally controlled tumor protein pro-

motes colorectal cancer invasion and metastasis through Cdc42/JNK/MMP9 signaling. Oncotarget 2016; 7: 50057-50073.
[32] Ma D, Cheng Y, Zhang Y, Guo Y, Li Z and Li G. Expression of CDC42 in cervical squamous cell carcinoma and its correlation with clinicopathologic characteristics. Chin J Cancer Res 2013; 25: 656-661.
[33] Yang JP, Yang XJ, Xiao L and Wang Y. Long noncoding RNA PVT1 as a novel serum biomarker for detection of cervical cancer. Eur Rev Med Pharmacol Sci 2016; 20: 3980-3986.
[34] Juan L, Tong HL, Zhang P, Guo G, Wang Z, Wen X, Dong Z and Tian YP. Identification and characterization of novel serum microRNA candidates from deep sequencing in cervical cancer patients. Sci Rep 2014; 4: 6277.
[35] Rykova EY, Wunsche W, Brizgunova OE, Skvortsova TE, Tamkovich SN, Senin IS, Laktionov PP, Sczakiel G and Vlassov VV. Concentrations of circulating RNA from healthy donors and cancer patients estimated by different methods. Ann N Y Acad Sci 2006; 1075: 328-333.
[36] McDonald JS, Milosevic D, Reddi HV, Grebe SK and Algeciras-Schimnich A. Analysis of circulating microRNA: preanalytical and analytical challenges. Clin Chem 2011; 57: 833-840.
[37] Ries J, Vairaktaris E, Agaimy A, Kintopp R, Baran C, Neukam FW and Nkenke E. miR-186, miR-3651 and miR-494: potential biomarkers for oral squamous cell carcinoma extracted from whole blood. Oncol Rep 2014; 31: 1429-1436.