Int J Clin Exp Pathol 2016;9(9):9491-9496
www.ijcep.com /ISSN:1936-2625/IJCEP0034798

Original Article

Upregulation of miR-150 expression as a poor prognostic marker for cervical cancer patients

Jiaying Lin[1], Ding Ding[2]

[1]*Department of Assisted Reproduction, Shanghai Ninth People's Hospital, Shanghai Jiao Tong University, School of Medicine, Shanghai, China;* [2]*Department of Gynecology, Obstetrics and Gynecology Hospital, Fudan University, Shanghai, China*

Received July 1, 2016; Accepted July 20, 2016; Epub September 1, 2016; Published September 15, 2016

Abstract: Background: Deregulation of miR-150 expression has been implicated in the development and progression of several types of human cancers. However, the clinical implications of miR-150 in human cervical cancer is still not clear. Here, we aimed to evaluate the prognostic values of miR-150 in cervical cancer. Methods: Quantitative real-time PCR was performed to determine the expression levels of miR-150 in cervical cancer and adjacent normal cervical tissues. Kaplan-Meier survival and multivariate analyses were then carried out to examine the correlation between miR-150 expression and overall survival of cervical cancer patients. Results: miR-150 expression significantly increases in cervical cancer tissues compared with that in corresponding adjacent normal tissues (P = 0.021). Moreover, increased miR-150 expression in cervical cancer is significantly correlated with worse histological grade (P = 0.012) and increased risks of lymph node metastasis (P = 0.003) and lymphatic invasion (P = 0.002). Kaplan-Meier analysis further showed that patients with higher levels of miR-150 expression exhibits significantly poorer 5-year OS (71.9%) than those with lower miR-150 expression (90.8%) (P = 0.048). Furthermore, multivariate analysis revealed that miR-150 expression was independently associated with the OS (HR = 2.323, 95% CI: 1.132-6.658; P = 0.028). Conclusion: Our findings demonstrated that upregulation of miR-150 expression in cervical cancer is associated with poor prognosis and miR-150may serve as a prognostic biomarker for patients with cervical cancer in the future.

Keywords: Cervical cancer, miR-150, prognosis, biomarker

Introduction

Cervical cancer is one of the leading causes of cancer deaths in women worldwide. The incidence of cervical cancer among women aged 15 and 39 years old is approximately 16 per 100,000 [1]. The malignant transformation of normal cervical epithelium to invasive cervical cancer is a multistep and multifactorial process [2]. Persistent infection with oncogenic human papillomavirus (HPV) is one of the most important risk factors in the development of cervical cancer [3]. Although the incidence of cervical cancer has remarkably decreased over the past 50 years due to the cervical cytology (Papanicolaou test) screening programs, substantial evidence suggests that, aside from HPV infection, some other factors such as host genetic variations also play important roles in the pathogenesis of cervical cancer [4]. Therefore, it is very important to identify additional biomarkers for early prediction and better management of cervical cancer patients.

MicroRNAs (miRNAs) are noncoding RNAs that are 18-23 nucleotides in length. They regulate the expression of their target genes by base-pairing with their target messenger RNAs (mRNAs) and then leading to translational repression or transcript degradation [5]. Recent studies have shown that deregulated expression of miRNAs have been involved in a wide range of pathological processes such as viral infection and the development and progression of human cancers [6].

Notably, recent evidence indicates that miR-150 may function as a tumor suppressor and inhibit the development and proliferation of lymphoma [7]. Furthermore, it has also been

Table 1. Association between miR-150 expression and different clinicopathological features of human cervical cancers

Clinicopathological features	No. of cases	miR-150 expression		P
		High (n, %)	Low (n, %)	
Age at diagnosis (years)				
< 50	56	38	18	0.254
≥ 50	60	34	26	
Tumor size (cm)				
< 4	70	46	24	0.335
≥ 4	46	26	20	
Histological grades				
Well/Moderate	64	33	31	0.012
Poor	52	39	13	
FIGO stage				
Ia1-Ib2	66	37	29	0.176
IIa1-IIa2	50	35	15	
Lymph node metastasis				
No	72	37	35	0.003
Yes	44	35	9	
Vascular space involvement				
No	64	40	24	0.534
Yes	52	32	20	
Histological type				
Squamous	85	49	36	0.207
AC/ASC	26	20	6	
Others	5	3	2	
Lymphatic invasion				
Yes	59	45	14	0.002
No	57	27	30	
HPV				
(-)	32	24	8	0.09
(+)	84	48	36	

Abbreviations: No. or n, number of patients; P, *P* value; AC/ASC, adenocarcinomas/adenosquamous carcinomas; HPV, human papilloma virus.

found that miR-150 displays potential prognostic values in several types of human cancers. For example, miR-150 expression is markedly down regulated in human esophageal squamous cell carcinoma and colorectal cancer, contributing to a poor prognosis [8, 9]. On the contrary, the upregulation of miR-150 expression in human prostate cancer and thyroid cancer is closely correlated with tumor recurrence or metastasis [10, 11]. However, the potential clinical implications of miR-150 in cervical cancer has not been investigated. Hence, in this study, we aimed to determine whether there is an association between miR-150 expression and the clinicopathologic features of human cervical cancers. Our findings may uncover novel biomarkers for the prognosis and effective management of cervical cancer patients.

Materials and methods

Clinical samples and patients characteristics

Primary cervical cancer and adjacent normal cervical tissues were resected from a total of 116 cervical cancer patients who underwent pelvic surgery at the Obstetrics and Gynecology Hospital of Fudan University between 2008 and 2012. The staging and clinicopathological classifications of cervical cancer were determined according to guidelines of the modified International Federation of Gynecology Obstetrics (FIGO) system for cervical cancer [12]. All patients enrolled in this study were found to suffer from only gynaecological tumor(s) and received no preoperative radiotherapy, chemotherapy, or hormonal therapy. Following the pelvic surgery, 89 patients further underwent adjuvant radiotherapy and/or chemotherapy. All resected specimens were immediately frozen in liquid nitrogen and stored at -80°C until RNA extraction was carried out as described below. The median follow-up time for the primary cervical cancer cohort was 51.7 months (range, 16 to 85 months). Written informed consent from the patients was obtained and all protocols involving this study were approved by the Research Ethics Committee of Fudan University (Shanghai, China).

RNA extraction and quantitative real-time PCR

Total RNA was extracted from the frozen tissues using TRIzol reagent (Invitrogen, Carlsbad, CA, USA). Quantitative real-time PCR (qRT-PCR) was then carried out on Applied Biosystems 7500 real-time PCR system (Applied Biosystems, Foster City, CA, USA). The U6 gene was used as an internal standard control for nor-

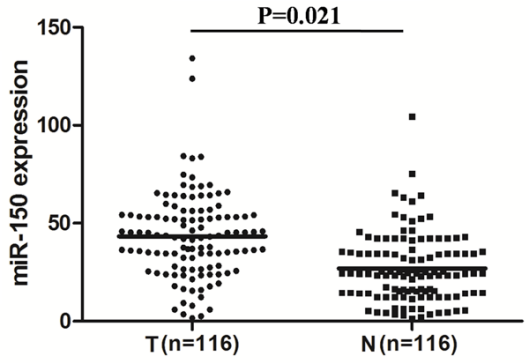

Figure 1. Upregulation of miR-150 expression in cervical cancer patients. Cervical cancer and matched adjacent normal tissues from 116 cervical cancer patients were collected during the pelvic surgery and frozen at -80°C. After RNA extraction from the frozen tissues, quantitative real-time -PCR was then performed to determine the expression levels of miR-150.

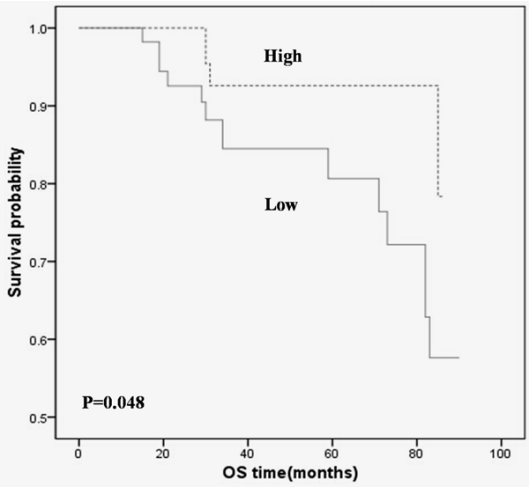

Figure 2. miR-150 expression is inversely correlated with 5-year overall survival (OS) of cervical cancer patients. The median expression levels of miR-150 were used to divide he patients into two groups with low and high levels of miR-150 expression. Kaplan-Meier analysis was then carried out to evaluate the correlation between the expression levels of miR-150 and 5-year OS of cervical cancer patients.

malization. The following primers were used to determine the levels of miR-150 and U6 snRNA expression: miR-150 forward (5'-CAGTATTCTCTCCCAACCCTTGTA-3') and reverse primers (5'-AATGGATGATCTCGTCAGTCTGTT-3'); U6 snRNA forward (5'-ATTGGAACGATACAGAGAAGATT-3') and reverse primers (5'-GGAACGCTTCACGAATTTG-3') (Invitrogen, Carlsbad, CA, USA). PCR amplification was performed with an initial denaturation step at 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 62°C for 30 s, and 72°C for 30 s. The relative expression levels of miR-150 were normalized to that of U6 snRNA and then calculated using the comparative cycle threshold (CT) method.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Services (SPSS, version 19.0) (Chicago, IL, USA). Overall survival time for all patients was censored during the last visit of regular follow-ups. Comparisons between groups were evaluated with the chi-squared test or Fisher's exact test. The hazard ratios and corresponding 95% confidence intervals (CIs) were calculated with Cox's proportional hazards model. Multivariate analysis of prognostic values was performed by multivariate Cox regression. Kaplan-Meier survival curves were constructed to illustrate the overall survival of cervical patients and the log-rank test was performed to compare the differences between survival curves of patients with low and high levels of miR-150 expression. Statistical results were considered significant if $P < 0.05$.

Results

Upregulation of miR-150 expression in cervical cancer patients

Cervical cancer and matched adjacent normal tissues were collected from a total of 116 cervical cancer patients. The demographic information and different clinicopathological parameters of the cervical patients were summarized in **Table 1**. To assess the potential clinical implications of miR-150 in human cervical cancer, we first performed qRT-PCR to compare the expression levels of miR-150 between cervical cancer tissues and neighboring non-cancerous cervical tissues. As shown in **Figure 1**, the expression levels of miR-150 are significantly elevated in cervical cancer tissues compared with those in adjacent normal cervical tissues ($P = 0.021$).

The correlation between miR-150 expression and clinicopathological features of cervical cancer patients

To examine the correlation between miR-150 expression and clinicopathological features of

Table 2. Multivariate Cox's hazards model analysis forprognostic factors

Factors	Hazard ratio	95% CI	*P* value
Age	0.582	0.743-1.564	0.634
Tumor size	0.854	0.503-1.432	0.438
Histologic type	0.437	0.164-1.498	0.276
LN metastasis	1.126	2.142	0.786
Vascular space involvement	0.563	0.201-1.327	0.475
Lymphatic invasion	1.198	0.583-2.098	0.723
HPV infection	0.423	0.176-1.462	0.285
Expression levels of miR-150	2.323	1.132-6.658	0.028

Abbreviations: CI, confidence interval; P, *P* value; LN, lymph node; HPV, human papilloma virus.

cervical cancer patients, we then used the median expression level of miR-150 as a cut-off point to divide the cervical cancer patients into two groups with low (n = 44) and high levels (n = 72) of miR-150 expression (**Table 1**). Remarkably, increased miR-150 expression in cervical cancer is significantly associated with worse histological grade (P = 0.012) and increased risks of lymph node metastasis (P = 0.003) and lymphatic invasion (P = 0.002) (**Table 1**). However, there is no significant correlation between miR-150 expression and other clinicopathologic parameters such as age, tumor size, FIGO stage, vascular space involvement, histological type, and HPV infection status (P > 0.05).

Inverse correlation between miR-126 expression and survival of cervical cancer patients

We next performed survival analysis to determine whether the levels of miR-150 expression can predict the prognosis of cervical cancer patients. As shown in **Figure 2**, our Kaplan-Meier analysis revealed that patients with higher levels of miR-150 expression (71.9%) exhibit significantly poorer 5-year OS than those with lower expression levels of miR-150 (90.8%) (P = 0.048).

In order to evaluate whether the expression levels of miR-150 and other clinicopathological features are independent prognostic factors for patients with cervical cancer, we then carried out a multivariate analysis using Cox proportional hazard regression. Our data demonstrated that miR-150 expression was independently associated with 5-year OS (HR = 2.323, 95% CI: 1.132-6.658; P = 0.028) (**Table 2**). By contrast, other clinicopathological parameters, including tumor size, histological type, lymph node metastasis and lymphatic invasion are not significantly correlated with the survival of patients with cervical cancer (P > 0.05) (**Table 2**).

Discussion

Cervical cancer remains one of the most common and deadly cancers in women worldwide [13]. Although advanced therapeutic strategies have been designed to fight cervical cancer, the prognosis of patients with cervical cancer is still very difficult to predict and varies significantly among patients, since individual patients with cervical cancer may display different responses to the same treatment protocol. Therefore, it is very important to identify novel biomarkers to predict the prognosis and help to guide the treatment of patients with cervical cancer. In this study, our data showed that the expression levels of miR-150 are significantly upregulated in cervical cancer tissues compared with those in adjacent normal cervical tissues. Additionally, upregulated expression of miR-150 in cervical cancer is significantly correlated with worse histological grade and increased risks of lymph node metastasis and lymphatic invasion. Moreover, our results further demonstrated that the expression levels of miR-150 are inversely correlated with the 5-year OS in patients with cervical cancer. These findings, for the first time, demonstrate that miR-150 may serve as a biomarker for predicting the prognosis of cervical cancer patients in the future.

miR-150 was initially identified as an important player in modulating the functions of hematopoietic and immune cells [14]. Recent studies have further demonstrated that the aberrant expression of miR-150 is involved in the development and progression of human cancers by regulating the expression of certain oncogenes and/or tumor suppressor genes [15-17]. Chang et al showed that the downregulation of miR-150 expression was mediated by c-Myc and miR-150 may function as a tumor suppressor in lymphoma [18]. It has also been reported that miR-150 mediate the degradation of its target ZEB1 mRNA, thereby inhibiting tumorigenicity and tumor cell growth in esophageal squamous

cell carcinoma (ESCC) [8]. Similarly, miR-150 can impede the proliferation and invasion of pancreatic cancer cells by downregulating the expression of MUC4 [19]. These findings indicate that miR-150 can serve as a tumor suppressor in lymphoma, ESCC and pancreatic cancer. Consistently, downregulated expression of miR-150 has been found to be correlated with poor prognosis in patients with chronic lymphocytic leukemia, colorectal cancer, and esophageal carcinoma [9, 20, 21].

However, consistent with previous studies [22, 23], our findings demonstrated that miR-150 expression is upregulated in cervical cancer tissues as compared to that in normal cervical tissues. Furthermore, we find that upregulation of miR-150 expression is significantly correlated with a poorer prognosis of cervical cancer patients. These data indicate that miR-150 can act as an oncogene and contribute to the development and progression of human cervical cancers, as opposed to its functions as a tumor suppressor in other types of human cancers as discussed above. It has been documented that miR-150 promotes the proliferation and survival of cervical cancer cells by targeting FOXO4, a well-known inhibitor of cancer cell growth and metastasis [24].

In conclusion, our data demonstrated that upregulation of miR-150 expression is associated with an aggressive phenotype and poor prognosis in cervical cancer patients and miR-150 may serve as an independent biomarker for predicting the clinical outcomes and providing guidelines for the treatment of cervical cancer patients in the future.

Acknowledgements

This study was supported by grants 814011-83 from the National Science Foundation of China, and grant 11ZR1404100 (DD) from the Shanghai Science and Technology Commission.

Disclosure of conflict of interest

None.

Address correspondence to: Ding Ding, Department of Gynecology, Obstetrics and Gynecology Hospital, Fudan University, 419 Fangxie Road, Shanghai 200011, China. Tel: 86-21-33189922; E-mail: dingdingGYN1208@163.com

References

[1] Llaguno AT, Parra VN, Fuenzalida GS and Ramos SA. Incidence of Cervical Dysplasia and Cervical Cancer in Patients Younger Than 25 Years in a Dysplasia Clinic. J Pediatr Adolesc Gynecol 2016; 29: 179.

[2] Lee JW, Choi JJ, Seo ES, Kim MJ, Kim WY, Choi CH, Kim TJ, Kim BG, Song SY and Bae DS. Increased toll-like receptor 9 expression in cervical neoplasia. Mol Carcinog 2007; 46: 941-947.

[3] Baseman JG and Koutsky LA. The epidemiology of human papillomavirus infections. J Clin Virol 2005; 32: 16-24.

[4] Schiffman M, Castle PE, Jeronimo J, Rodriguez AC and Wacholder S. Human papillomavirus and cervical cancer. Lancet 2007; 370: 890-907.

[5] He L and Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. Nat Rev Genet 2004; 5: 522-531.

[6] Xie H, Lee L, Scicluna P, Kavak E, Larsson C, Sandberg R and Lui WO. Novel functions and targets of miR-944 in human cervical cancer cells. Int J Cancer 2015; 136: E230-E241.

[7] Ito M, Teshima K, Ikeda S, Kitadate A, Watanabe A, Nara M, Yamashita J, Ohshima K, Sawada K and Tagawa H. MicroRNA-150 inhibits tumor invasion and metastasis by targeting the chemokine receptor CCR6, in advanced cutaneous T-cell lymphoma. Blood 2014; 123: 1499-1511.

[8] Yokobori T, Suzuki S, Tanaka N, Inose T, Sohda M, Sano A, Sakai M, Nakajima M, Miyazaki T and Kato H. MiR-150 is associated with poor prognosis in esophageal squamous cell carcinoma via targeting the EMT inducer ZEB1. Cancer Sci 2013; 104: 48-54.

[9] Ma Y, Zhang P, Wang F, Zhang H, Yang J, Peng J, Liu W and Qin H. miR-150 as a potential biomarker associated with prognosis and therapeutic outcome in colorectal cancer. Gut 2012; 61: 1447-53.

[10] Dezhong L, Xiaoyi Z, Xianlian L, Hongyan Z, Guohua Z, Bo S, Shenglei Z and Lian Z. miR-150 is a factor of survival in prostate cancer patients. J BUON 2015; 20: 173-179.

[11] Dettmer MS, Perren A, Moch H, Komminoth P, Nikiforov YE and Nikiforova MN. MicroRNA profile of poorly differentiated thyroid carcinomas: new diagnostic and prognostic insights. J Mol Endocrinol 2014; 52: 181-189.

[12] Pecorelli S. Revised FIGO staging for carcinoma of the vulva, cervix, and endometrium. Int J Gynaecol Obstet 2009; 105: 103-104.

[13] Maher DM, Bell M, Schaefer A, Jaggi M and Chauhan SC. Benzo (a) pyrene exposure increases expression of HPV oncoproteins: a po-

tential co-factor for increased cervical cancer among Northern Plains American Indian women. Cancer Res 2013; 73: 3611-3611.
[14] He Y, Jiang X and Chen J. The role of miR-150 in normal and malignant hematopoiesis. Oncogene 2014; 33: 3887-3893.
[15] Jiang X, Huang H, Li Z, Li Y, Wang X, Gurbuxani S, Chen P, He C, You D and Zhang S. Blockade of miR-150 maturation by MLL-fusion/MYC/LIN-28 is required for MLL-associated leukemia. Cancer Cell 2012; 22: 524-535.
[16] Watanabe A, Tagawa H, Yamashita J, Teshima K, Nara M, Iwamoto K, Kume M, Kameoka Y, Takahashi N and Nakagawa T. The role of microRNA-150 as a tumor suppressor in malignant lymphoma. Leukemia 2011; 25: 1324-1334.
[17] Zhao JJ, Lin J, Lwin T, Yang H, Guo J, Kong W, Dessureault S, Moscinski LC, Rezania D, Dalton WS, Sotomayor E, Tao J, Cheng JQ. microRNA expression profile and identification of miR-29 as a prognostic marker and pathogenetic factor by targeting CDK6 in mantle cell lymphoma. Blood 2010; 115: 2630-2639.
[18] Chang TC, Yu D, Lee YS, Wentzel EA, Arking DE, West KM, Dang CV, Thomas-Tikhonenko A, Mendell JT. Widespread microRNA repression by Myc contributes to tumorigenesis. Nat Genet 2008; 40: 43-50.
[19] Srivastava SK, Bhardwaj A, Singh S, Arora S, Wang B, Grizzle WE and Singh AP. MicroRNA-150 directly targets MUC4 and suppresses growth and malignant behavior of pancreatic cancer cells. Carcinogenesis 2011; 32: 1832-1839.
[20] Mraz M, Chen L, Rassenti LZ, Ghia EM, Li H, Jepsen K, Smith EN, Messer K, Frazer KA and Kipps TJ. miR-150 influences B-cell receptor signaling in chronic lymphocytic leukemia by regulating expression of GAB1 and FOXP1. Blood 2014; 124: 84-95.
[21] Stamatopoulos B, Van Damme M, Crompot E, Dessars B, El Housni H, Mineur P, Meuleman N, Bron D and Lagneaux L. Opposite Prognostic Significance of Cellular and Serum Circulating MicroRNA-150 in Patients with Chronic Lymphocytic Leukemia. Mol Med 2015; 21: 123.
[22] Wilting S, Snijders P, Verlaat W, Jaspers A, Van De Wiel M, Van Wieringen W, Meijer G, Kenter G, Yi Y and Le Sage C. Altered microRNA expression associated with chromosomal changes contributes to cervical carcinogenesis. Oncogene 2013; 32: 106-116.
[23] Lee JW, Choi CH, Choi JJ, Park YA, Kim SJ, Hwang SY, Kim WY, Kim TJ, Lee JH and Kim BG. Altered MicroRNA expression in cervical carcinomas. Clin Cancer Res 2008; 14: 2535-2542.
[24] Li J, Hu L, Tian C, Lu F, Wu J and Liu L. microRNA-150 promotes cervical cancer cell growth and survival by targeting FOXO4. BMC Mol Biol 2015; 16: 1.