European Review for Medical and Pharmacological Sciences 2016; 20: 1740-1744

Down-regulation of miR-664 in cervical cancer is associated with lower overall survival

Y.-X. ZHANG, L.-L. QIN, S.-Y. YANG

Department of Obstetrics and Gynecology, Rizhao People's Hospital, Rizhao, Shangdong, China

Abstract. – OBJECTIVE: The aim of our study was to explore the clinicopathologic and prognostic significance of microRNA-664 expression in human cervical cancer.

PATIENTS AND METHODS: qRT-PCR was used to determine the expression of miR-664 in cervical cancer tissues. The relationship between miR-664 expression levels in cervical cancer tissues and clinicopathological characteristics was estimated respectively. The survival curves of the patients were determined using the Kaplan-Meier method. Univariate and multivariate Cox regression analyses were performed.

RESULTS: The expression of miR-664 is down-regulated in human cervical cancer tissues when compared to the corresponding non-cancerous tissues ($p < 0.01$). Low miR-664 expression was significantly associated with lymphatic invasion ($p = 0.000$), distant metastasis ($p < 0.000$), FIGO stage ($p = 0.001$), and histological grade ($p = 0.008$). Kaplan-Meier analysis demonstrated that low levels of miR-664 expression were associated with poorer overall survival ($p < 0.001$). In the multivariate analysis, low miR-664 expression was an independent prognostic factor for OS ($p = 0.005$).

CONCLUSIONS: MiR-664 may be a promising biomarker for the detection and prognosis evaluation of cervical cancer.

Key Words:
miR-664, Cervical cancer, Lymphatic invasion, Prognosis.

Introduction

Cervical cancer is a gynecological malignancy with the second most common malignancy in female worldwide[1]. It was reported in Lancet that global cervical cancer incidence increased at 0.6% annual rate from 1980 to 2010[2,3]. Recent progress in diagnosis and therapy methods has helped to cure this disease of many patients at early stages, but the outcome of cervical cancer patients with a poor response to chemotherapy is not encouraging. Early diagnosis and prognostic evaluation of cervical cancer are crucial for timely and appropriate treatment. Therefore, the identification of novel biomarkers of cervical cancer progression is crucial to improve the patient outcome.

Micro(miR)RNAs are small RNA molecules (18-25 nucleotides in length) that function as posttranscriptional regulators of gene expression in various species[4,5]. It is recognized that miRNAs are involved in the regulation of a variety of biological processes including cell proliferation, differentiation, and apoptosis[6,7]. Furthermore, the functions of miRNAs involved in the initiation and progression of human cancers have been extensively verified. Recent studies have proved that miRNAs are aberrantly expressed in various human cancers and exert important regulations on tumor biology by acting as oncogenes or tumor suppressors. For example, hsa-miR-206 may repress the tumor proliferation and invasion in breast cancer by targeting Cx43[8]. MiR-150 function as a tumor promoter in prostate cancer by suppressing p27[9]. MicroRNA-664 (miR-664) has shown to be involved in many aspects of carcinogenesis. miR-664 was found to be upregulated in hepatocellular carcinoma tissues and to be down-regulated in breast carcinoma tissues[10,11]. Those results informed that miR-664 may function as a different role in different cancer. As for cervical cancer, the clinical significance and prognostic value of miR-664 in cervical cancer have not been investigated.

Patients and Methods

Patients and Tissue Samples

186 pairs of cervical cancer tissues and adjacent noncancerous tissues were obtained from Department of Obstetrics and Gynecology, Rizhao People's Hospital. None of the patients

Corresponding Author: Li-li Qin, MD; e-mail: qinliop0@163.com

received radiotherapy and chemotherapy before the tissues were obtained. The corresponding adjacent normal tissues were obtained 3 cm beyond the boundary of cervical cancer tissues. Tumor differentiation was graded following WHO criteria. After surgical removal, the tissues were frozen immediately in liquid nitrogen until use.

Our study protocol was recognized by Research Ethics Committee in Rizhao People's Hospital. We obtained written informed consents from each participant. All specimens were handled and made anonymous according to the ethical and legal standards.

RNA Extraction and qRT-PCR Analyses

Total RNA was extracted from fresh tissues and cells using Trizol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The quantity and quality of the extracted RNA were determined spectrophotometrically by measurement of absorbance at 260 and 280nm using a DU530 UV-VIS spectrophotometer (Beckman Coulter, Fullerton, CA, USA). The TaqMan microRNA assay and TaqMan universal PCR master mix were used to detect the expression of miR-664, and the U6 gene was used as an internal control to normalize variances. All samples were carried out in triplicate. The cycle threshold (CT) value was calculated. The $2^{-\Delta CT}$ ($\Delta CT = C_{T\ miR664}-C_{TU6\ RNA}$) method was used to quantify the relative amount of miR-664.

Statistical Analysis

Statistical analyses were performed using the SPSS 17.0 statistical software package (SPSS Inc., Chicago, IL, USA). Associations between clinicopathological parameters and miR-664 expression were evaluated using χ^2 tests. Overall survival was calculated and survival curves were plotted using the Kaplan-Meier method; differences between groups were compared using log-rank tests. The significance of survival variables was evaluated using a multivariate Cox proportional hazards regression analysis. $p < 0.05$ was considered to indicate statistical significance.

Results

The Expression Levels of miR-664 in Cervical Cancer

Taqman qRT-PCR assay was performed to detect the expression level of miR-664 in cervical cancer tissues and adjacent non-tumorous tissues. Our results showed that miR-664 expression was decreased in cervical cancer tissues compared with that in adjacent normal tissues ($p < 0.01$, shown in Figure 1).

miR-664 Expression and Clinicopathologic factors in Cervical Cancer

Cervical cancer tissue samples were classified into low expression group (n = 93) and high expression group (n = 93), according to the median expression level of all cervical cancer samples. Table I summarized the correlation between miR-664 expression and clinicopathological features of patients with cervical cancer. the low level of miR-664 expression was significantly associated with lymphatic invasion ($p = 0.000$), distant metastasis($p = 0.000$), FIGO stage ($p = 0.001$), and histological grade ($p = 0.008$). However, there were no correlations between miR-664 expression and other clinicopathologic variables, such as age, HPV infection, tumor histology, and tumor size.

Low Expression Level of miR-664 was Associated with Shorter OS

To further investigate the correlation between miR-664 expression level and prognosis of cervical cancer, survival curves were calculated by Kaplan-Meier method and compared by the log-rank test. As shown in Figure 2, our data showed that low levels of miR-664 expression were associated with poorer overall survival ($p < 0.001$). A

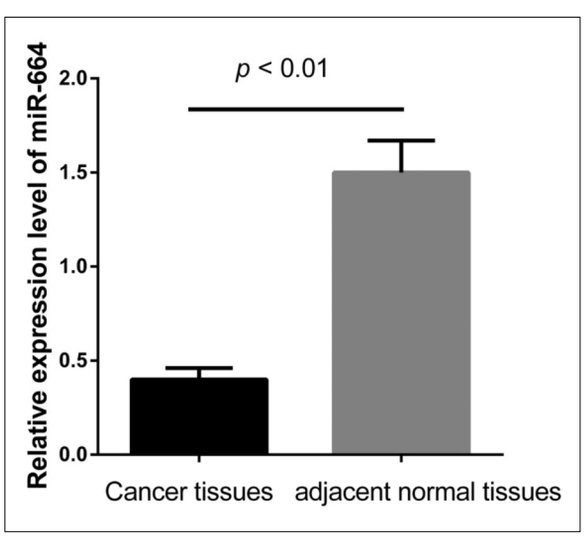

Figure 1. Relative expression of miR-664 was lower in cervical cancer tissues than in matched adjacent non-tumor tissues. miR-664 expression was deter¬mined by qRT-PCR and normalized against U6 RNA (an endogenous control).

Table I. Correlations between miR-664 expression and clinicopathological characteristics in cervical cancer.

Variables	Cases (n = 186)	miR-664 expression		p-value
		Low	High	
Age (years)				0.657
< 65	81	39	42	
≥ 65	10554	51		
HPV				0.518
(+)	132	68	64	
(–)	54	25	29	
Tumor histology				0.236
Squamous	137	71	66	
Adenocarcinoma	35	14	21	
Clear cell	14	8	6	
Tumor Size (cm)				0.075
< 4	83	35	48	
≥ 4	103	58	45	
Lymphatic invasion				0.000
Yes	104	66	38	
No	82	27	55	
Distant metastasis				0.000
Yes	46	35	11	
No	140	58	82	
FIGO stage				0.001
I/II	97	37	60	
III/IV	89	56	33	
Histological grade				0.008
Well/moderate	98	40	58	
Poor	88	53	35	

Cox proportional hazards analysis was used to further evaluate the potential of miR-664 expression as a prognostic biomarker. Multivariate analysis revealed that miR-664 expression (p = 0.005) were independently associated with the overall survival (shown in Table II).

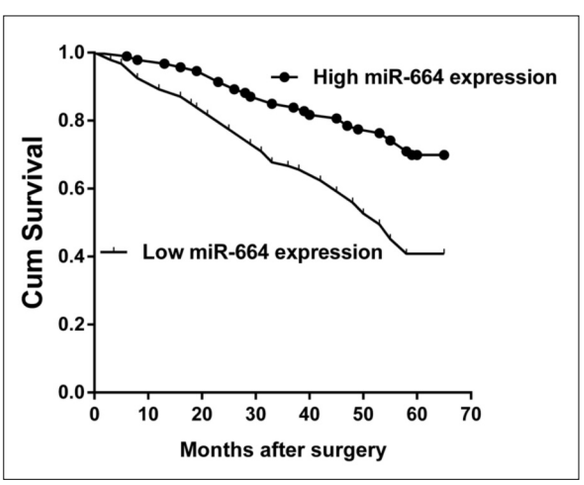

Figure 2. Kaplan-Meier postoperative survival curve for patterns of patients with cervical cancer and miR-664 expression.

Discussion

Recent advances in genomics, proteomics, and metabolomics technologies have identified key molecular events during lung cancer carcinogenesis. More novel cancer biomarkers like mRNA, non-coding RNA and metabolites were identified[12]. However, the biomarkers used in this tumor group today are not satisfactory. MicroRNAs have increasingly been recognized as major players in the development of cancer[13,14]. Compared with other biomarkers. miRNAs may be ideal biomarkers. In the present study, we focus on the prognostic value of miR-664 in cervical cancer.

Several studies[15,16] have reported that miR-664 is specifically expressed at higher levels in cardiac tissue and is dysregulated in various cardiovascular and diabetic diseases. For cancer, Bao et al[17] found that miR-664 promote cell invasion and migration in Osteosarcoma by suppressing SOX7 expression. Chen et al[18] showed that miR-664 functioned as an oncogene miRNA and promoted human osteosarcoma cell proliferation by suppressing FOXO4 expression. On the contrary, Yang et al[19] informed that miR-664 may act as a

Table II. Multivariate Cox's hazards model analysis for prognostic factors.

Variable	Hazard ratio	95% CI	*p*-value
Age (years)	1.35	0.41-2.69	0.54
HPV	1.58	0.61-2.74	0.18
Tumor size (cm)	2.41	0.77-3.34	0.11
Tumor histology	0.87	0.37-2.21	0.72
Lymphatic invasion	3.56	0.72-21.45	0.09
Distant metastasis	4.47	2.55-18.81	0.002
FIGO stage	2.34	1.91-16.63	0.001
Histological grade	4.32	1.21-15.39	0.08
miR-664 expression	4.21	2.36-17.32	0.005

tumor suppressor to modulate cervical cancer cell growth and migration. Those results revealed that miR-664 may serve as tumor promoter or tumor suppressor in different cancer, suggesting miR-664 may be associated with prognosis in cervical cancer.

In the present work, we found that the expression level of miR-664 was reduced in cervical cancer tissues in comparison to normal matched tissue. miR-664 expression was negatively correlated with lymphatic invasion, distant metastasis, FIGO stage, and histological grade, suggesting that miR-664 might be involved in the carcinogenesis and metastasis of cervical cancer. Moreover, Kaplan-Meier analysis showed that cervical cancer patients with low miR-664 expression tend to have shorter overall survival. Moreover, multivariate Cox analysis proved that miR-664 was an independent prognostic indicator for cervical patients.

Conclusions

Our findings demonstrated for the first time that cervical cancer patients with low miR-664 expression had a poor OS. Moreover, the down-regulation of miR-664 may be significantly associated with tumor progression and prognosis in patients with cervical cancer.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

References

1) Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA Cancer J Clin 2014; 64: 9-29.
2) Forouzanfar MH, Foreman KJ, Delossantos AM, Lozano R, Lopez AD, Murray CJ, Naghavi M. Breast and cervical cancer in 187 countries between 1980 and 2010: a systematic analysis. Lancet 2011; 378: 1461-1484.
3) Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA Cancer J Clin 2011; 61: 69-90.
4) Ueda T, Volinia S, Okumura H, Shimizu M, Taccioli C, Rossi S. Relation between microRNA expression and progression and prognosis of gastric cancer: a microRNA expression analysis. Lancet Oncol 2010; 11: 136-146.
5) Farazi TA, Hoell JI, Morozov P, Tuschl T. MicroRNAs in human cancer. Adv Exp Med Biol 2013; 774: 1-20.
6) Calin GA, Croce CM. MicroRNA signatures in human cancers. Nat Rev Cancer 2006; 6: 857-866.
7) Ying SY, Chang DC, Lin SL. The microRNA. Methods Mol Biol 2013; 936: 1-19.
8) Fu Y, Shao ZM, He QZ, Jiang BQ, Wu Y, Zhuang ZG. Hsa-miR-206 represses the proliferation and invasion of breast cancer cells by targeting Cx43. Eur Rev Med Pharmacol Sci 2015; 19: 2091-2104.
9) Liu DZ, Zhang HY, Long XL, Zou SL, Zhang XY, Han GY, Cui ZG. MIR-150 promotes prostate cancer stem cell development via suppressing p27Kip1. Eur Rev Med Pharmacol Sci 2015; 19: 4344-4352.
10) Yang H, Cho ME, Li TW, Peng H, Ko KS, Mato JM and Lu SC. MicroRNAs regulate methionine adenosyltransferase 1A expression in hepato¬cellular carcinoma. J Clin Invest 2013; 123: 285-298
11) Bueno RC, Canevari RA, Villacis RA, Domingues MA, Caldeira JR, Rocha RM, Drigo SA, Rogatto SR. ATM down-regulation is associated with poor prognosis in sporadic breast carcino¬mas. Ann Oncol 2014; 25: 69-75.
12) Kim B, Lee HJ, Choi HY, Shin Y, Nam S, Seo G, Son DS, Jo J, Kim J, Lee J. Clinical validity of the lung cancer biomarkers identified by bioinformatics analysis of public expression data. Cancer Res 2007; 67: 7431-7438.
13) Ruan K, Fang X, Ouyang G. MicroRNAs: novel regulators in the hallmarks of human cancer. Cancer Lett 2009; 285: 116-126.

14) Xia M, Duan ML, Tong JH, Xu JG. MiR-26b suppresses tumor cell proliferation, migration and invasion by directly targeting COX-2 in lung cancer. Eur Rev Med Pharmacol Sci 2015; 19: 4728-4737.

15) Lu Y, Zhang Y, Wang N, Pan Z, Gao X, Zhang F, Zhang Y, Shan H, Luo X, Bai Y, Sun L, Song W, Xu C, Wang Z, Yang B. MicroRNA-328 contributes to adverse electrical remodeling in atrial fibrillation. Circulation 2010; 122: 2378-2387.

16) Zhang Q, Xiao X, Li M, Li W, Yu M, Zhang H, Wang Z, Xiang H. Acarbose reduces blood glucose by activating miR-10a-5p and miR-664 in diabetic rats. PLoS One 2013; 8: e79697.

17) Bao Y, Chen B, Wu Q, Hu K, Xi X, Zhu W, Zhong X, Chen J. Overexpression of miR-664 is associated with enhanced osteosarcoma cell migration and invasion ability via targeting SOX7. Clin Exp Med 2015; 15: 257-266.

18) Chen B, Bao Y, Chen X, Yi J, Liu S, Fang Z, Zheng S, Chen J. Mir-664 promotes osteosarcoma cells proliferation via downregulating of FOXO4. Biomed Pharmacother 2015; 75: 1-7.

19) Yang Y, Liu H, Wang X, Chen L. Up-regulation of microRNA-664 inhibits cell growth and increases cisplatin sensitivity in cervical cancer. Int J Clin Exp Med 2015; 15; 8: 18123-18129.