

The association of miR-138 variants with the prognosis of cervical cancer

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Abstract

Background MicroRNA-138 (miR-138) is shown to inhibit tumor growth and played a critical role in tumor pathogenesis, the present study aimed to investigate the prognostic value of miR-138 in cervical cancer. Methods Quantitative real-time polymerase chain reaction (qRT-PCR) assay was used to detect the expression of miR-138 in the tissues of cervical cancer and adjacent normal tissues. The association of miR-138 expression with clinical characteristic was analyzed via χ^2 test. Then Kaplan-Meier analysis was performed to analyze the association of miR-138 expression with the overall survival of cervical cancer patients. The multivariate cox analysis was used to evaluate the prognostic value of miR-138. Results In the current study, we found the expression level of miR-138 was significantly downregulated in the most cervical cancer patients tissues compared with that in the adjacent normal tissues ($P < 0.001$). And its expression was closely affected by TNM stage ($P = 0.043$), lymph node metastasis ($P = 0.011$) and FIGO stage ($P = 0.002$). Kaplan-Meier analysis result showed that the decreased expression level of miR-138 expression was associated with poor overall survival of patients. The cox regression analysis result indicated that miR-138 expression was independently associated with the overall survival. Conclusions The expression of miR-138 is down-regulated and involved in the development of cervical cancer. Moreover, it may serve as a prognostic marker for patients with cervical cancer.

Background

Cervical cancer is the most common gynecological malignant tumor affecting women worldwide with an estimated 12,900 new cases of cancer and 4,100 cancer-related deaths in USA in 2015 [1]. Vaccination against high risk human papilloma virus (HPV) types, population screening, and early-stage diagnosis are the major strategies for cervical cancer control in the world [2]. Studies have suggested that HPV plays an important role in the occurrence of cervical cancer [3-5]. The spread of cervical cancer is usually through direct invasion of the surrounding anatomical structures or through the lymphatics and circulatory system [6]. Although current treatments, including screening, that can lead to the early diagnosis of cervical cancer, this cancer in those who are too young to benefit from screening or who are no longer offered screening is usually diagnosed at advanced stage and has poor prognosis. Thus, it is crucial to explore novel cancer-related genes that may serve as diagnosis or prognostic markers in cervical cancer therapy.

MicroRNAs (miRNAs) are small (19-25 nucleotides) noncoding RNAs that regulate target gene expression by affecting mRNA translation and stability or by modulating the promoter activity of target genes [7-9]. MiRNAs was initial identified from *Caenorhabditis elegans*, and may control the majority of human genes, that play crucial role in the various physiological processes [10, 11]. Recent researches have identified miRNAs involved in various cancers that function as novel biomarkers for diagnosis and potential therapeutic targets [12-14]. MiR-138, one member of miRNAs, has been shown to play a critical role in tumor pathogenesis and found that it inhibited cervical cancer cells proliferation via c-Met [15]. However, the clinical significance of miR-138 in the prognosis of cervical cancer was still unclear.

In the present study, we investigated the expression level of miR-138 in clinical cervical cancer tissues and adjacent normal tissues to investigate its role in cervical cancer, and its correlation with clinicopathological variables.

Methods

Patients and specimens

A total of 149 cervical cancer tissues and matched adjacent normal tissues were obtained from patients who underwent surgery in Harrison International Peace Hospital. Patients were diagnosed with cervical cancer based on histopathological evaluation. Clinicopathological characteristics of the tumor cases are listed in **Table 1**. All of the patients never underwent the chemotherapy and radiotherapy before surgery. After surgical resection, the fresh cervical cancer tissues and adjacent normal tissue specimens were immediately put into liquid nitrogen and then stored at -80 °C until use, respectively. A 5-years' follow-up was conducted.

The study protocol was approved by the Medical Ethics Committee of Harrison International Peace Hospital. All patients provided written informed consents in the study prior to surgery.

RNA extraction and quantitative real-time RT-PCR (qRT-PCR)

Total RNA from all cervical cancer tissues and matched adjacent normal tissues were extracted using the TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and cDNA was reversely transcribed using TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA) according to manufacturer's instructions. QRT-PCR was performed with a Taqman MicroRNA Assay Kit (Applied Biosystems, Foster City, CA, USA) on the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). The relative mRNA expression of target gene was calculated by $2^{-\Delta\Delta CT}$ method and U6 was used as endogenous control. Each sample was examined in triplicate.

Statistical analysis

All statistical analyses were carried out using the SPSS 21.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism 5 statistical software package (GraphPad Software Inc., San Diego, CA, USA). The correlation between miR-138 expression and other clinicopathological variables were analyzed by χ^2 test. Student's t test was used to examine the differences of miR-138 expression between tumor and adjacent normal groups. The survival rates were computed by the Kaplan-Meier method and the method of log-rank tests were used to calculate the differences between the survival curves. A multivariate analysis with cox regression analysis was conducted to evaluate the prognostic value of miR-138 in cervical cancer. When $P < 0.05$, the difference was considered to be statistical significant.

Results

The expression of miR-138 was decreased in cervical cancer

In the cervical cancer samples, the miR-138 levels were quantified using qRT-PCR assay with U6 as the internal control. As shown in **Figure 1**, the relative miR-138 expression was significantly lower in cervical cancer samples compared with adjacent normal controls ($P < 0.001$).

Relationship between miR-138 and clinicopathological characteristics of cervical cancer

To assess the association between miR-138 expression and the clinicopathological parameters, 149 cervical cancer tissue samples were classified into low expression group ($n = 79$) and high expression group ($n = 70$), according to the median expression level of all cervical cancer samples. As displayed in **Table 1**, the expression of miR-138 found to be significantly associated with TNM stage ($P = 0.043$), lymph node metastasis ($P = 0.011$) and FIGO stage ($P = 0.002$). However, there was no significant association was observed between miR-138 expression and other parameters including age, tumor size and differentiation (all $P > 0.05$).

Association of miR-138 expression with prognosis in cervical cancer patients

The prognostic performances of tissue miR-138 for cervical cancer were detected. To investigate the correlations between the miR-138 down-expression and prognosis in cervical cancer patients, a 5-years' follow-up was conducted. As shown in **Figure 2**, Kaplan-Meier analysis showed that patients with low expression of miR-138 had shorter overall survival time than those with high expression (log rank test, $P = 0.000$). Then multivariate analysis using the cox regression analysis adjusted for all variables was performed which manifested that the low expression of miR-138 (**Table 2**, HR = 2.011, 95% CI = 1.027-3.937, $P = 0.042$) was important factor for predicting poor outcome and it might be an independent prognostic bio-marker.

Discussion

Cervical carcinoma is a common cancer with complex development and progression, it remains the leading cause of death of female malignancy. The infection of HPV, especially the high risk type HPV virus, is the main cause of cervical cancer [16, 17]. Despite cervical cancer having a good prognosis through early detection, the morbidity and mortality of this cancer are still increasing in the developing countries [18]. The accurate bio-markers combining with Pap testing are meaningful for the prediction of the prognosis of cervical cancer.

Accumulating evidences have been identified that molecular genes may be responsible for tumor initiation, metastasis and recurrence, which playing important role in the detection and treatment of patients with several different cancer types, including cervical cancer [19-22]. Li et al. suggested that fotillin-1 facilitates cervical cancer cell metastasis through Wnt/ β -catenin and NF- κ B pathway-regulated EMT and the increased fotillin-1 expression is associated with lymph node metastasis and poor prognosis in early-stage cervical cancer [19]. HOXA9 is found downregulated by Liliana Alvarado-Ruiz et

al. in cervical cancer and controlling HOXA9 expression appears to be a necessary step during cervical cancer development [20]. Torres-Poveda K et al. conducted a case-control study paired by age and found the serum levels of Th2 and Th3 cytokines were higher in cervical cancer cases than the controls, representing a risk allelic load for cervical cancer and can be used as a biomarker of susceptibility to this disease [21]. MALAT1 expression was found by Yang et al. significantly increased in cervical cancer than in normal tissues that might be an important marker of prognosis and a potential therapeutic target of cervical cancer [22].

Aberrantly expression of miRNA is observed in various cancers, holding potential as therapeutic targets and novel biomarkers [23-27]. Xin et al. demonstrated that miR-22 directly downregulated ATP citrate lyase (ACLY), which yields promising therapeutic effects in osteosarcoma, prostate, cervical and lung cancers [23]. Jiang et al. revealed that HPV16 E6 promoted EMT and invasion in cervical cancer via the repression of miR-218, while miR-218 inhibited EMT and invasion in cervical cancer by targeting SFMBT1 and DCUN1D1 [24]. MiR-138, belonging to miRNAs family, was found dysexpressed in many diseases and cancers. Xiao et al. investigated the regulatory mechanism of miR-138 in non-small cell lung cancer (NSCLC) and found its expression level was significantly decreased in NSCLC tissues compared to their matched adjacent normal tissues, suggesting that miR-138 may play a suppressive role in the growth and metastasis of NSCLC cells partly at least by targeting YAP1 [25]. Stojcheva N et al. identified that miR-138 overexpression increased TMZ resistance in long-term glioblastoma cell lines and glioma initiating cell cultures, defining miR-138 as a glioblastoma cell survival-promoting miRNA associated with resistance to TMZ therapy in vitro and with tumor progression in vivo [26]. Zhang et al. indicated that miR-138 overexpression inhibited metastasis of breast cancer cells, proposing that miR-138 might be used as therapeutic agent for breast cancer [27].

In the current study, we have studied miR-138 expression in 149 specimens of cervical cancer patients by qRT-PCR. The expression of miR-138 in cervical cancer was reduced compared with adjacent normal control. The result suggests that miR-138 may therefore function as a tumor suppressor gene. Then we further explored the role of miR-138 in the development of cervical cancer via investigating the relationship between its expression and clinical factors. The result revealed the miR-138 was involved in the progression of cervical cancer due to its tightly correlation with TNM stage, lymph node metastasis and FIGO stage. The Kaplan–Meier analysis showed that patients with a low miR-138 expression had a shorter overall survival compared to those with high expression, revealing miR-138 was related to the prognosis of cervical cancer. Multivariate analysis (Cox proportional hazard model) revealed that miR-138 expression was an independent prognostic factor and provided a promising therapeutic strategy for cervical cancer.

Conclusions

In conclusion, miR-138 is downexpressed in cervical cancer tissues and its expression is influenced by some clinical factors, which is consistent with the previous study [15]. Our study provides support for the

down-regulation of miR-138 is correlated with the progression and poor prognosis of cervical cancer patients. Further studies are ongoing to assess its prognostic utility in this malignancy.

List Of Abbreviations

MicroRNA-138 (miR-138)

Quantitative real-time polymerase chain reaction (qRT-PCR)

human papilloma virus (HPV)

MicroRNAs (miRNAs)

ATP citrate lyase (ACLY)

non-small cell lung cancer (NSCLC)

Declarations

Ethics approval and consent to participate

This study was supported by the Ethics Committee of Harrison International Peace Hospital and also has been carried out in accordance with the World Medical Association Declaration of Helsinki.

The subjects had been informed the objective. Certainly, written consents were signed by every subject in this study.

Consent for publication

We obtaining permission from participants to publish their data.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

S.C., L.Z. design of the work; S.C., L.Z. the acquisition, analysis, S.C., L.Z. interpretation of data; S.C., L.Z. the creation of new software used in the work; S.C., L.Z. have drafted the work or substantively revised it. All authors read and approved the final manuscript.

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Tables

Table 1. The relationship between miR-138 expression and clinicopathological features of cervical cancer patients

Parameters	Cases (n = 149)	miR-138 expression		χ^2	P
		Low	High		
Age				0.181	0.671
< 50	76	39	37		
≥ 50	73	40	33		
Tumor size				2.954	0.086
< 4 cm	74	34	40		
≥ 4 cm	75	45	30		
TNM stage				4.114	0.043
I-II	72	32	40		
III	77	47	30		
Lymph node metastasis				6.499	0.011
Negative	75	32	43		
Positive	74	47	27		
FIGO stage				9.316	0.002
Ib-IIa	76	31	45		
IIb-IIIa	73	48	25		
Differentiation				1.880	0.170
Poor	83	45	32		
Moderate+well	66	34	38		

Table 2. Multivariate analysis adjusted for clinical variables for the prognostic value of miR-138 in cervical cancer patients

Variable	HR	95% CI	P value
Lymph node metastasis	4.083	1.691-9.858	0.002
Positive vs. Negative			
FIGO stage	3.224	1.465-7.097	0.004
Ib-IIa vs. IIb-IIIa			
MiR-138 expression	2.011	1.027-3.937	0.042
Low vs. High			

HR: hazard ratio, 95%CI: 95% confidence interval. $P < 0.05$ was considered to be statistically significant.

Figures

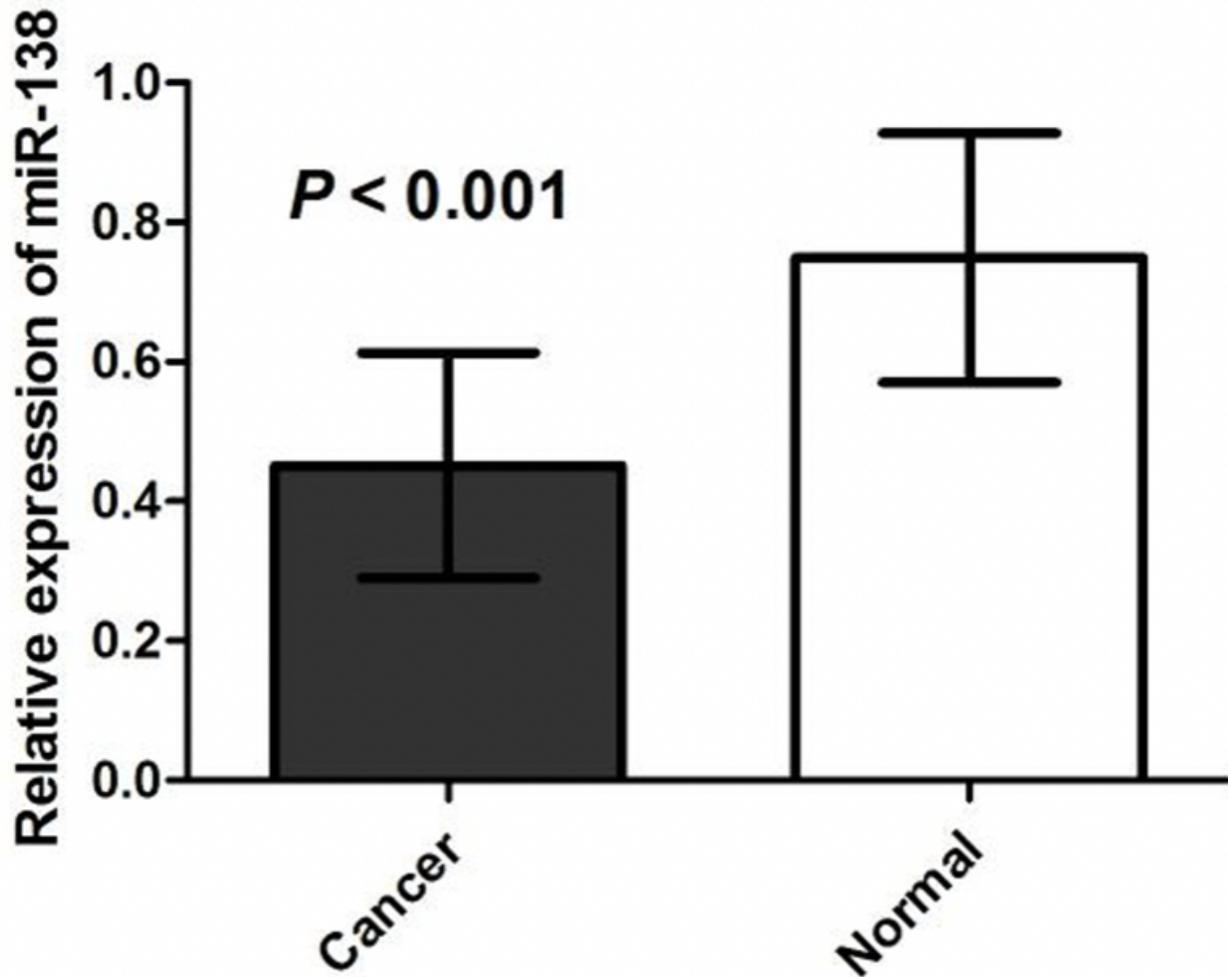


Figure 1

Relative expression of miR-138 in cervical cancer tissues and adjacent normal tissues. The expression of miR-138 in cervical cancer tissues was lower than that in adjacent normal tissues ($P < 0.001$).

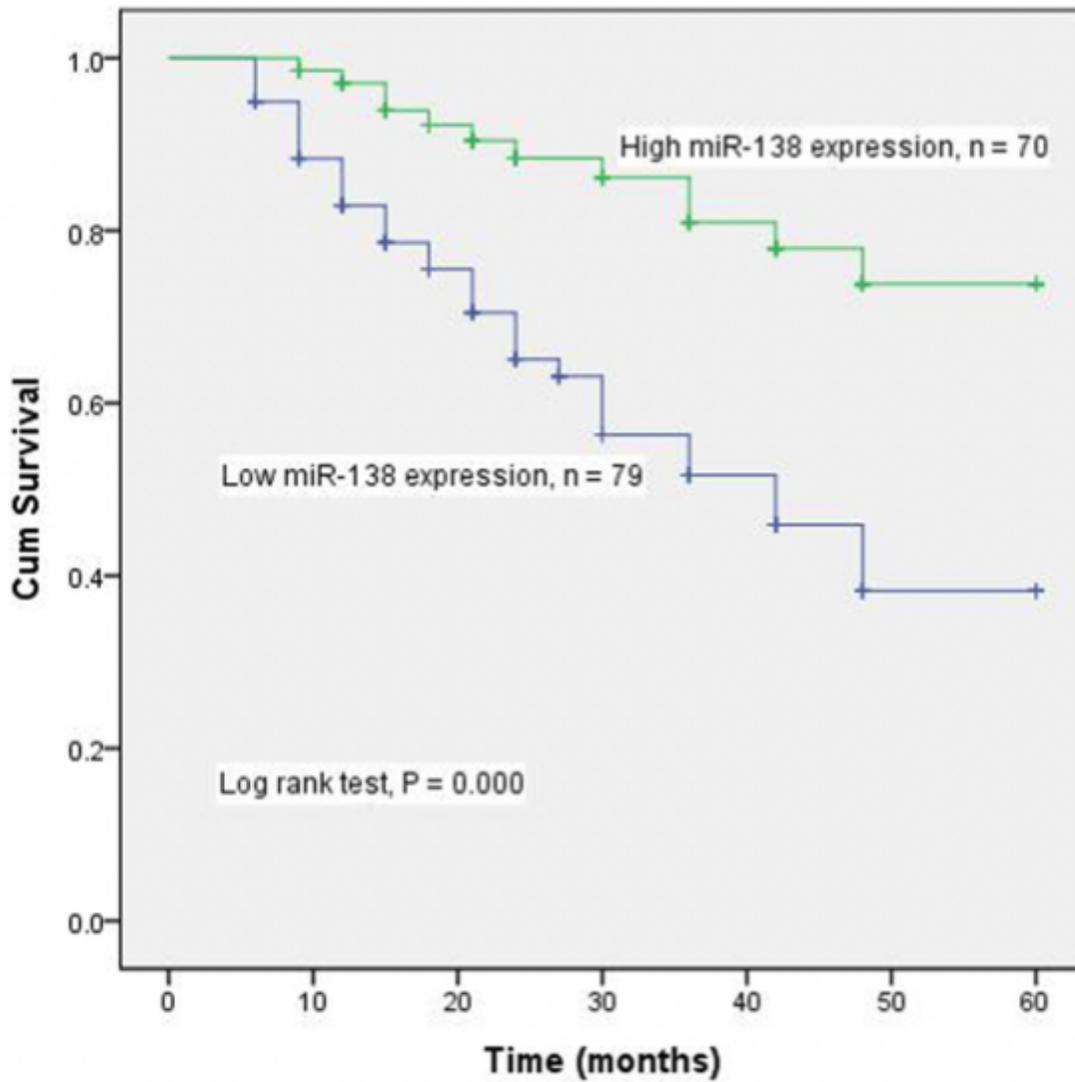


Figure 2

Kaplan-Meier analysis for analyzing the overall survival of patients with cervical cancer. Patients with low miR-138 expression had a shorter overall survival time than those with high expression (log rank test, $P = 0.000$).