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ORIGINAL PAPER

Molecular identification of miR-145 and miR-9 expression level as prognostic biomarkers for early-stage cervical cancer detection

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Summary

Background: MicroRNAs (miRNAs) may act as carcinogen or tumor suppressor genes by targeting various biological molecules. Therefore, it is important to identify significant markers for prognosis, diagnosis treatment strategies of cancers.
Objective: To evaluate the clinical importance and prognostic value of miR-9 and miR-145 in cervical cancer.
Method: miRNAs expression was detected using quantitative real-time polymerase chain reaction in cervical cancer specimens and adjacent normal tissues.
Results: MiR-9 up-regulated in cervical cancer specimens than adjacent normal tissues (9.743 ± 2.172 vs. 2.131 ± 1.083; $P < 0.05$). MiR-145 was decreased in cervical cancer specimens compared to corresponding normal tissues (2.189 ± 0.724 vs. 7.173 ± 1.558 $P < 0.05$). In addition, increased expression of miR-9 was strongly linked to lymph node metastasis ($P = 0.017$) and vascular invasion ($P = 0.011$). On the other hand, the low expression of miR-145 was related to advanced FIGO stage ($P = 0.007$), lymph node metastasis ($P = 0.02$) and vascular invasion (0.026). Kaplan–Meier survival and log-rank analysis suggested that patients with high expression of miR-9 had shorter overall survival compared with those with low expression (log-rank test $P = 0.028$; $P < 0.001$). In addition, shorter overall survival time was remarkably linked to decreased expression of miR-145 (log-rank test $P < 0.001$). Multivariate Cox proportional hazards model analysis of miR-9 and miR-145 showed that FIGO stage ($P = 0.011$) high expression of miR-9 and low expression of miR-145 ($P = 0.023$; $P = 0.031$) were independent prognostic factors for overall survival of patients.
Conclusions: miRNA-145 and 9 may be as potential prognostic marker in patients suffering from cervical cancer.

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Introduction

Cervical cancer is known to be the third usual malignancy among women all over the world. Persistent infection of the human papillomavirus is correlated with transformation in cervical cells. It is worth noting that human papillomavirus vaccine may be effective for prevention of cervical cancer.[1,2] Moreover, other factors from host cells can be important in transformation process of cervical malignant.[3] MicroRNAs (miRNAs) are small non-coding RNA molecules that are capable to regulate cellular proliferation, differentiation and apoptosis via targeting specific genes. In addition, miRNAs are significant markers for prognosis, diagnosis of cancer[4,5] and may act as carcinogen or tumor suppressor.

Previous studies reported a number of dysregulated miRNAs in cervical carcinomas.[9–11] *MiR-9* is a highly conserved gene that there are in primates and insects. Three independent *miR-9* gene loci is known in human including, *miR-9-1, miR-9-2* and *miR-9-3*, these *miR-9* gene loci are located at chromosomes 1, 5 and 15, respectively. Up-regulation of miR-9 has reported in cervical cancer. MiR-9 may act as tumor suppressor genes by targeting PTEN, P53INP1 and TP53INP2. MiR-145 is located on chromosome 5q32-33, which was down-regulated in cervical cancer.[6] It has been previously suggested that miR-145 is involved in cell migration and invasion in HeLa cells.[7] On the other hand, Zhang et al.[8] indicated that miR-145 can act as suppressor of cyclin-dependent protein kinase, target gene of miR-145 and HeLa cells proliferation. These findings suggested that miR-145 may be linked to poor prognosis in cervical cancer. Therefore, this study aimed to evaluate the clinical importance of miR-9 and miRNA-145 in cervical cancer and we investigate the prognostic value of these miRNAs.

Materials and methods

Ethic statement

All participating patients signed the consent forms and all protocols in this study were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice Guidelines. All procedures and treatments were reviewed and approved by the Ethics Committees (Reference Number, EC;2036/109) .

Patients and data source

In this study, cancer tissues and adjacent normal tissues were obtained from 35 cases who undergoing surgical resection between April 2009 and March 2013 in Tehran and Mashhad hospitals, Iran. None of the patients received chemotherapy or radiotherapy history or other treatment history. The tissues were immediately snap frozen in liquid nitrogen and were stored at −80 °C until use. The stage of tumors was determined based on the 2009 FIGO criteria. The clinical features of the patients are listed in Table 1.

RNA extraction and quantitative real-time polymerase chain reaction

In this study, we evaluated the expression of miRNAs in the cervical cancer tissues and corresponding non-cancer tissues using quantitative real-time polymerase chain reaction (PCR) assay. Briefly, total RNA was extracted from the tissues using TRIzol reagent. Gene-specific primers were applied to synthesize cDNA from the TaqMan MicroRNA Assays. The TaqMan MicroRNA assay and TaqMan universal PCR master mix were applied for evaluation of miRNAs expression, as well as the U6 gene, an internal standard control, was applied for normalization. Moreover, relative miRNAs expression was analyzed using the comparative cycle threshold procedure.

Statistical analysis

Differences between expression levels were evaluated using the Student's *t*-test in the cervical cancer and corresponding non-cancer tissues. Association between miRNAs expression and the clinicopathological characteristics were also evaluated using the chi-square test. Survival evaluation was performed using the log-rank test and Kaplan–Meier method. Multivariate analyses of prognostic values were evaluated by Cox proportional hazards model. $P < 0.05$ was considered to be statistically significant

Results

The age of patients ranged from 26 to 80 years with a median of 53 years in this study. miRNAs expression were detected using quantitative real-time PCR. As shown in Figure 1, our results demonstrated that miR-9 up-regulated in cervical cancer specimens than adjacent normal tissues (9.743 ± 2.172 vs. 2.131 ± 1.083; $P < 0.05$). MiR-145 was decreased in cervical cancer specimens compared to corresponding normal tissues (2.189 ± 0.724 vs. 7.173 ± 1.558 $P < 0.05$). In our study, the expression levels of miRNAs were classified into low- and high-expression groups based on the median expression level. The patients who had the expression level less than median expression were assigned as low-expression group and those with high expression was divided as high-expression group. The clinical stage based on FIGO staging system and the clinicopathological parameters of miRNAs is listed in Table 1.

Our findings showed that miR-9 was significantly higher in patients with progressed FIGO stage ($P = 0.002$). In addition, increased expression of miR-9 was strongly linked to lymph node metastasis ($P = 0.017$) and vascular invasion ($P = 0.011$). On the other hand, the low expression of miR-145 was related to advanced FIGO stage ($P = 0.007$), lymph node metastasis ($P = 0.02$) and vascular invasion (0.026).

Correlation of miRNAs with prognosis

Kaplan–Meier survival and log-rank analysis suggested that patients with high expression of miR-9 had shorter overall survival compared with those with low expression (log-rank test $P = 0.028$; $P < 0.001$, Figure 2). On the other hand, shorter overall survival time was remarkably linked to decreased expression of miR-145 (log-rank test $P < 0.001$) (Figures 2 and 3).

Multivariate Cox proportional hazards model analysis of miR-9 and miR-145 showed that FIGO stage ($P = 0.011$), high expression of miR-9 and low expression of miR-145 ($P = 0.023$; $P = 0.031$) were independent prognostic factors for overall survival of patients (Table 2).

Discussion

Previous studies have indicated that miRNAs are significant markers for prognosis, diagnosis of cancer.[4,5] Dysregulation of different miRNAs have been demonstrated in cervical carcinomas.[9–11] In this study, miR-9 was up-regulated in cervical cancer specimens compared with adjacent normal tissues. Our results showed that high expression of miR-9 was significantly

Table 1. Association of miRNAs expression with clinicopathological features

Variables	No. of cases	No. expression of miR-9		No. expression of miR-145		*P* values of miR-9	*P* values of miR-145
		High = 20	Low = 15	Low = 18	High = 17		
Age (years)							
<50	21	12	9	9	12	0.512	0.608
≥50	14	8	6	9	5		
Tumor size (cm)							
<4	16	9	7	7	9	0.438	0.437
≥4	19	11	8	11	8		
Histological grades							
Well/moderate differentiated	17	10	7	10	7	0.61	0.56
Poorly differentiated	18	10	8	8	10		
FIGO stage							
Ib–IIa	20	8	12	5	15	0.002	0.007
IIb–IIIa	15	12	3	13	2		
Vascular invasion							
No	11	3	8	2	9	0.011	0.026
Yes	24	17	7	16	8		
Lymph node metastasis							
No	21	7	14	8	13	0.017	0.201
Yes	14	13	1	10	4		

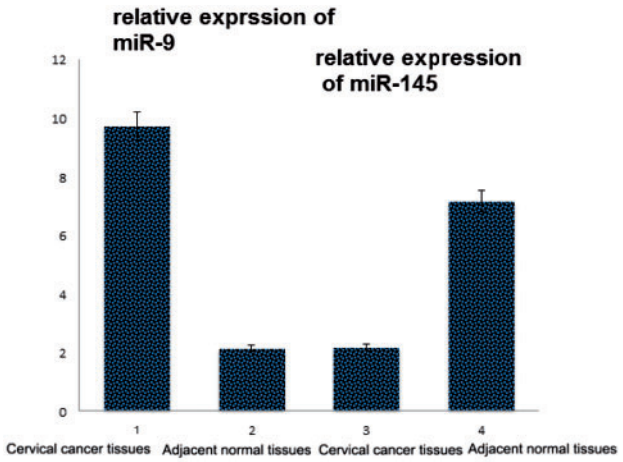

Figure 1. miRNAs expression level in cervical cancer and adjacent normal tissues.

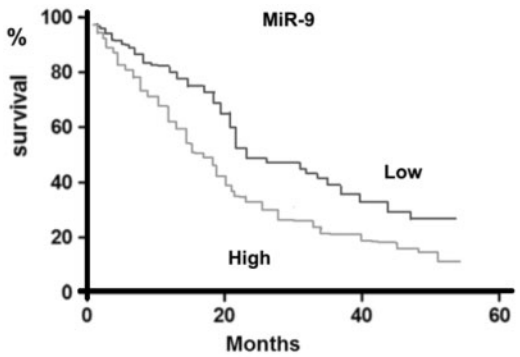

Figure 2. Kaplan–Meier survival curves of patients with cervical cancer based on miR-9 expression status.

related to higher FIGO, lymph node metastasis and vascular invasion. We found that patients with high expression of miR-9 had shorter overall survival time in comparison with patients with low expression. MiR-9 has been reported to act as

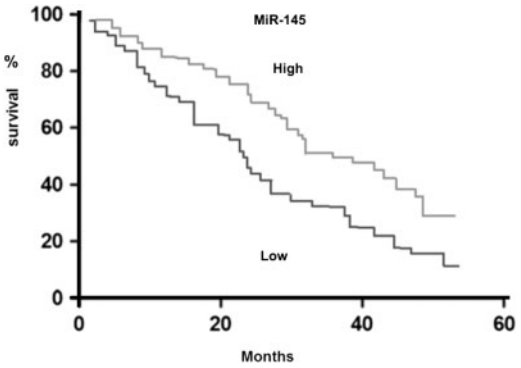

Figure 3. Kaplan–Meier survival curves of patients with cervical cancer based on miR-145 expression status.

'carcinogenic' agent in various biological processes. Previous studies showed that miR-9 expression is correlated to metastasis in various kinds of cancer such as breast, cervix, liver, ovarian, gastric, thyroid and colon cancer.[12–18] Decreased MiR-9 levels have been shown in colorectal cancer cell line,[15] renal cell carcinoma, breast cancer, primary CRC tumors and gastric cancer.[19–22] Decrease expression of miR-9 may have proliferative role in tumor cells, and it was possibly involved in pathogenesis of disease. In contrast, up-regulation of miR-9 has been reported in many kinds of tumor such as gastric, gliomas and HCC cancer.[23–25]

It is worth noting that miR-9 has heterogeneous expression within a given tissue. As matter of fact, miR-9 might have various functions in several kinds of cancers and can function as tumor specific. Recently, MiR-9 has also been described to act as a prognostic factor in many kinds of tumors including, colon cancer and acute lymphocytic leukemia.[26,27] In our study, the decreased expression of the miR-9 was linked to aggressive clinicopathological factors, but its involved mechanism is still not known. Therefore, further studies in needed to clarify the role of this miRNA. A previous study indicated that miR-9 is able to directly target CDH1 (the E-cadherin-encoding mRNA) and contribute to down-regulation of E-cadherin, as well as it can

Table 2. Multivariate analysis of clinicopathological parameters

Clinicopathological characteristics	HR	95% CI	P
Age	0.643	0.782–1.621	0.514
Tumor diameter (cm)	0.839	1.316–2.371	0.498
Histological grades	0.912	1.314–2.157	0.473
FIGO stage	3.414	3.238–8.123	0.011
Lymph node metastasis	2.226	0.695–4.521	0.064
Vascular invasion	2.136	0.462–2.348	0.058
miR-9 expression (high/low)	2.735	1.248–7.192	0.023
miR-145 expression	2.62	1.134–6.362	0.031

enhance motility and invasiveness in cancer cell.[17] Our result demonstrated that miR-145 was down-regulated in cervical cancer specimens compared with corresponding normal tissues. The low expression of miR-145 was related to advanced FIGO stage, lymph node metastasis and vascular invasion. Moreover, shorter overall survival time was remarkably related to low expression of miR-145. Down-regulation of miR-145 has been demonstrated in cervical cancer cell line.[9] MiR-145 strongly inhibits proliferation and motility of cancer cell and has been previously described as a novel tumor suppressive.[28] It has been previously suggested that down-regulation of miR-145 can be associated with lymph node metastasis, advanced stage and poor prognosis in small cell cervical carcinoma.[25] The results of previous studies indicated that miR-145 expression has important role in cervical carcinogenesis, though further studies are required to demonstrate its role in adenocarcinoma as one of the most prevalent type of cervical cancer.

It has been described that wild-type p53 banded to the p53 response element in promoter of miR-145 and effectively provides its transcription.[29] The loss of p53 might result in the reduction of miR-145 in cervical cancer. Vascular invasion plays an important role in the progression of cervical cancer,[30] and miR-145 was strongly related to proliferative vascular diseases.[31] Therefore, it can be interpreted that decreased expression of miR-145 in vessels may enhance progression of tumor in cervical cancer and may partly describe association between lower miR-145 levels and vascular invasion. Multivariate Cox proportional hazards model analysis showed that FIGO stage and high expression of miR-9 and low expression of miR-145 were independent prognostic factors for overall survival of patients.

In conclusion, our results demonstrated that miR-9 up-regulated in cervical cancer specimens than those adjacent normal tissues, while miR-145 was down-regulated. We have shown the association of miR-9 and miR-145 expression levels with clinical features. Moreover, it can be also concluded that these miRNAs may be as potential prognostic marker for cervical cancer.

Conflict of interest: None declared.

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