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Expression of miRNAs dysregulated by Human papillomavirus 16 E5/E6/E7 oncoproteins in cervical carcinogenesis

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Expression of miRNAs dysregulated by Human papillomavirus 16 E5/E6/E7 oncoproteins in cervical carcinogenesis

Directed by Professor Hyon-Suk Kim

The Doctoral Dissertation
submitted to the Department of Medicine,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

Mi-Soon Han

June 2017

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June 2017

Mi-Soon Han

연세대학교
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<TABLE OF CONTENTS>

연세대학교
YONSEI UNIVERSITY

LIST OF FIGURES

LIST OF TABLES

연세대학교
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ABSTRACT

Expression of miRNAs dysregulated by Human papillomavirus 16 E5/E6/E7 oncoproteins in cervical carcinogenesis

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(Directed by Professor Hyon-Suk Kim)

Cervical cancer is the third most common malignancy in women worldwide. Almost all cervical cancers are associated with human papilloma viruses (HPV), but the majority of women infected with HPV do not develop cervical cancer. We aimed to find putative microRNAs (miRNAs) that serve as diagnostic biomarkers for cervical cancer in high-risk HPV positive patients.

We analyzed the miRNA expression profiles of 12 cervical tissues using the hybridization method (nCounter Human v3 miRNA Expression Assay). Significant miRNAs, based on arbitrary |fold change| ≥ 2 ($p \leq 0.01$), were verified by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) using StepOnePlus^{TM} Real-time PCR System (Applied Biosystems, Foster City, CA, USA). We additionally performed the same qRT-PCR analysis on 29 cervical tissues. For verified miRNAs by qRT-PCR,

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we investigated the effects of HPV16 E5/E6/E7 on dysregulated miRNAs through cervical cancer cell experiments.

Upon screening of 800 human miRNAs, eight genes (miR-9-5p, -136-5p, -148a-3p, -190a-5p, -199b-5p, -382-5p, -597-5p, -655-3p) showed significant differences in the HPV16-positive cervical cancer (PC) group compared to the HPV16-positive normal (PN) group. QRT-PCR analysis showed miR-148a-3p, -190a-5p, -199b-5p and -655-3p were significantly decreased in the PC group compared to the PN group ($p < 0.05$).

In the silencing experiment of HPV16 E5 and HPV16 E6/E7, miR-148a-3p was inversely increased in both cervical cancer cell lines at silencing 72 hrs. The silencing of HPV16 E6/E7 in SiHa cells induced the increase of miR-199b-5p and miR-190a-5p.

In conclusion, three miRNAs (miR-148a-3p, miR-199b-5p, miR-190a-5p) might be serve as early biomarkers in women with HPV16 infection for cancer development. Especially, all three oncoproteins of HPV16 were associated with down-regulation of miR-148a-3p and HPV16 E6/E7 reduced miR-199b-5p and miR-190a-5p expression in cervical carcinoma.

Key words: microRNA, Human papillomavirus, E5, E6, E7, cervical cancer, biomarker

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Expression of miRNAs dysregulated by Human papillomavirus 16 E5/E6/E7 oncoproteins in cervical carcinogenesis

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(Directed by Professor Hyon-Suk Kim)

I. INTRODUCTION

Cervical cancer is the third most common malignancy among females worldwide, with a global incidence of >500,000 diagnosed new cases and ~260,000 mortalities each year.[1] In Korea, cervical cancer is the fifth common cancer in women. In 2013, there were 3,633 newly diagnosed cases and 892 death cases.[2] Recently, the guidelines for cervical cancer screening in Korea have intensified, with asymptomatic women over the age of 20 recommended to undergo cervical cancer screening with a Pap smear or liquid-based cytology every three years until the age of 74 when three-consecutive cytology screenings show negative results within the last ten years.[3]

Several factors are required for cervical cancer development, including

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the interaction of viral, environmental, and host-dependent factors, which trigger tumor growth, invasion, and metastasis. Among other factors, the human papillomavirus (HPV) infection has known to be the most important factor in cervical carcinogenesis. The transformation of the normal cervical epithelium to cervical cancer is most likely caused by HPVs, which are episomal, double-stranded DNA viruses that induce epithelial lesions. Even if more than 150 HPV types have been reported, only parts of them are highly associated with cervical cancers. As oncogenic viruses, high-risk HPV16 is the most prevalent type, followed by types 18, 31, 33, and 35.[4,5] The oncogenic potential of high-risk HPV is mostly attributed to three early genes: E5, E6, and E7. E6 and E7 exert their oncogenic effect by destabilizing and degrading pRB and p53.[6-11] E5 is believed to play a role during the early steps of transformation in the basal layers of the epithelium and enhance the oncogenic effect of E6 and E7.[12,13]

We need to find a more sensitive and reliable marker to detect cervical cancer development in high-risk patients because the majority of women infected with HPV do not develop cervical cancer. Evidence of the importance of epigenetic regulation mechanisms has steadily increased over the past two decades and has focused on the dysregulation of oncogenes and tumor-suppressor genes as the main generators of the malignant phenotype. In this regard, microRNAs (miRNAs) have an important role as regulators of cell processes such as apoptosis, cell cycle progression, metastases, and both

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chemo- and radio-resistance.[14,15]

However, the interactions between viral factors (early oncoproteins) and host factors (dysregulated miRNAs) in cervical carcinogenesis are still poorly understood.[16-18] We aimed to find putative miRNAs that serve as diagnostic biomarkers to detect the early development of cervical cancer in high-risk HPV positive patients. In addition, we investigated the association between high-risk HPV and dysregulated human miRNAs in cervical cancer.

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II. MATERIALS AND METHODS

1. Study samples and Nucleic acid extraction

We randomly collected 27 cervical tissues, composed of 3 HPV16-negative normal (NN), 8 HPV16-positive normal (PN), 3 HPV16-negative cervical carcinoma (NC), and 13 HPV16-positive cervical carcinoma (PC) samples from the Department of Pathology, Yonsei University College of Medicine. In addition to these samples, 30 cervical cancer tissues, which were 20 frozen tissues and 10 formalin-fixed paraffin-embedded (FFPE) tissues, were obtained from the Korean Gynecological Cancer Bank, Yonsei University College of Medicine. Cancer samples were histologically classified as squamous cell carcinoma, which make up about 80% of all cervical cancers. HPV infection status was tested with the Abbott Real*Ti*me High Risk HPV PCR assay (Abbott Molecular, Abbott Park, IL, USA).

Total RNA was extracted from the frozen tissues using Labozol reagent™ (CosmoGenetech, Seoul, Korea) and from the FFPE tissues by a miRNeasy FFPE kit (Qiagen, Valencia, CA, USA). RNA was quantitated using the NanoDrop and the DS-11 spectrophotometer (DeNovix, Wilmington, DE, USA) and the amount and quality were confirmed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA).

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2. Screening of miRNAs by Hybridization method

We prepared the RNA extract from 12 cervical tissues, composed of 3 NN, 3 PN, 3 NC, and 3 PC samples. Total RNA from each sample (100 ng) was added as instructed in the miRNA sample preparation protocol. Mature miRNAs were ligated to a species-specific tag sequence (miRtag). After enzymatic purification of unligated miRtags, prepared samples were hybridized with an nCounter Human v3 miRNA Expression Assay CodeSet (NanoString Technologies, Seattle, WA, USA) for 800 human miRNAs overnight at 65℃ for 16–20 hrs.

After completion of hybridization, excess probes were removed using two-step magnetic bead-based purification on the nCounter prep station. The abundance of specific target molecules was quantified on the digital Analyzer by counting the individual fluorescent barcodes and assessing the target molecules. For each sample, a scan encompassing 280 fields of view was performed. The data was collected using the digital analyzer after taking images of the immobilized fluorescent reporters in the sample cartridge with a charge-coupled device camera.

MiRNA data analysis was performed using the nSolver software analysis, freely available from NanoString Technologies. The miRNA profiling data was normalized using five housekeeping genes (ACTB, B2M, GAPDH,

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RPL19, and RPL10) included in the panel and the combination of miRNA-23a and-191 as reference miRNA gene.[19] Significant miRNAs were selected based on arbitrary |fold change| ≥ 2 and p value ≤ 0.01.

3. Real-time qRT-PCR in clinical samples

To verify the reliability of the miRNA expression profile from our NanoString nCounter analysis, we performed real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) on the same set of samples. In addition, we validated 8 miRNAs targeting 29 additional clinical tissues (25 PC and 4 PN samples) by qRT-PCR. Reverse transcription was performed using the miScript II RT Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Real-time PCR was performed on the StepOnePlus™ Real-Time PCR System (Applied Biosystems, CA, USA) using 2X QuantiTect SYBR Green PCR Master Mix (Qiagen). Thermal cycling conditions were 95℃ for 15 min followed by 40 cycles of 94℃ for 15 s and 30 s at 55℃, and 70℃ for 30 s. The data was analyzed using the StepOne software v2.2.2 (Applied Biosystems). All PCR reactions were run in triplicates and gene expression levels of each miRNA were normalized to an endogenous control small RNA, U6, and calculated using the $2^{-\Delta Ct}$ method. We compared relative expression of each miRNA to an internal control between the PC group and PN group ($p < 0.05$).

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4. Silencing of HPV16 E5/E6/E7 in cancer cell lines

We purchased two HPV16 positive cervical cancer cell lines (CaSki cell and SiHa cell) to investigate the action of HPV16 oncoproteins on human miRNAs. Cervical cancer cell lines were cultured in RPMI 1640 and Dulbecco`s-formulated Eagle's Minimum Essential Medium, supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37℃ and 5% CO2, respectively.

To verify the roles of HPV16 E5, E6, and E7 in dysregulating miRNAs during cervical carcinogenesis, each gene was modulated in cervical cancer cell lines using small hairpin RNA (shRNA) vectors for the E5 gene and short interfering RNAs (siRNA) for the bicistronic E6/E7 genes as done by previous stuides.[20,21] Scrambled shRNA or siRNA was used as a negative control.

After 0, 24, 48, 72 hrs from transfection, the cell lines were seeded on 12-well plates. Total RNA was extracted with the TRIzol reagent (Invitrogen). Transfection efficiency of each oncoprotein mRNA was measured three times by qRT-PCR, 72 hrs after transfection. The primer sets were used to amplify each mRNA according to previous studies.[20] The GAPDH was used as an internal reference.

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5. Real-time qRT-PCR in cancer cell lines

Significantly dysregulated miRNAs in clinical tissues were measured by qRT-PCR on all silencing time points with the RNA extract from each cervical cancer cell line (CaSki cell and SiHa cell). The procedure of this experiment was the same as described for clinical samples.

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III. RESULTS

1. MiRNA profiling data in NanoString nCounter system

Among 800 human miRNAs, 99 miRNAs were upregulated and 9 miRNAs were downregulated in the PC group compared with the PN group using the 2-fold change ($p < 0.01$) cut-off. Compared with the pooled control group (NN + NC + PN), 8 miRNAs among them showed significant change in the PC group (upregulated: miR-9-5p, -136-5p, -148a-3p, -190a-5p, -199b-5p, -382-5p; downregulated: miR-597-5p, -655-3p) (Figure 1). These 8 miRNAs showed more than 2-fold change in PN group compared with the NN group, but there were no statistical significance.

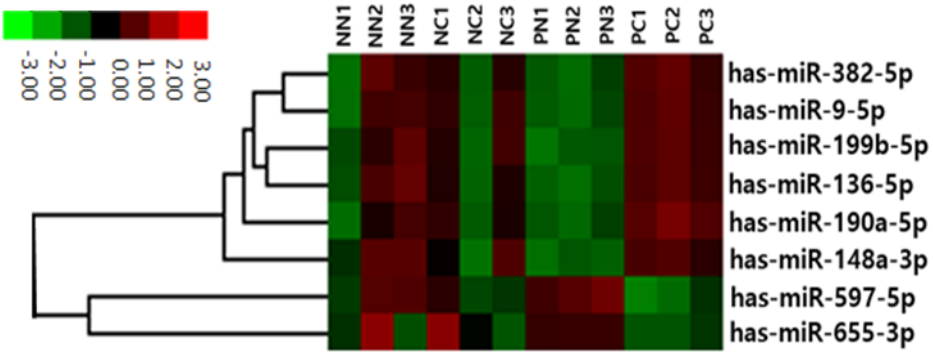

Figure 1. Differential miRNA expression in cervical tissues by the NanoString nCounter system (NN, HPV16-negative normal cervix; NC, HPV16-negative cervical carcinoma; PN, HPV16-positive normal cervix; PC, HPV16-positive cervical carcinoma).

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2. Relative expression of dysregulated miRNAs by qRT-PCR

In the qRT-PCR results with the same samples as NanoString nCounter analysis, miR-148a-3p, miR-190a-5p, miR-199b-5p, and miR-655-3p were significantly downregulated in PC group, but there were no significant differences in other miRNAs between the PC and PN groups ($p < 0.05$) (Figure 2(A)). Only miR-655-3p showed a regulation pattern consistent with the results of the NanoString nCounter analysis. Additional 29 clinical samples showed that miR-190a-5p, miR-199b-5p, and miR-655-3p were downregulated in the PC group compared to the control group ($p < 0.05$) (Figure 2(B)).

These 4 miRNAs, significant by qRT-PCR, were analyzed based on the International Federation of Gynecology and Obstetrics (FIGO) staging system (Figure 3). Four miRNAs showed significant reduction of expression in almost all FIGO stages in the PC group, except for the stage IB group of miR-148a-3p. Furthermore, their expression levels tended to decrease as the disease progressed.

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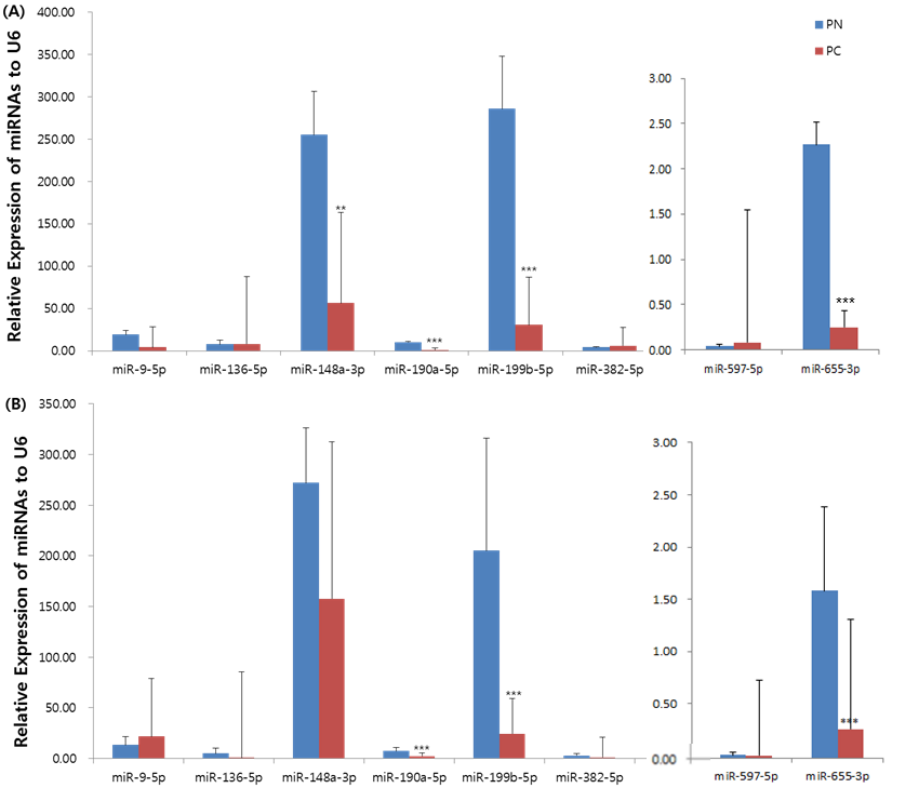

Figure 2. Relative expression of 8 miRNAs in clinical samples (A) In same set of samples of NanoString nCounter analysis (B) In additional 29 samples composed of HPV16-positive normal cervix (PN) group and HPV16-positive cancer cervix (PC) group (*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$).

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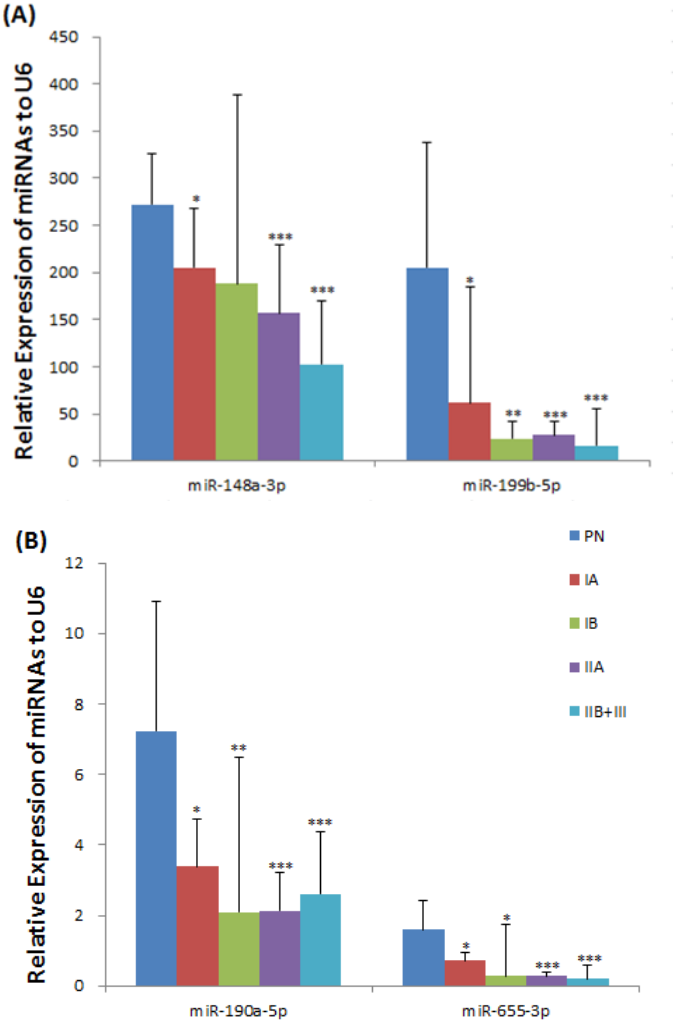

Figure 3. Relative miRNA expression based on FIGO stages. (A) miR-148a-3p and 199b-5p. (B) miR-190a-5p and miR-655-3p of HPV16-positive cancer cervix (PC; IA (n = 2), 1B (n = 12), IIA (n = 5), IIB+III (n = 6)) group compared to HPV16-positive normal cervix (PN) group by qRT-PCR (*$p < 0.05$, **$p < 0.001$, ***$p < 0.0001$).

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3. Effects of HPV16 E5/E6/E7 on the miRNA expression

Transfection efficiency of HPV16 E5 and E6/E7 modulation was measured by qRT-PCR. Using the E5-shRNA and E6/E7-siRNA, mRNAs expression of E5, E6, E7 were reduced respectively by 0.53 fold, 0.85 fold, and 0.49 fold in CaSki cells and by 0.74 fold, 0.62 fold, and 0.50 fold in SiHa cells transfected compared to the scramble control (Figure 4).

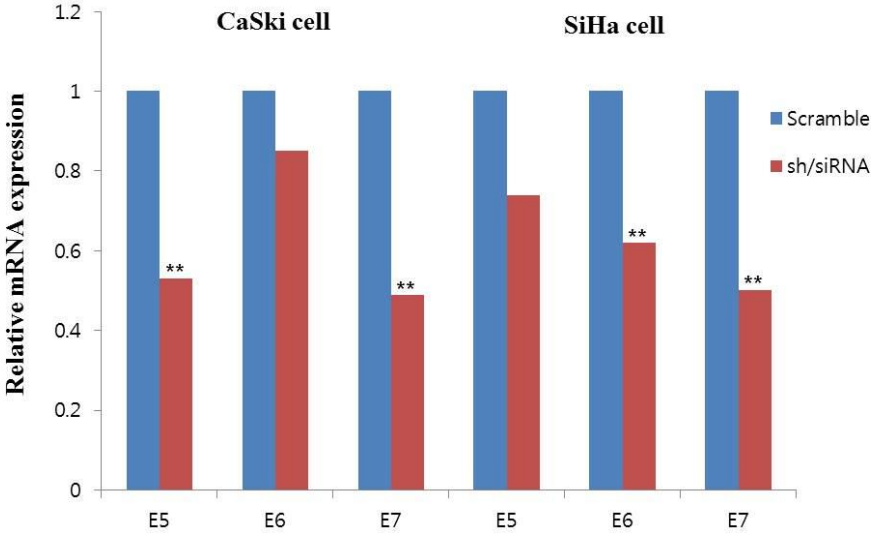

Figure 4. Relative mRNA expression levels of E5, E6, E7 upon shRNA or siRNA-mediated silencing at 72 hrs both CaSki and SiHa cells. GAPDH was used for normalization and relative expression was compared to that of the scramble control (*$p < 0.05$, **$p < 0.01$).

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We determined relative expression levels for selected 4 miRNAs in cervical cancer cell lines. The expression of selected miRNAs in cell lines was compared with the scramble control according to the time of silencing after normalization (Table 1).

Table 1. Relative level of miRNAs to U6 in sequential HPV16 E5, E6/E7 silencing time points

Human miRNAs	Cell lines	$2^{-\Delta CT}$	Scramble	24 hr	48 hr	72 hr
miR-148a-3p	CaSki	E5 silencing	0.11875	0.07204	0.15763	0.25467
		E6/E7 silencing	0.07533	0.13196	0.13196	0.21795
	SiHa	E5 silencing	0.09888	0.05795	0.12688	0.18166
		E6/E7 silencing	0.07956	0.07421	0.08990	0.18596
miR-199b-5p	CaSki	E5 silencing	0.01767	0.01163	0.00942	0.00892
		E6/E7 silencing	0.00691	0.00906	0.00779	0.00489
	SiHa	E5 silencing	0.00394	0.00719	0.00396	0.00613
		E6/E7 silencing	0.00135	0.00177	0.00331	0.00700
miR-190a-5p	CaSki	E5 silencing	0.01017	0.00779	0.01486	0.08737
		E6/E7 silencing	0.04701	0.11044	0.00810	0.04134
	SiHa	E5 silencing	0.14137	0.00857	0.02657	0.02126
		E6/E7 silencing	0.02202	0.03099	0.05756	0.10799
miR-655-3p	CaSki	E5 silencing	0.00238	0.00441	0.00370	0.00659
		E6/E7 silencing	0.00385	0.00420	0.00264	0.00343
	SiHa	E5 silencing	0.00134	0.00259	0.00305	0.00256
		E6/E7 silencing	0.00328	0.00319	0.00225	0.00253

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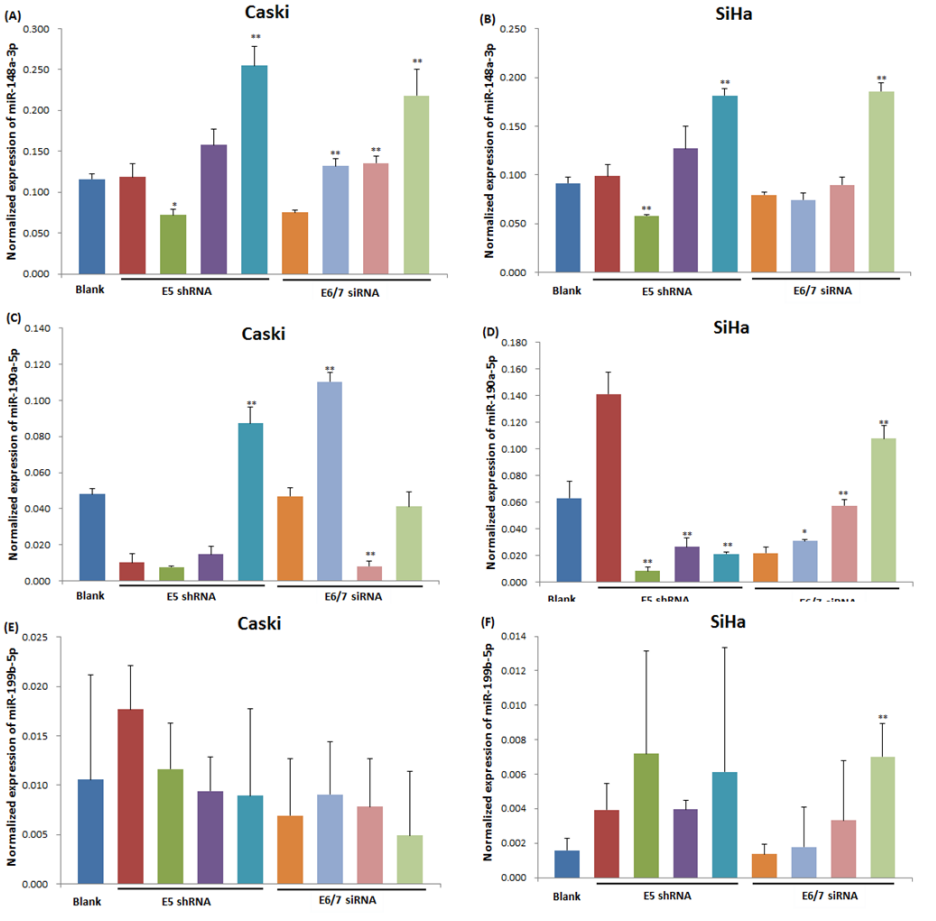
The miR-148a-3p was significantly increased in both cell lines at 72 hrs after silencing, especially E6/E7 knockdown. And miR-199b-5p showed variable expression pattern and significantly increased only in SiHa cells by E6/E7 silencing at 72 hrs. MiR-190a-5p increased significantly in CaSki cells by E5 silencing and in SiHa cells by E6/E7 silencing at 72 hrs. But there was no significant change of the miR-655-3p (Figure 5).



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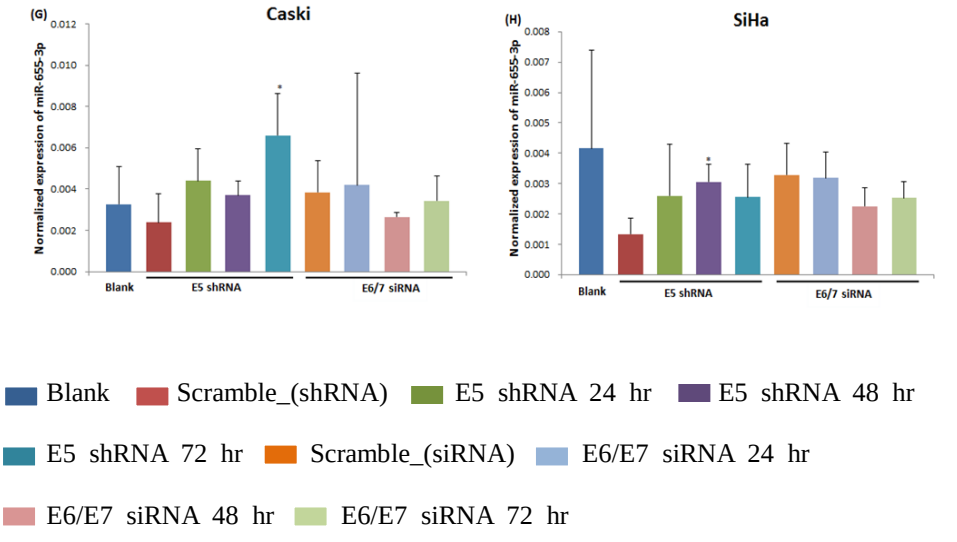

Figure 5. Alteration of human miRNAs by HPV16 E5, E6/E7 silencing time points in cervical cell lines. miR-148a-3p; (A) and (B). miR-190a-5p; (C) and (D). miR-199b-5p; (E) and (F). miR-655-3p; (G) and (H). *$p < 0.05$, **$p < 0.01$, significant difference from the scramble control.

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IV. DISCUSSION

MiRNAs are small (21-23 nucleotide long) endogenous, non-protein-coding, single-stranded RNAs that control gene expression by binding to the 3′ untranslated region (3′UTR) of messenger RNA (mRNA), leading to mRNA degradation or protein translation inhibition.[22,23] Over 1,600 miRNAs are present in the human genome, each of which potentially regulates hundreds of mRNAs. MiRNAs with altered expression patterns have been found to have oncogenic (oncomirs) or tumor-suppressing (anti-oncomirs) functions present in the pathogenesis of most malignancies.

Evidence supporting the correlation between miRNA expression and cervical cancer related processes was initially described in 2009.[24] The study′s findings demonstrated that the expression of miR-21 promoted cell proliferation in HeLa cervical cancer cells, while its inhibition suppressed cell proliferation by overexpression of the tumor-suppressor gene PDCD4, a related programmed cell death protein. It was demonstrated that miR-21 is a major oncomir, overexpressed in a wide variety of cancers including cervical cancer.[25]

Upregulated or downregulated miRNAs, such as miR-34a, miR-886-5p, miR-143, miR-203, and miR-155, with differential expression in cervical cancer versus normal samples were known.[26-30] Similarly, miRNAs have been studied as diagnostic biomarkers in cancer development and progression, and as a

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therapeutic target for cervical cancer.[31-33] Sharma G, et al[34] reviewed 246 differentially expressed miRNAs in cervical cancer progression using PubMed in 2014.

However, no certain miRNA has been applied for clinical use as a tumor marker for the diagnosis of cervical cancer so far. Inconsistent reports for miRNA expression in cervical carcinogenesis could be attributed to patient-intrinsic variation, time, and temperature from sample collection to storage processing, processing methods, contamination by cells and blood components, RNA extraction method, normalization, and storage time and conditions.[35]

To minimize the effect of variables on our results, we focused on the most prevalent HPV type (HPV16) and squamous cell carcinoma of the cervix (FIGO stages IB1 ~ III). There are many miRNAs that have not yet been studied in cervical cancer because various miRNAs are continuously being discovered. This study, which comprehensively screened 800 human miRNAs, was a chance to find a novel marker. And the effects of all well-known HPV16 oncoproteins were investigated on altered expression of significant miRNAs. Even if other similar studies commonly used the HPV negative normal group as the control group, we compared the HPV16 positive cancer group to the HPV16 positive normal group to identify early diagnostic markers for cancer development of high-risk HPV infected patients.

As a result, we identified 8 putative miRNAs as biomarkers in

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HPV16-positive cervical cancer tissues using the NanoString nCounter system (upregulated: miR-9-5p, -136-5p, -148a-3p, -190a-5p, -199b-5p, -382-5p; downregulated: miR-597-5p, -655-3p). According to previous reports for cervical cancer, miR-148a expression was altered in cancer development and served as a specific marker for HPV induced malignancy.[36,37] MiR-136 was downregulated in minimal deviation adenocarcinoma in the uterine cervix.[38] Also miR-9 upregulated in cervical cancer specimen, especially linked to lymph node metastasis and vascular invasion.[39] MiR-199b-5p downregulated in squamous cell carcinoma and associated with poor prognosis.[40]

Especially among them, miR-148a, miR-190a, miR-199b, and miR-655 were reduced significantly in the PC group in qRT-PCR analysis. There is a difference in the results between the two methods for miRNA profiling. Mestdag P et al[41] reported that concordance of differential miRNA expression was lower by 70% between hybridization and qPCR among the platforms. Particularly, the average validation rate of miRNA expression between any platform combination was only 54.6% (95% confidential interval, 52.5 – 56.7%). When it comes down to low input RNA samples, qPCR platforms showed superior sensitivity by amplification technology.

Transfection efficiency of HPV16 oncoproteins was determined to be about 50 ~ 60% for E5 and E7 mRNA in CaSki cells and E6 and E7 mRNA in SiHa cells. This was due to the difference of HPV16 copy numbers per cell and

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characteristics (race and histologic type) by cancer cell lines. MiR-148a-3p showed that the modulation of HPV16 E5 and E6/E7 induced inverse response on dysregulated miRNAs in cervical cancer at 72 hrs. Silencing of HPV16 E6/E7 in SiHa cells induced the increase of miR-199b-5p and 190a-5p.

Several limitations, such as the difficulties of relying on banked samples, prevented us from completely being able to control pre-analytical factors. This study used two sample types such as frozen and FFPE tissues, processed by different methods of storage and RNA extraction. Also, sample collection time, which is an important determinant of miRNA stability, varied depending on the sample from May 2012 to March 2017.

Finally, we suggest that miR-148a, miR-199b, and miR-190a might be serve as early diagnostic biomarkers in cervical carcinogenesis after the HPV16 infection. Three oncoproteins of HPV16 were associated with downregulation of miR-148a-3p in cervical carcinogenesis. HPV16 E6/E7 specifically reduced miR-199b-5p and miR-190a-5p in cervical carcinoma.

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V. CONCLUSION

Cervical cancer is a common malignancy in women worldwide and Korea. Almost all cervical cancers are associated with high-risk HPV, but the majority of women infected with HPV do not develop cervical cancer. We needed a sensitive and accurate biomarker for the early detection of cervical cancer development in high-risk HPV positive patients.

We comprehensively screened human miRNAs using the NanoString nCounter system and found putative dysregulated 8 miRNAs (miR-9-5p, -136-5p, -148a-3p, -190a-5p, -199b-5p, -382-5p, miR-597-5p, -655-3p) in cervical cancer tissues. Upon the qRT-PCR, miR-148a, miR-190a, miR-199b, and miR-655 were significantly downregulated in HPV16 positive cervical cancer compared HPV16 positive normal cervix. We confirmed that HPV16 E5, E6 and E7 were associated with the dysregulation of miR-148a-3p, miR-199b-5p and miR-190a-5p in cervical carcinoma.

These three miRNAs (miR-148a-3p, miR-199b-5p, miR-190a-5p) might be serve as early biomarkers in women with HPV16 infection for cancer development. We believe that three oncoproteins of HPV16 contributed to the downregulation of miR-148a-3p, miR-199b-5p and miR-190a-5p in cervical carcinogenesis.

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ABSTRACT(IN KOREAN)

자궁경부암 발생에서 인두유종바이러스16 E5/E6/E7 종양단백에 의한 miRNAs 발현 양상의 변화

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한 미 순

자궁경부암은 전세계적에서 세번째로 흔한 여성암이다. 거의 모든 자궁경부암이 인유두종바이러스(HPV)와 관련되어 있다고 알려져 있지만 대부분의 감염된 여성들에서 암이 발생하는 것은 아니다. 본 연구는 자궁경부암 발생과정에서 조기 진단표지자로서 microRNAs (miRNAs)의 역할을 규명하고자 하였다.

부합반응(nCounter Human v3 miRNA Expression Assay)을 통해 12개 자궁경부조직의 miRNAs발현양상을 분석하고 HPV16양성-자궁경부암군에서 의미 있는 발현변화를 보인 miRNAs들을 실시간 역전사효소중합효소연쇄반응(이하 실시간중합연쇄반응으로 축약)으로 검증하였다. 또한 암세포주를 이용하여 miRNAs의 발현양상에 있어 HPV16 종양단백의 영향을 평가하였다.

부합반응에서 HPV16-양성 정상군에 비해 HPV16-양성 자궁경

연세대학교
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부암군에서 8개 miRNAs(miR-9, -136, -148a, -190a, -199b, -382, -597, -655)가 유의미한 변화를 보였다(2배 이상 증가, $p \leq 0.01$). 32개 자궁경부조직에서 실시간 중합연쇄반응을 시행한 결과 그 중 miR-148a, -190a, -199b 와 miR-655가 HPV16-양성 정상군에 비해 HPV16-양성 자궁경부암군에서 의미 있게 감소하였다($p < 0.05$).

암세포주에 대해 HPV16 E5, E6, E7 유전자 silencing 실험을 진행한 결과 miR-148a는 CaSki와 SiHa 세포 모두, miR-199b 와 miR-190a는 SiHa 세포에서 조작 72 시간 후에 자궁경부조직에서의 결과와 반대로 발현증가를 보여 이러한 miRNAs의 암발생과정에서의 발현변화에 HPV16 종양단백이 의미있게 작용함을 확인하였다.

따라서, miR-148a, miR-199b와 miR-190a는 HPV16-양성 환자에서 자궁경부암 발생의 조기 진단표지자로의 가능성이 있다고 판단된다. 또한 이러한 변화는 자궁경부암발생의 가장 중요한 병인자인 HPV 종양단백에 의한 변화임을 확인할 수 있었다.

핵심되는 말: microRNA, 인두유종바이러스, E5, E6, E7, 자궁경부암, 진단표지자