ORIGINAL ARTICLE

ROLE OF MICRORNA-155 AS A DIAGNOSTIC BIOMARKER FOR HUMAN PAPILLOMAVIRUS ASSOCIATED CERVICAL CANCER

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ABSTRACT

The aim: This study was designed to investigate the potential role of miRNA-155 in the pathogenesis of HPV-induced cervical cancer.

Materials and methods: A total of 42 formalin-fixed paraffin-embedded (FFPE) cervical cancer tissue samples and 38 FFPE normal cervical tissue samples were used (they were collected at the Department of Pathology, Baghdad teaching hospital, Baghdad, Iraq, between January 2019 to January 2021). Following HPV testing and genotyping, the expression of miRNA-155 were evaluated by real-time PCR (qPCR).

Results: A statistically significant up-regulation of miRNA-155 expression was observed in cervical cancer tissues compared to results in control group, regardless of HPV status and clinical grading.

Conclusions: These data suggest that overexpression of miRNA-155 can delineate cervical cancer tissues from normal and may be a useful diagnostic biomarker for early detection of cervical cancer.

KEY WORDS: miRNA-155, Cervical cancer, High-risk HPV, qPCR

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INTRODUCTION

Cervical cancer represents one of the fatal malignant neoplasms with about 570,000 new cases annually and around 311,000 deaths a year [1]. Infection with high-risk human papillomavirus (HPV) is recognized as the major risk factor of cervical cancer. Persistent over-expression of HPV oncogenic proteins, E6 and E7, plays a critical role in the development of cervical cancer by causing genomic instability [2-3]. Specifically, E6 degrades p53, which is a critical tumor suppressor that impairs DNA repairing and inhibits apoptosis. Furthermore, HPV E7 binds and deactivates another important tumor suppressor, the retinoblastoma protein (Rb), thereby, interfering with cell cycle regulation [4].

High-risk HPV infection alone is insufficient to induce malignant transformation, other genetic and epigenetic alterations are also involved. In the field of cancer research, the focus has turned to regulatory networks, especially microRNAs, which are a small group of non-coding RNAs that negatively regulates the expression of their target genes post-transcriptionally [5]. They affect numerous developmental processes, including differentiation, inflammation, apoptosis, and cell cycle regulation.

MiRNAs can function as oncogenes or tumor suppressors and lead to tumorigenesis and cancer development. These oncogenic miRNAs are not only therapeutic targets but also important biomarkers for the detection and management of cancer [6]. Among the known oncomiRs, miRNA-155 represents a typical multifunctional miRNA that has been linked to the development of leukemia, breast, colorectal and cervical tumors [7]. Accumulating evidence demonstrates that miRNA-155 is one of the most commonly up-regulated miRNAs in solid and hematological malignancies that provokes tumor growth, invasion, and metastasis by targeting downstream genes including SOCS1, C/EBPbeta, and SHIP1 [8]. For that, it is an important target for diagnosis, prognosis, and therapy. Therefore, the present study is designed to explore the clinical relevance of miRNA-155 in cervical cancer.

MATERIALS AND METHODS

SUBJECTS

A total of 42 formalin-fixed paraffin-embedded (FFPE) cervical cancer tissue samples and 38 FFPE normal cervical tissue samples were used (they were collected at the Department of Pathology, Baghdad teaching hospital, Baghdad, Iraq, between January 2019 to January 2021) (Table I). The study was ethically approved by the Ministry of Health in Iraq (order N 42341 dated 25/11/2018) and all subjects provided written informed consent. The 42 cervical cancer samples consisted of tissue samples from 40 squamous cell carcinomas and 2 adenocarcinomas.

Table I. Information of samples with cervical cancer and normal

Variables	Cancer cases N. (%)	Normal N. (%)
Age		
≥ 30	30 (71.5)	24 (63.2)
< 30	12 (28.5)	14 (36.8)
Histology		
SCC	40 (95.2)	
ADC	2 (4.8)	
HPV infection		
HPV- positive	16 (38)	
HPV- negative	26 (62)	
Total	42 (100)	38 (100)

Table II. Sequences of microRNA primers and reference gene

Gene	Primer	Sequence (5' 3')
MiRNA-155	Stem-loop	GTC GTA TCC AGT GCA GGG TCC GAGGTATTC GCACTG GAT ACG ACA ACCCC
	Forward	CGC GCG TTA ATG CTA ATC
U6	Forward	GCT TCG GCA GCA CAT ATA CTA AAAT
	Universal reverse	CGC TTC ACG AAT TTG CG TGT CAT

Table III. Expression data of miRNA-155 in cervical cancer patients and control

Groups	N	MiRNA-155 ΔCt (Mean± SEM)	*P-value*	MiRNA-155 ΔΔCt	Fold change
Controls	38	-24.1±2.362			1
Patients	42	-89.1± 2.146	0.002	198.24	1.5943

DEPARAFFINIZATION OF FFPE TISSUES AND TOTAL RNA EXTRACTION

Three sections 10-µm thick of FFPE cervical tissue were used for total RNA extraction. A volume of 160 µl of deparaffinization solution (Qiagen, Hilden, Germany) was added to remove paraffin from FFPE tissue. Extraction of RNA was performed using the Qiagen RNeasy FFPE kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Total RNA purity and concentration were determined by measuring the ratio of the absorbance at 260 and 280 nm. Isolated total RNA was stored at –70 °C until used.

TWO STEPS RT-PCR

The expression of miRNA-155 was determined by two steps RT-PCR technique. Complementary DNA (cDNA) was synthesized using GoScript™ reverse transcription system kit (Promega, USA) and miRNA-155 specific stem-loop primer (Integrated DNA Technologies, USA) (Table 2). Reverse transcription was carried out under thermal-cycling conditions (annealing at 16 °C for 30 min, extension at 42 °C for 30 min, enzyme inactivation at 85 °C for 5 min and holding at 4 °C). Quantitative Real-time PCR (qPCR) was performed using BRYT Green Go Taq® qPCR fluorescent dye (Promega, USA) under the following thermal cycling conditions (GoTaq DNA Polymerase activation 1 cycle for 5 min at 95 °C, Denaturation of double-stranded cDNA 40 cycle for 20 s at 95 °C, then Primer annealing and extension for 40 cycles for 20 s at 60 °C).

Ct value of target miRNA-155 was normalized to U6 reference gene and the expression was determined by the relative quantitative method using the comparative Ct formula: Folding = 2^-ΔΔCT where, ΔCT= CT (target gene) – CT (reference gene), ΔΔCT ΔCT (Patients) – ΔCT (Control). Control value was considered as 1, the samples that are more than 1 are up-regulated, while values less than 1 are down-regulated (Table II).

STATISTICAL ANALYSIS

Data were statistically analyzed using SPSS program version 23 and GraphPad prism software version 6. Results were expressed using simple statistical parameters such as mean and standard error. Differences between means were assessed by independent samples of T-test. A probability of ≤ 0.05 was considered significant.

RESULTS AND DISCUSSION

The rate of high-risk HPV in SCC was 38%, which was lower than previously reported. While the two cases of

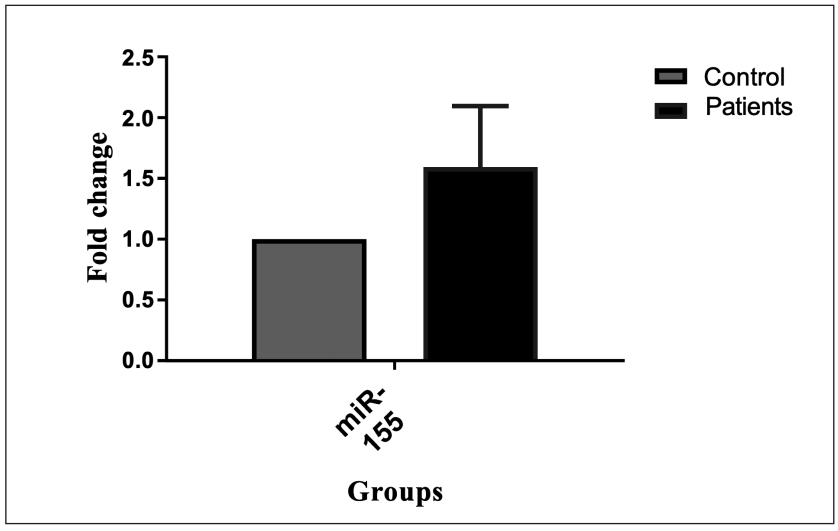

Fig. 1. Fold expression of miRNA-155 in cervical cancer patients and control

ADC were HPV-negative. Out of the high-risk HPV types, 16 and 18 were the most frequently detected with an overall prevalence of 62.5% and 37.5%, respectively.

Relative analysis of miRNA-155 data after normalization with the U6 gene revealed a significant up-regulation in miRNA-155 expression level in cervical cancer tissues compared to normal samples (P=0.002) (Fig. 1) (Table III).

Cervical cancer represents a serious aggressive gynecological malignancy, which metastasis results in an unsatisfactory overall survival of patients. Therefore, the assessment of metastatic-associated biomarkers will contribute to improving cervical carcinoma patient outcomes. An association between dysregulation of several miRNAs, including miRNA- 155, miRNA-21, miRNA-503, miRNA-224, and miRNA-1246, and prognosis of cervical cancer patients have been identified in several studies [9]. Specifically, miRNA-155 is a metastasis-related miRNA that plays a vital role in the regulation of metastasis and growth of cervical cancer cells through targeting certain oncogenes such as E2F transcription factor 2 (E2F2) [10]. Up-regulation of miRNA-155 levels in tumors decreases the expression of target genes [11]. Previous research reported over-expression of miRNA-155 in cervical cancer tissues compared to normal. This subsequently, increases the proliferation of cervical cancer cells via suppressing its target liver kinase B1 gene [12]. Another study proved the up-regulation of miRNA-155 expression in both cervical cancer cell lines and tissues by direct targeting of the TP53INP1 gene that promoted the progression of cervical carcinoma. On the other hand, it has been demonstrated that down-regulation of miRNA-155 induces cell cycle arrest and apoptosis through regulating many anti-apoptotic genes [13]. Consistently, Li et al. (2019) observed raised apoptosis in SiHa cervical cancer cells during down-regulation of miRNA-155 expression.

In addition, aberrant expression of miRNA-155 has been reported in different types of malignancies, such as breast, colon cancer, hepatocellular carcinoma, and gastric cancer [13]. In hepatocellular carcinoma, miRNA-155 regulates tumor cell malignant phenotypes by regulating the glycogen synthase kinase-3β-involved Wnt/β-catenin signaling and collagen triple helix repeat [14]. Additionally, overexpression of miRNA-155 increases the growth and metastasis of colorectal cancer cells. While, down-regulation of miRNA-155 reduces the expressions of matrix metalloproteinase-2 (MMP-2), MMP-9, and vascular endothelial growth factor, thereby inhibiting the metastasis of gastric cancer cells [14].

CONCLUSIONS

In conclusion, these results verified the crucial role of miRNA-155 in the progression of cervical cancer, and miRNA-155 could be a potential biomarker and a therapeutic target for the treatment of cervical cancer.

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