

Original Article

Significance of high YKL-40 expression regulated by miR-24 in cervical cancer progression and prognosis

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Abstract: Background: Cervical cancer (CC) is a prevalent disease which ranks as the third leading cause of cancer-related death in females for developing countries. MicroRNAs (miRNAs) are small non-coding RNAs that regulate the expression of target proteins. YKL-40 plays an important part in inflammation and tissue remodeling. High YKL-40 serum levels may indicate the presence of cancer and poor cancer prognosis. In this study, we validated the association between serum YKL-40 level and CC along with the potential relationship between miR-24 and YKL-40 in CC cells. Methods: A total of 59 cervical cancer subjects were enrolled in this study. Serum YKL-40 levels were tested by ELISA. RT-PCR and Western blot were performed to study the expression of miR-24 and YKL-40 in CC cells. Luciferase reporter assay was performed to investigate the association between YKL-40 and miR-24. The influence of YKL-40 level on CC cells was assessed by the cell proliferation assay, cell migration assay and cell invasion assay. Results: YKL-40 serum level is an efficient biomarker in CC diagnosis and prognosis. MiR-24 could directly regulate the expression of YKL-40 by binding to its 3'-UTR. YKL-40 regulated by miR-24 also exacerbates proliferation, migration and invasion of CC cells. Conclusion: The over-expression of YKL-40 regulated by miR-24 is correlated with CC progression and prognosis.

Keywords: YKL-40, miR-24, cervical cancer, proliferation, invasion, migration

Introduction

Cervical cancer (CC) has been described as the second most frequently diagnosed cancer and ranks as the third leading cause of cancer-related death occurred in females for developing countries. Approximately 527,600 new cervical cancer cases and 265,700 deaths were annually reported worldwide [1]. Adenocarcinoma and squamous carcinoma are the two most frequent histologic types of CC. The incidence rate of CC is closely related to the prevalence of human papillomavirus (HPV) infection and it has been decreased by more than 80% over the last 70 years since the establishment of HPV infection screening programs [2]. Other than HPV infection, the underlying mechanism behind CC carcinogenesis has not been elucidated yet. Although the incidence rate has been dramatically decreased as a result of the utility of Pap smear screening for HPV infection, the available treatment for CC is rather invasive. Up to now, the primary treatment for patients with early CC is radical surgery.

However, the pelvic recurrence rate after surgery is approximately 8%, which remained to be relatively high [3]. The invasion and metastasis of CC cells remained to be a major challenge which significantly influences its mortality. Identification of related genes and pathways that may play critical roles in CC carcinogenesis and progression can help us to understand the underlying mechanism of CC and provide novel therapeutic target and biomarkers for its diagnosis and prognosis.

MicroRNAs (miRNAs) are small non-coding RNAs that target at a majority of protein-coding transcripts and act as guiding molecules in RNA silencing. Furthermore, miRNAs play critical roles in wide-ranging pathological processes, particularly carcinogenesis and they may exert their influence by regulating the expression of target protein via base-pairing to the 3' untranslated region (3'-UTR) of target mRNAs. For example, it has been manifested that microRNA-15b/16 enhanced the induction of regulatory T Cells by regulating the expression of

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Table 1. Relationship of YKL-40 level with CC clinic pathologic feature

Characteristics	Case (n)	YKL-40 (ng/ml)	P*	YKL-40 ≥ 133 ng/ml (n)	P#
Age (years)			0.592		0.3686
≤ 46	23	173.68 (21.63-755)		20 (86.9%)	
> 46	36	175.84 (65-996.17)		34 (94.4%)	
Tumor size			0.511		1
≤4 cm	33	183 (21.63-592.99)		30 (90.9%)	
> 4 cm	26	181 (65-996.17)		24 (92.3%)	
FIGO stage			0.003		1
I-II	41	163.62 (21.61-592.99)		37 (90.2%)	
III-IV	18	246.06 (65.66-996.17)		17 (94.9%)	
Invasion			0.792		0.6056
Negative	16	189.64 (21.62-592.99)		14 (87.5%)	
Positive	43	198.53 (44.87-996.17)		40 (93.0%)	
Lymph node metastasis			0.501		1
No	43	154.035 (21.61-592.99)		39 (90.6%)	
Yes	16	210.84 (44.87-996.17)		15 (93.7%)	

*Rank-sum test was used to compare the different levels of YKL-40 in clinic pathologic feature. #Two-sided χ^2 test was performed to evaluate the distribution of high level YKL-40 in clinic pathologic feature.

Rictor and mTOR [4] and up-regulated miR-21 expression is found to be significantly associated with both cervicitis and HPV infection together with the process of CC progression [5]. Besides that, miR-24 was also reported to be able to function as a tumor suppressor through altering FSCN1 expression in nasopharyngeal carcinoma [6]. Previous research has manifested that aberrant expression of miR-24 is critical to cancer cell growth in CC [7] whereas no further studies on miR-24 and CC have been conducted.

YKL-40, also known as chitinase 3-like 1 (CHI3L1) and cartilage glycoprotein-39 (hCGP-39), encodes a glycoprotein related to the chitinase family. YKL-40 is primarily secreted by activated macrophages, neutrophils, and chondrocytes. YKL-40 is considered to play an important part in inflammation and tissue remodeling process. Apart from that, YKL-40 is thought to be a biomarker for angiogenesis and extracellular matrix degradation due to its critical role in inflammation [8, 9]. Accumulating studies revealed that high serum level of YKL-40 is related to progression and poor prognosis of cancer. Circulating YKL-40 was found to be a valuable tumor biomarker for endometrial carcinoma diagnosis [10]. It is also indicated that YKL-40 expression is up-regulated in esophageal squamous cell carcinoma [11]. Researcher observed that YKL-40 serum levels are associ-

ated with the presence of bladder cancer and poor prognosis [12] and similar results were also observed in patients with breast cancer [13]. Additionally, YKL-40 is found to be a good biomarker for predicting nodal metastasis in anal carcinoma [14]. However, the detailed mechanisms underlying the carcinogenic effect of YKL-40 have not been elucidated yet. Since YKL-40 is a potential target of miR-24 [15], we suspected that miR-24 might play an important part in YKL-40 related to carcinogenic process. In this study, we aimed at validating the association between serum YKL-40 level and CC progression and prognosis. The potential relationship between miR-24 and YKL-40 in CC cells was also assessed by this study.

Materials and methods

Study subjects

A total of 59 cervical cancer subjects who were admitted in the department of gynecology of The First Hospital of Qinhuangdao between January 2010 and September 2012 were enrolled in this study. All patients were diagnosed and confirmed by two pathologists. None of the participants had received immunotherapy, chemotherapy, radiotherapy, or hormone therapy before surgery. The histological grades and clinical stages were classified according to the International Federation of Gynecology and

Obstetrics (FIGO) grade. Forty age-matched females who participated in normal health examination in the same hospital during approximately the same time were recruited as the control group. None of them had been confirmed with cervical cancer or other cancer on enrollment. The follow-up data of all case subjects in this study were complete and available. Clinical and pathological characteristics of patients are showed in **Table 1**. This research on human subjects was approved by the ethics committee of The First Hospital of Qinhuangdao. All participants have signed the informed consent and all experiments were conducted according to relevant approved guidelines and regulations of The First Hospital of Qinhuangdao.

Serum assays

Blood samples of 3-5 mL were collected from all participants before chemoradiation or surgery and serum were stored at -80°C for experiments. Hemolysis and repeated frozen thawing were avoided for all serum samples. The concentrations of YKL-40 in serum were measured using a commercial ELISA kit (Y-Y Chemical reagent, Shanghai, China) under the manufacturer instructions.

Cell culture

Cervical cancer cells and CaSki (Epidermoid carcinoma) cell lines were obtained from Y-Y Chemical reagent (Shanghai, China). Human cervical epithelial cells were purchased from CHI Scientific, Inc (Maynard, MA, USA). Cells were cultured in DMEM high-glucose (DMEM-HG) containing 10% fetal bovine serum (FBS) and 1% Penicillin/Streptomycin and incubated in a humidified chamber with 5% CO₂ at 37°C.

Transfection and generation of stable cell lines

MiR-24 mimics and miR-24 inhibitors used in this study were purchased from Biomics Biotech (Nantong, China). Cells were transfected with miR-24 mimics, miR-NC (a scrambled mimic), miR-24 inhibitors or anti-miR-24-NC (a scrambled inhibitor) at a 50 nM concentration using oligofectamine (Invitrogen) under the manufacturer guidelines. The YKL-40 RNA interference (RNAi) sequence was designed, short hairpin RNA (shRNA)-YKL-40 sequences of 5'-AAGACTCTCTGTCTGTCGGA-3' were constructed using BLOCK-iT U6 RNAi Entry Vector kit (Invitrogen, Carlsbad, CA), and 5'-TCTCCG-AACGACTCAGACCGTG-3' sequence was served as negative control. Full-length human YKL-40 cDNA was purchased from Open Biosystems (Huntsville, AL) and transferred into pcDNA3.1 vector (Invitrogen). Then cells were transfected by pcDNA3.1/YKL-40 or YKL-40 shRNA with SuperFect® reagent (Qiagen, Valencia, CA) in accordance with the manufacturer's protocols. After 72-hour transfection, cells were harvested for measuring mRNA and protein.

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RNA extraction and real-time PCR

RNA was isolated from CaSki cells using TRIzol reagent (Invitrogen) in accordance with the manufacturer's protocols and cDNA was synthesized from the total RNA through reverse transcription. Moreover, cDNA was served as a transcription template in polymerase chain reaction (PCR) using Taq polymerase (Takara, Japan). The primers for PCR were: human YKL-40 forward, 5'-GACCAGGAAAGCGTCAAAAG-3'; reverse, 5'-GGGCAGGGAGTTGAAGAAAT-3'; GAPDH (internal control) forward, 5'-AGCTGAACGGGAAGCTCACT-3'; reverse, 5'-TGC-TTAGCCAAATTCGTTG-3'; miR-24 forward, 5'-TTCTCCGGGCTGTCGATTGG-3'; reverse, 5'-CA-AGGGCTCGACTCCTGTTC-3'; U6 (internal control) forward, 5'-CTCGCTTCGGCAGCAC-3'; reverse, 5'-AACGCTTCACGAATTGCGT-3'. The relative mRNA expression of miR-24 and YKL-40 were calculated using the 2^{-ΔΔCt} method and normalized to the threshold cycle (Ct) value of the internal control.

Western blot analysis

Cells were digested in buffer with 0.25 mM HEPES, 10 mM NaF, 14.9 mM NaCl, 2 mM MgCl₂, 0.5% NP-40, 20 μM pepstatin A, 20 μM leupeptin and 0.1 mM PMSF. After centrifugation, cell lysate supernatant was collected for measuring protein concentration using a DC protein assay kit (Bio-Rad, Richmond, CA, USA). The proteins contained in cells supernatant were separated on a 12% SDS-PAGE and transferred onto a PVDF membrane. Then membrane was blocked with 5% skim milk at room temperature for 1 h and then was incubated with proper primary antibody anti-YKL-40 (1:1000; Quidel, CA) or anti-β-actin (1:2500; Abcam) at 4°C overnight. Appropriate peroxidase-coupled secondary antibodies were used

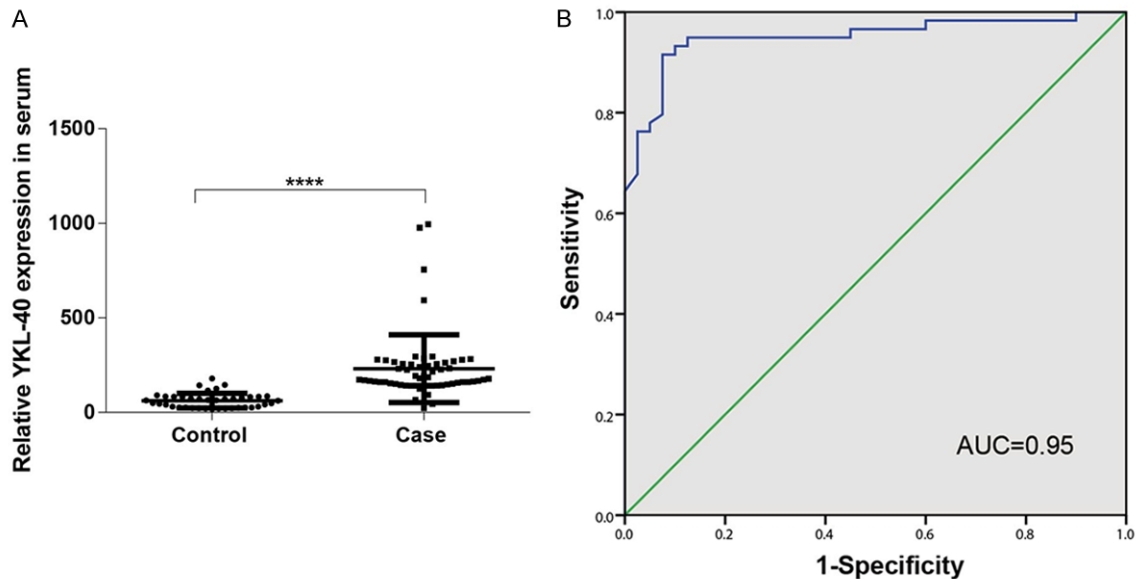


Figure 1. Expression of YKL-40 in serum of patients with cervical cancer A. Elisa assay of YKL-40 expression in serum of patients with cervical cancer and healthy control. B. Receiver operating characteristic curves for YKL-40. **** $P < 0.0001$ versus with control.

to detect antibodies, and proteins were visualized using ECL chemiluminescence detection reagents (LAS-1000, Fuji, Japan) in which β -actin was served as a control.

Luciferase reporter assay

Briefly, CaSki cells were seeded in 12-well plates (1×10^5 cells/ml) and allowed to be cultured for 12 h. Then cultured cells were co-transfected with wild-type or mutant YKL-40 3'-UTR reporter constructs and miR-24 mimic or scrambled sequences (negative control, miR-NC) using Lipofectamine 2000. The luciferase activity of cells was measured after 48-hour transfection using the Dual Luciferase Reporter Assay Kit (Promega Corporation, Beijing, China) in accordance with the manufacturer's protocols.

Cell proliferation assays

The logarithmic growth phase cells were seeded into 96-well plates at 2000 cells per well. Cell proliferation was assayed at 0, 24, 48, 72 and 96 h using the cell counting kit-8 (CCK-8) (Beyotime, Beijing) following the manufacturer's protocols. Briefly, 10 μ l CCK-8 was added to each well after cells have been cultured for 1, 2, 3, 4, and 5 days in the 100 μ l culture medium. After incubation for another 4 h at 37°C, optical density (OD) of each well was detected

at 490 nm. Cell viability was measured using OD CaSki cells/OD normal. Experiments were independently performed for three times.

Cell invasion assay and migration assay

Cells invasion and migration assay in vitro were performed using transwell chambers (Costar, Corning, NY, USA). For the migration assay, 100 μ L cell suspension with a density of 5×10^5 cells/mL were added to the top chamber without matrigel coating, and high-glucose DMEM supplemented with 20% FBS was placed to the lower chamber. After cells were incubated for 24 h, non-moving cells were cleared by cottons and cells that migrated to the underlying membrane surface were fixed with 4% paraformaldehyde and stained with crystal violet. Stained cells were counted for three randomly selected fields under a microscope. For cells invasion assay, cells were seeded on the upper chamber coated with Matrigel (500 ng/mL; BD, Franklin Lakes, NJ, USA). After 24-hour incubation, cells that invaded through the Matrigel were fixed and stained in a similar ways to the migration assay. Invaded cells were observed in three randomly chosen microscopic fields.

Statistical analysis

All experiments were independently performed for three times. Measurement data was

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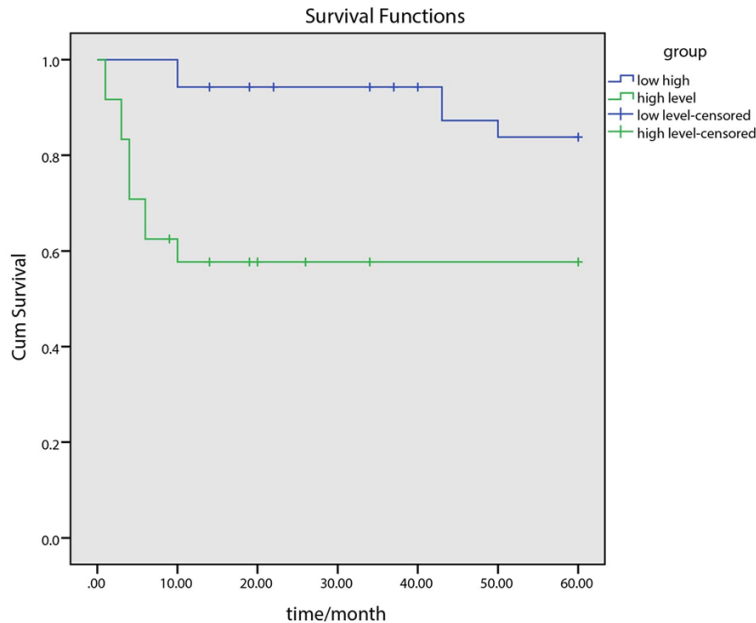


Figure 2. Overall survival curves plotted using the Kaplan-Meier method based on YKL-40 serum levels.

expressed in the form of median or mean \pm standard deviation (SD), and group differences were analyzed by the rank-sum test, t-test or two-sided χ^2 test. All statistical analysis was performed using SPSS21.0 software (IBM) and $P < 0.05$ provided evidence for statistically significant difference.

Results

Expression of YKL-40 in serum of patients with cervical cancer

We firstly measured the expression of YKL-40 contained in serum from 59 patients with cervical tumors and 40 healthy subjects to investigate the potential function of YKL-40 in CC. Compared with the control group, YKL-40 expression was significantly over-expressed in cervical tumor patients ($P < 0.001$) (**Figure 1A**) with a median of 173.8 ng/ml (21.63-996.17) in the patient group and 62.07 ng/ml (17.77-178.75) in the control group. The utility of YKL-40 serum level for detecting cervical cancer was examined using ROC analysis: YKL-40 had superior diagnostic performance for cervical cancer detection with an area under curve (AUC) of 0.95 (**Figure 1B**). The ROC curve showed that a precise cut-off value for distinguishing CC patients from healthy individuals

(133 ng/ml). As suggested by the cut-off value of 133 ng/ml, the specificity and sensitivity of YKL-40 for diagnosing CC were 92.5% and 91.5%, respectively (**Figure 1B**).

Relationship of YKL-40 level with CC clinic pathologic feature and prognosis

We then evaluated the relationships of YKL-40 expression level in serum of CC patients with clinical characteristics. Results showed that YKL-40 level was significantly associated with FIGO stages ($P = 0.003$, **Table 1**). However, there was no association between YKL-40 level and age, tumor size, lymph node metastasis or invasion. To further study the relationship

between YKL-40 and clinic pathological characteristics, we divided the CC serum samples into subgroups based on their YKL-40 expression levels. As a result, high expression of YKL-40 was evenly distributed across each clinical characteristic (**Table 1**). Kaplan-Meier survival analyses showed that CC patients with highly expressed YKL-40 had a significantly less unfavorable 5-year overall survival rate (OS) (58.33% versus 85.71%, $\chi^2 = 6.938$, $P = 0.003$) than those with low YKL-40 expressions (**Figure 2**). Furthermore, multivariate Cox regression model demonstrated that high YKL-40 expression was a significant predictor of poor prognosis (OR: 3.759; 95% CI: 1.244-11.359; $P = 0.019$).

Expression of YKL-40 and miR-24 in cervical cancer cells

RT-PCR and western blot results both revealed that the expression levels of YKL-40 mRNA and protein are higher in CaSki cell lines compared to normal cervical epithelial cells (**Figure 3A**), which suggested that YKL-40 expression was up-regulated in cervical cancer cell lines. To further explore the function of miR-24 in CC, we measured its expression in both CaSki cell lines and normal cervical epithelial cell lines. Expression of miR-24 was lower in CC cells than

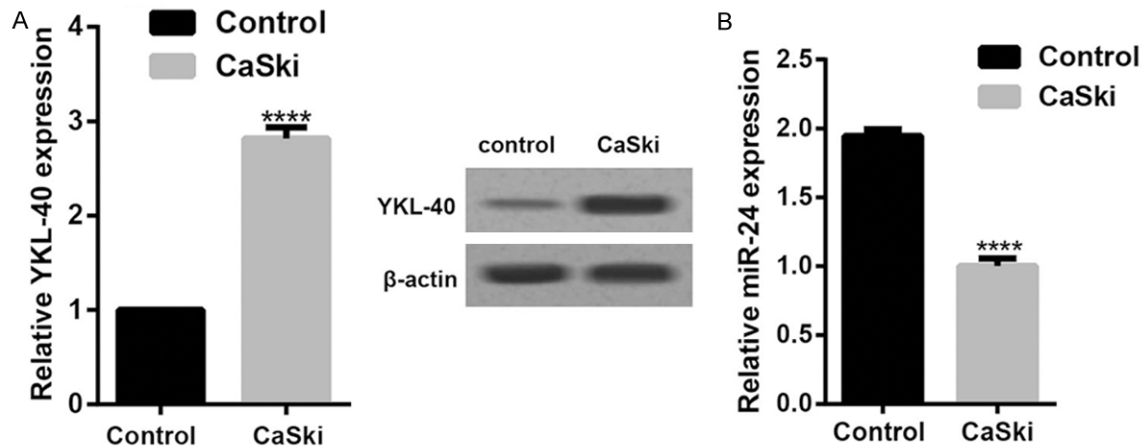


Figure 3. Expression of YKL-40 and miR-24 in cervical cancer cells. A. The expression of YKL-40 protein and mRNA in normal cervical epithelial cell and cervical cancer cell line were measured by Western blot and qPCR assay, protein expression was normalized to β -actin, mRNA expression was normalized to GAPDH. B. miR-24 expression levels were analyzed by qPCR assay, and U6 was as an internal control. **** $P < 0.0001$ when compared with control.

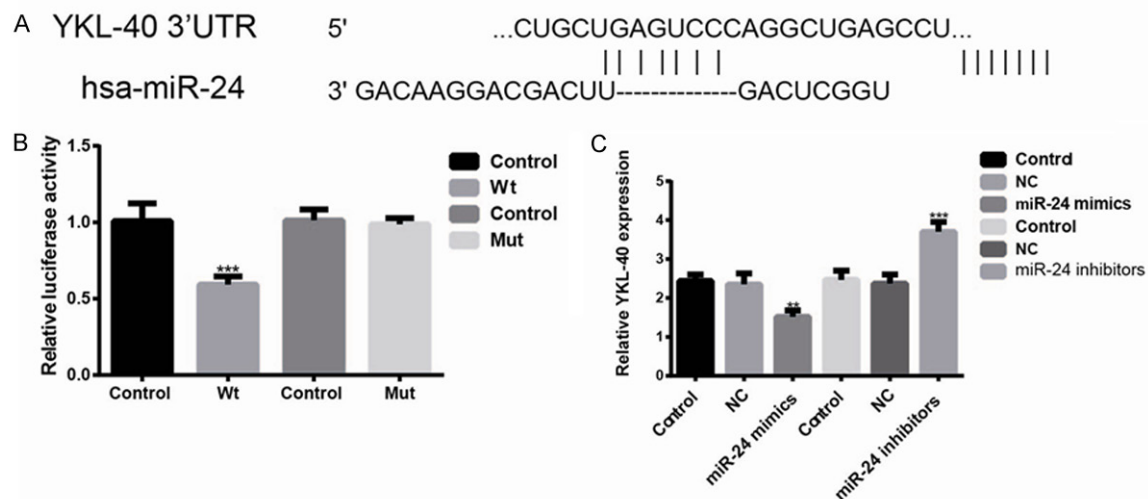


Figure 4. MiR-24 directly targets YKL-40 in cervical cancer cells. A. The locations of the predictive miR-24 target site and luciferase insert in the 3'-UTR of YKL-40. B. Relative luciferase activity of CaSki cells showed significantly reduction with insertion of the wild-type (wt) 3'-UTR of YKL-40, but not with the insertion of the mutant (mut) 3'-UTR of YKL-40. C. The mRNA level of YKL-40 was up-regulated by miR-24 inhibitor and down-regulated by miR-24 mimic; *** $P < 0.001$ when compared with control; ** $P < 0.01$ when compared with control or NC.

that in normal control cervical epithelial cells (Figure 3B). Our findings implied that miR-24 or YKL-40 might be associated with CC progression and metastasis.

MiR-24 directly targets YKL-40 in cervical cancer cells

A synthetic mimic of miR-24 or miR-NC was transfected into CaSki cells and qRT-PCR was

carried out to examine the potential relationship of miR-24 with YKL-40 expression. Results indicated that over-expression of miR-24 could effectively down-regulate YKL-40 expression compared with miR-NC ($P < 0.01$, Figure 4C). When CaSki cells were transfected with miR-24 inhibitor, YKL-40 expressions were significantly higher than that in anti-miR-NC ($P < 0.0001$, Figure 4C). This finding demonstrated that miR-24 could inhibit YKL-40 expressions in

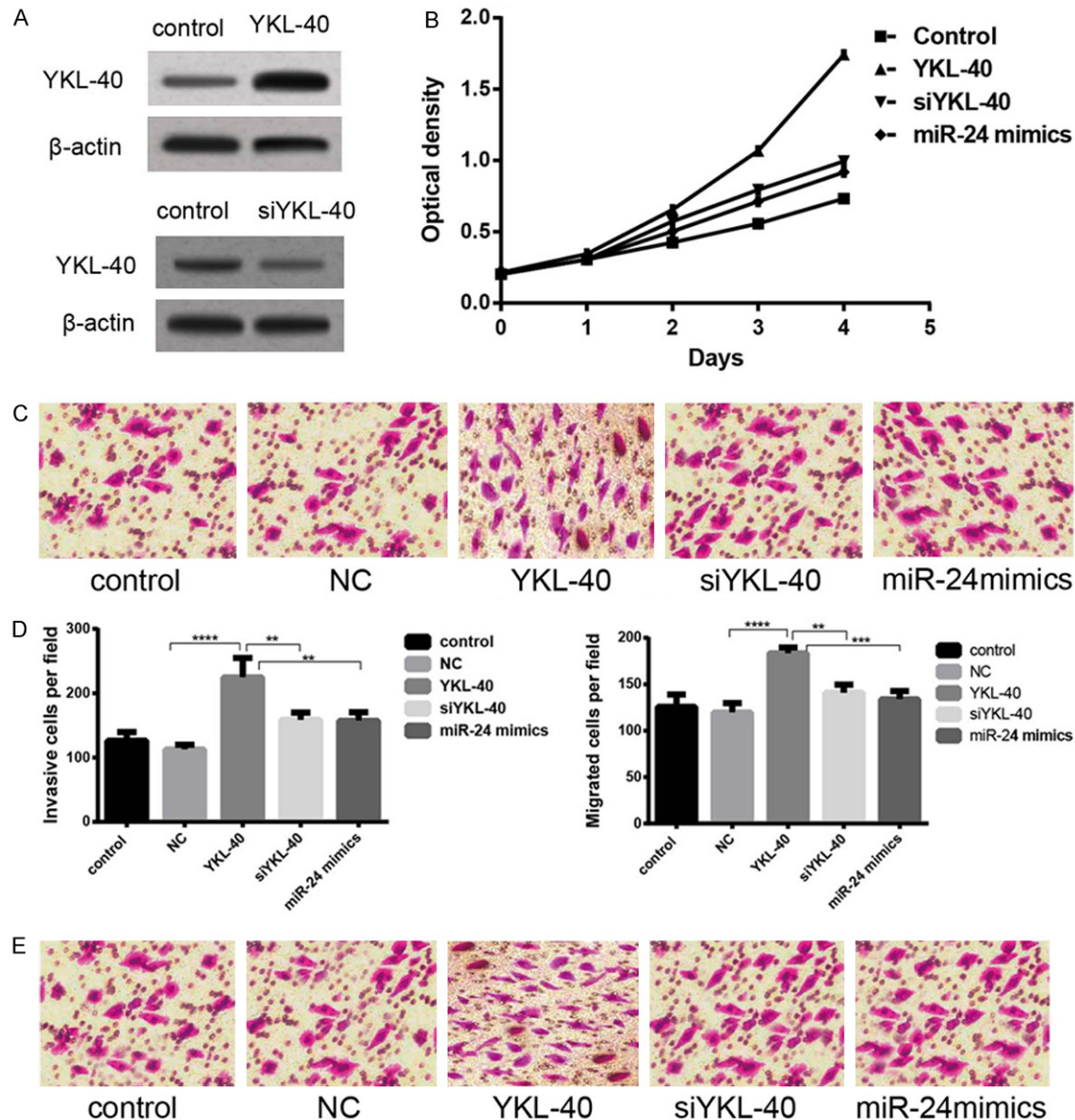


Figure 5. YKL-40 promotes proliferation, invasion and migration of CC cells while regulating by miR-24. A. YKL-40 protein levels after transfection full-length YKL-40 cDNA or short hairpin RNA (shRNA)-YKL-40. B. Cell proliferation assays were performed using a CCK-8 kit at the indicated times. C. Cell invasion of CaSki cells was evaluated using a Matrigel invasion chamber. D. Invasion cells number and migration cells number. E. Transwell migration assays. ** $P < 0.01$ when compared with control, *** $P < 0.001$, **** $P < 0.0001$.

CaSki cells. We also performed a luciferase reporter assay in order to validate whether miR-24 could directly target YKL-40. A dual-luciferase reporter vector with the wild-type YKL-40 3'-UTR (Figure 4A) was co-transfected into CaSki cells together with miR-24 mimics or miR-24 NC. As suggested by the experiment, the relative luciferase activity in CaSki cells transfected with miR-24 mimics was signifi-

cantly decreased compared with cells transfected with miR-NC ($P < 0.0001$, Figure 4B). Furthermore, the sequence of YKL-40 3'-UTR mutant was constructed to verify that the decreased luciferase activity was contributed by miR-24 binding to the 3'-UTR site of YKL-40. Results showed that the relative luciferase activity in CaSki cells transfected with the mutant reporter construct was about the same

as those in cells treated with miR-24 mimics or miR-NC (**Figure 4B**). These results indicated that miR-24 could directly bind to YKL-40 3'-UTR.

YKL-40 promotes proliferation, migration and invasion in CC cells while regulated by miR-24

CaSki cells were treated with YKL-40 and conducted with cell proliferation analyses to explore the functions of YKL-40 on CC cells. CCK-8 assays showed that over-expression of YKL-40 significantly decreased the growth ability of CaSki cells compared with the control group whereas silencing the expression of YKL-40 exacerbated the proliferation of CaSki cells (**Figure 5B**). On the other hand, over-expression of miR-24 by transfection of miR-24 mimic reversed the impact of YKL-40 on the proliferation of CaSki cells (**Figure 5B**). These results revealed that YKL-40 regulated by miR-24 exacerbated the proliferation of CC cells.

Next, we performed a Transwell assay to examine the effects of YKL-40 expression on CaSki cell invasion and migration. As shown in **Figure 5C**, YKL-40 noticeably increased CaSki cells invasion, while silencing the expression of YKL-40 significantly decreased cells invasion. Consistent results were obtained from the cell migration assay (**Figure 5E**). Over-expression of miR-24 reversed the exacerbation effects of YKL-40 on CaSki cell invasion and migration (**Figure 5C and 5E**). These results in vitro justified the hypothesis that high YKL-40 expressions were correlated with metastasis of CC cells.

Discussion

In the present study, we demonstrated that YKL-40 serum level was an efficient biomarker for CC diagnosis with relatively high sensitivity (92.5%) and specificity (91.5%). This result is consistent with previous studies on cervical adenocarcinoma, which identified a sensitivity of 78% and a specificity of 89% for diagnosing cervical adenocarcinoma [16]. CC progression begins with local cancer cell growth and it may extend to paracervical tissues. As a result, pelvic organs are likely to be invaded and cancer cells will eventually affect lymph nodes as CC progresses. Our result discovered that YKL-40 level was significantly associated with FIGO stages ($P = 0.003$), whereas no association

between YKL-40 and age, tumor size, lymph node metastasis or invasion was observed. Since significantly elevated YKL-40 expressions along with down-regulated miR-24 expressions were observed in CC cells, we suspected that YKL-40 is an oncogene whereas miR-24 is considered to be tumor suppressive.

Similar results have also been obtained in vivo or in vitro for other types of cancer. Results from luciferase reporter assay indicated that miR-24 could directly bind to YKL-40 3'-UTR and alter its expression. We also manifested that YKL-40 regulated by miR-24 exacerbates proliferation, migration and invasion of CC cells. Therefore, YKL-40 has the potential to be both an important biomarker and treatment target for CC.

MiRNAs are a group of non-coding RNAs that can regulate the expression of target genes by binding to the 3'UTR of mRNAs. In the current study, we explored the correlation between miR-24 and YKL-40. MiR-24 has been reported to participate in the carcinogenesis of cancer by targeting various proteins. For instance, elevated plasma level of miR-24 was proposed to have promising potential to serve as diagnostic biomarker for colorectal cancer [17]. A recent study provided evidence that deregulation of miR-24 is associated with tumor progression and miR-24 induces cell survival and cisplatin resistance by targeting PTEN [18]. Moreover, MiR-24 might inhibit cell growth, induce apoptosis, and reverse radio-resistance in laryngeal squamous cell carcinoma by targeting X-linked inhibitor of apoptosis proteins [19]. This study demonstrated that miR-24 could regulate the progression of CC by altering YKL-40 expression.

YKL-40 is a glycoprotein that plays an important part in the process of inflammation. Serum YKL-40 level has been found to be a potential biomarker for the diagnosis of various cancer, including endometrial cancer, renal cell cancer and non-small cell lung cancer [20-22]. Several studies have proposed that it may exert its influence by enhancing epithelial-mesenchymal transition or angiogenesis [22, 23]. Since YKL-40 is primarily secreted by activated macrophages and neutrophils, it is also suspected that it contributes to inflammation which is closely related to cancer progression. The proliferation, invasion and metastasis of tumor cells can be regulated by inflammatory media-

tors produced by cancer cells and the immune system. Apart from that, chronic inflammation induced by HPV infection may further advance to cervical intraepithelial neoplasia (CIN) or CC and YKL-40 has been observed to be up-regulated in disease related to inflammation [24-26]. Therefore, it is hypothesized that elevated YKL-40 expressions in CC tissues are closely related to inflammation and it further contributes to angiogenesis and epithelial-mesenchymal transition of CC cells.

Infection of HPV type 16 and type 18 accounts for the majority of HPV-related CC. Oncoproteins encoded by HPV type 16 and 18 may also be involved in the carcinogenesis of CC. It has also been suggested that both HPV type 16 E5 and E6 proteins could modulate the expression of host microRNAs that might be related to CC progression. Researchers observed that miR-21 was induced by HPV type 16 E6 protein whereas the expression of miR-27a and miR-218 was suppressed by HPV type 16 E6 [27]. E5 proteins have different effects on various microRNAs, for instance, E5 proteins contributes to an upregulated expression of miR-146a and a down-regulated expression of miR-324-5p [28]. Further studies are required to confirm the influence of HPV proteins on miR-24.

This is the first study concerning the potential role of miRNAs in cancer associated with YKL-40. These results enhanced our understanding of the underlying mechanism behind CC and the role of miRNAs in carcinogenesis. Limitation may arise from the fact that we did not further investigate the association of HPV infection with YKL-40 and miR-24 expressions. The CaSki cell line used in this study is HPV 16 positive and it would be interesting to assess the influence of HPV on YKL-40.

This study enabled us to validate that YKL-40 serum level is an efficient biomarker for CC diagnosis and prognosis. Over-expression of YKL-40 regulated by miR-24 is associated with CC cell proliferation, invasion and metastasis. Further studies based on our results may provide clues for novel therapeutic targets.

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Disclosure of conflict of interest

None.

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