Transcriptome changes induced by RUNX3 in cervical cancer cells in vitro

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Abstract. Runt-related transcription factor 3 (RUNX3) is a member of Runt domain family that is known to play key roles in various different types of tumor. It was recently demonstrated that RUNX3 may also be associated with cervical cancer. The aim of the present study was to investigate the potential association between transcriptome changes and RUNX3 expression in cervical cancer. A RUNX3 overexpression model was constructed using cervical cancer cell lines by RUNX3 plasmid transfection. It was demonstrated that the upregulated expression of RUNX3 inhibited proliferation of cervical cancer cell lines, particularly SiHa cells, and was associated with the expression of the IL-6, PTGS2, FOSL1 and TNF genes. In addition, it was revealed that the TNF and FoxO pathways may also be affected by RUNX3. Therefore, the expression of the RUNX3 gene may be involved in the occurrence and progression of cervical cancer.

Introduction

Cervical cancer was reported as the most common gynecological tumor in developing countries in 2012 (1). It was also estimated that 527,600 women were first diagnosed with cervical cancer and 265,700 women succumbed to this disease worldwide in the same year (1). In recent years, cervical cytology screening and vaccination have been used to prevent cervical cancer development due to human papillomavirus (HPV) infection (2). However, thousands of women are still affected by cervical cancer, with detrimental effects on their quality of life (3). Alongside the increased amount of research that makes the molecular mechanism underlying cervical cancer more clear, particularly with the knowledge that HPV and E6 oncoproteins potentially affect the progress of cervical cancer, an increasing number of genes have been demonstrated to be implicated in cervical cancer (4,5). Identifying new molecular targets for the prevention and treatment of cervical cancer would further improve the quality of life of the patients.

Runt-related transcription factor 3 (RUNX3), along with RUNX1 and RUNX2, are members of the Runt domain family (6). RUNX3 is involved in the transforming growth factor β (TGF-β) signaling pathway, which is a key downstream effector affecting the progression of tumors (7). In a number of previous studies on gastric, hepatocellular and breast cancer, RUNX3 has been revealed to play a significant role in tumor suppression (8-11). Recent research has demonstrated that RUNX3 may also act as a tumor suppressor gene in cervical cancer (12). RUNX3 expression may be suppressed by promoter hypermethylation, gene deletions, inactivating mutations and protein mislocalization (8,11,13-16). By contrast, other researchers concluded that RUNX3 acts as an oncogene in skin, ovarian and head and neck cancer, where its expression level was observed as significantly increased (17-20). In addition, Lotem et al (21) hypothesized that this gene plays important roles in immunity and inflammation, and may affect the development of epithelial tumors.

In a previous study, it was observed that the polymorphisms of RUNX3 may be associated with cervical cancer, and the mRNA expression of RUNX3 was significantly different between the cervical cancer group and the healthy female subjects (22). However, the specific molecular mechanism through which RUNX3 regulates cancer-associated signaling pathways and affects tumorigenesis remains elusive. The aim of the present study was to investigate the effect of RUNX3 on cervical cancer cell lines, and to identify the transcriptome changes in cervical cancer.

Materials and methods

Cell culture. SiHa, HeLa and C33A cells were obtained from the Laboratory of Molecular Translational Medicine,
West China Institute of Women and Children's Health, Key Laboratory of Obstetric & Gynecological and Pediatric Diseases and Birth Defects of Ministry of Education, West China Second University Hospital, Sichuan University (Sichuan, China). SiHa is an HPV-16 infected cervical cancer cell line, HeLa is an HPV-18 infected cervical cell line, and C33A is an HPV-negative cervical cancer cell line. The cells were cultured in DMEM high-glucose medium at 37°C in 5% CO₂, supplemented with 10% fetal bovine serum and penicillin/streptomycin (50 U/ml) (both Thermo Fisher Scientific, Inc.). There was no contamination of SiHa and C33A cells by HeLa cells.

**Construction of plasmids, RNA interference and transfection.** Homo sapiens RUNX3 mRNA sequence (23) was used to design and identify the RUNX3 overexpression model and empty plasmid (plasmid EX-NEG-M98, Guangzhou Fulengen Co., Ltd.), was used as the control; in addition, RUNX3 short-hairpin RNA (shRNA) was designed and identified (Guangzhou Fulengen Co., Ltd.). The following base pairs of shRNA (shRNA31, shRNA32, shRNA33 and shRNA34) and the non-targeting sequence control shRNA (shRNA001) were used for RUNX3 gene interference (Table I). After the single clones were obtained, the extracted plasmids were identified and sequenced via digestion, following the instructions of endotoxin-free plasmid extraction kit (Tiangen Biotech Co., Ltd.). Briefly, 8 ml buffer P1 with RNase A was added to the collected bacteria and vortexed. Subsequently, 8 ml buffer P2 was added, and the samples were shaken gently and left to stand at 25°C for 5 min. This was repeated with buffer P4, after which the samples were centrifuged at 8,228 g for 10 min at 25°C. Following which, 0.3 of the volume of isopropanol and 2.5 ml buffer BL was added, and the samples were transferred to CP6 columns and centrifuged at 8,228 g for 2 min at 25°C, twice. A total of 10 ml buffer PW with absolute ethanol was added and the samples were centrifuged at 8,228 g for 2 min at 25°C, twice. Subsequently, cervical cancer cells were transfected with RUNX3 overexpression plasmid or shRNA (0.5 µg plasmid) by X-tremeGene HP DNA Transfection Reagent (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference (Roche Diagnostics). In the present study, cells with overexpression model and RUNX3 shRNA interference

**RNA isolation and reverse transcription-quantitative PCR (RT-qPCR) analysis.** Total RNA was extracted from cervical cancer cells and purified using TRIzol® reagent according to the manufacturer's protocol (Thermo Fisher Scientific, Inc.). RT-PCR was performed using a One-Step RT-PCR kit (Bioneer), according to the manufacturer's protocol. RT-qPCR was performed using the SYBR Green PCR Master Mix (Roche Diagnostics). The samples of all genes were amplified in a thermocycler as follows: 95°C for 10 min (1 cycle), 95°C for 15 sec and 60°C for 1 min (48 cycles). The primer sequences are presented in Table II. Data were normalized against β-actin expression with the comparative quantification cycle method. Triplicate Cq values were averaged and the relative expression levels were determined as 2^ΔΔCq (24).

**WST-1 measurement and flow cytometry.** WST-1 measurement was used to detect cell viability using a WST-1 cell proliferation and cytotoxicity assay kit (Boster Biological Technology Co., Ltd.) according to the manufacturer's protocol. Briefly, the cervical cancer cells were seeded at a density of 10^4 cells/ml into 96-well plates and incubated overnight at 37°C. Subsequently, the plasmids were transfected into cancer cells for 24/48/72 h 3 times, as aforementioned. WST-1 (10 µl) was added, followed by incubation for a further 2 h at 37°C. In order to exclude the effect of the WST-1 reagent, the same concentrations of transfection reagent were added to the cells directly when the WST-1 measurement was performed. The absorbance of cells was monitored at 450 nm.

Apoptosis was also analyzed via flow cytometry. First, the transfected tumor cells in each group were lysed with trypsin without EDTA (HyClone; GE Healthcare Life Sciences), and the cells were stained using the FITC Annexin V Apoptosis Detection kit with propidium iodide (PI; both BestBio, http://bestbio.bioon.com.cn/). Finally, for the cell cycle analysis, cells in each group were stained with PI and then analyzed by flow cytometry (Guava® easyCyte™), using nCyte v2.7 software (both EMD Millipore; Merck KGaA).

**Transcriptome sequencing.** A total of 3 µg RNA from each sample was used, which was prepared for input material of the RNA samples. Sequencing libraries were generated, using the NEBNext® Ultra™ RNA Library Prep kit for Illumina® (New England Biolabs) according to the manufacturer's protocol. The index codes were added to assign sequences to each sample. Briefly, mRNA was purified from total RNA using poly-T oligo-attached magnetic beads NEBNext® Ultra™ Directional RNA Library Prep kit for Illumina® (New England Biolabs, Inc.) according to the manufacturer's protocol. Fragmentation was then performed using divalent cations at increasing temperatures of 25°C for 10 min, 42°C for 15 min, and 70°C for 15 min after which the samples were held at 4°C, in NEBNext First-Strand Synthesis Reaction Buffer (5X) (New England Biolabs, Inc.). Finally, the PCR products were purified (AMPure XP system), and the library quality was assessed on the Agilent Bioanalyzer 2100 system. According to the manufacturer's instructions, clustering of the index-coded samples was performed on a cBot Cluster Generation System, which used a TruSeq PE Cluster kit v3-cBot-HS (Illumina, Inc.). The library preparations were then sequenced on an Illumina Hiseq 2000/2500 platform, and 100/50 bp single-end reads were generated. Gene Ontology (GO; http://geneontology.org) enrichment analysis of differentially expressed genes was implemented by the GOseq R package (clusterProfiler v3.4.4; http://bioconductor.org/), in which gene length bias was corrected. GO terms with corrected P<0.05 were considered significantly enriched by differentially expressed genes. The Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg) is a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism and the ecosystem, from
molecular-level information, particularly from large-scale molecular datasets generated by genome sequencing and other high-throughput experimental technologies. The present study used clusterProfiler v3.4.4 software (http://bioconductor.org/) in order to test the statistical enrichment of differentially expressed genes in the KEGG pathways. Through this analysis, different genes were identified as potential regulators of cervical cancer, which may be downstream or upstream of \(\text{RUNX3}\).

**Statistical analysis.** GraphPad Prism software (version 5.01; GraphPad Software, Inc.) was used for the data analysis and to assess the normal distribution and equal variance of all data. The baseline characteristics of the participants were assessed by Student's t-test and single-factor Pearson's \(\chi^2\) test. Only the difference between two groups were evaluated by Student's t test. Differences among multiple groups were evaluated by the one-way ANOVA, followed by Bonferroni's multiple comparisons test. The \(\text{RUNX3}\) mRNA expression levels were compared between transfected cells, or between groups of cervical cancer cells and controls using Bonferroni's multiple comparisons test. \(p<0.05\) was considered to indicate a statistically significant difference. According to transcriptome sequencing, differential expression analysis of two groups was performed using the DESeq (DESeq) R package (v1.10.1; https://www.bioconductor.org/). DESeq provides statistical analysis for determining differential expression in digital gene expression data using a model based on the negative binomial distribution. The resulting P-values were adjusted using the Benjamini and Hochberg's approach for controlling the false discovery rate. Genes with an adjusted P-value (q value) found by DESeq were classified as differentially expressed. q<0.05 was considered to indicate a statistically significant difference.

**Results**

**Expression of \(\text{RUNX3}\) in cervical cancer lines.** The efficiency of exogenous \(\text{RUNX3}\) expression and \(\text{RUNX3}\) shRNA in cervical cancer cells was verified via RT-qPCR analysis. The mRNA levels of \(\text{RUNX3}\) were markedly higher in ORF \(\text{RUNX3}\) cells compared with the NEG control group of the three cervical cancer cell lines (\(p<0.001\); Fig. 1), which were also extremely significantly higher than the 'Control' and 'cell name' groups (\(p<0.001\); Fig. 1). It also demonstrated the low expression levels of \(\text{RUNX3}\) in all the control groups, with or without intervention. In addition, the shRNA \(\text{RUNX3}\) and control groups (shRNA001) were significantly different in the three cell lines, particularly in SiHa cells (\(p<0.001\) and \(p<0.05\), respectively; Fig. 2). As the \(\text{RUNX3}\) gene was successfully suppressed by shRNA34 in SiHa, HeLa and C33A cells, particularly the SiHa cell line at 24 (\(p<0.001\)) and 48 h (\(p<0.05\)), shRNA34 was selected as the interference plasmid for subsequent experiments (Fig. 2).

### Table I. shRNAs used in the present study.

| Clone name | Symbol | Chromosome location | Length | 5'-3' |
|------------|--------|---------------------|--------|-------|
| HSH021393-31-LVRH1GP(OS503663) | RUNX3 | 815 | 21 | GGCAATGACGAGAACTACTCC |
| HSH021393-32-LVRH1GP(OS545091) | RUNX3 | 159 | 21 | GGAATCCAAATTCTTGGGTAC |
| HSH021393-33-LVRH1GP(OS503664) | RUNX3 | 2895 | 21 | GTTCCTTACAGGTATAGTTC |
| HSH021393-34-LVRH1GP(OS545092) | RUNX3 | 3858 | 21 | GGGATAGTAAATATAATGTGCT |
| CSHCTR001-1-LVRH1GP(OSNEG20) | RUNX3 | 19 | | GCTTCGCACCCGTAGTCTTA |

shRNA, short hairpin RNA; \(\text{RUNX3}\), runt-related transcription factor 3.

### Table II. Primer sequences used in the present study.

| Product | Gene | Gene ID | Forward (5'-3') | Reverse (5'-3') | Product size, bp |
|---------|------|---------|----------------|----------------|-----------------|
| TNC     | NM_002160.3 | TCGCTACAAGCTGAAGGTGG | GTTAACGCCTGCCTGTTG | 214 |
| PTGS2   | NM_000963.3 | CAAATTGCTGCGAGGTTGC | AGGGCTTCAGCATAAAGCGT | 139 |
| ICAM1   | NM_000201.2 | ATGCGAAACACTCCCTTCTCG | GCCGAAACAGTGATAGTAC | 142 |
| TNF SF10| NM_001199042.1 | TGGCTTGCTATCGTGTATCTT | TCTTGGAGCTCTTCTAAGGAG | 234 |
| IL6     | NM_000600.3 | TTCAATGAGGAGACCTTGCGTTG | CTGGCATTTTGTTGTTGCT | 206 |
| IL7R    | NM_002185.3 | TGAAATATTGCGGGCCCTCG | GTCACTGCTTCCTCCCGAT | 223 |
| FOSL1   | NM_005438.4 | AGCCACGCAAGTTCCCAC | CCTCTTCCTCCGGGCTGAT | 227 |
| IL32    | NM_001308078 | AGACAGTGGCGCGCTTATATGAGG | GCCCTCGACCGTAATCC | 86 |
| TGF-β   | NM_000660.4 | TATGCAGATGAGGCTGTTGAGG | CAGCCTGAGCAAGGCTGTCG | 67 |
| β-actin | NM_001101.3 | TGACGCTGGACATCCGCAAG | CTGGGAAGTGAGACGCGAGG | 205 |
Role of RUNX3 in proliferation and apoptosis of cervical cancer cells. The WST-1 results suggested that there were significant differences among ORF and NEG plasmid transfection groups and shRNA34 and shRNA001 groups in the SiHa cell line (P<0.05; Fig. 3A). However, the shRNA34 and shRNA001, and ORF and NEG groups exhibited less prominent differences in HeLa and C33A cell lines, respectively, although the results were still significant; (Fig. 3B and C).
There was no significant difference between the groups of cervical cancer cells with WST-1 or without WST-1 (Fig. 3). In addition, the results revealed that there were significant differences at different transfection times; the ORF and NEG groups were significantly different at 24, 48, 72 and 96 h in the SiHa cell line, the shRNA34 and shRNA001 groups were significantly different at 72 and 96 h in the HeLa cell line and the ORF and NEG groups were significantly different at 24, 48, 72 and 96 h in the C33A cell line.

RUNX3 mRNA expression levels in shRNA RUNX3 and control groups (shRNA001) differed significantly in SiHa, HeLa and C33A cell lines, particularly between the shRNA34 and shRNA001 groups in SiHa cell. *P<0.05 and **P<0.001 vs. shRNA001. RUNX3, Runt-related transcription factor 3; shRNA, short hairpin RNA.
48 and 96 h in the C33A cell line (P<0.05). The results also demonstrated that the effects on different cervical cancer cells varied and indicated that RUNX3 may inhibit the proliferation of cervical cancer cells.

PL/Annexin V flow cytometry analysis was performed in the present study in order to detect the apoptosis rates in RUNX3-transfected cell lines. The apoptosis rates of different cervical cancer cell groups were significantly different, especially in SiHa cells (Fig. 4). The apoptosis rate of the ORF group was significantly higher compared with that in the control group in SiHa cells at 24, 48 and 72 h (P<0.001), indicating an apoptosis-promoting role of RUNX3 in cervical cancer. Furthermore the apoptosis rate of the shRNA34 group was significantly higher compared with that in the control group at 24, 48 and 72 h, which were inconsistent as expected (P<0.001, Fig. 4). In addition, the apoptosis ratios of HeLa and C33A cells were not significantly higher in the ORF group compared with that in the control group at 24 and 48 h, which were not consistent with those of the SiHa cells in ORF and control group, but in the 48 and 72 h time periods, shRNA34 group was significantly higher compared with that in the control group, in both the HeLa and C33A cells, which is consistent with the SiHa cells (P<0.05; Fig. 4). Finally the apoptosis ratios of ORF group were significantly lower compared with that in the shRNA34 group at 48 h in SiHa, HeLa and C33A cell lines (P<0.05; Fig. 4), and at 72 h in SiHa and HeLa cell lines (P<0.05; Fig. 4). The results may suggest the low expression of RUNX3 in cervical cancer is complexity to interfere and further research should be preceded.

**Effects of RUNX3 on cervical cancer cells by transcriptome sequencing.** The molecular mechanisms through which RUNX3 may affect cervical cancer were investigated in SiHa cells in vitro. Relative transcript levels were tested by transcriptome sequencing in order to determine the differences in transcripts mediated by RUNX3 in SiHa cells. The present study identified 31 genes that were differentially expressed in RUNX3-overexpressing SiHa cells. A total of 9 genes showed differences in the three groups simultaneously, including *IL-6*, *PTGS2*, *FOSL1*, *TNC*, *ICAM1*, *IL-7R*, *IL-32*, *TGF-β* and *TNFSF10* (Figs. 5 and 6), which may be potential regulators of cervical cancer combined with RUNX3. The mRNA expressions of the 9 genes confirmed the results of the transcriptome sequencing (Fig. 7), although further research is required to clarify the connection between RUNX3 and these genes. However, in the shRNA34 groups, except with the *TNC* and *IL-6* gene, the expression levels of these genes were not changed, which may be due to the low expression of RUNX3 mRNA in cervical cancer and limited interference of shRNA34. Additional research is required in order to develop an improved understanding. Furthermore, following verification of the mRNA expression levels, there was no significant difference between the control groups. In addition, the GO enrichment analysis of biological processes revealed that certain genes were enriched, such as those involved in the regulation of carboxypeptidase, exopeptidase and hydrolase activity. Furthermore, the GO enrichment analyses of the KEGG pathways concluded that these genes were enriched in signaling pathways such as the tumor necrosis factor (*TNF*) pathway, Forkhead box O (*FoxO*) pathway, African trypanosomiasis and malaria (Fig. 8). Transcription factors and proto-oncogenes, such as *HMGA1* and *FOSL1*, *IL7R* and *MALAT1*, were demonstrated as likely to be associated with RUNX3 in cervical cancer (Fig. 6) and may be associated with microfollicular thyroid adenoma, various benign mesenchymal tumors and renal cell carcinoma (25,26). Finally, the transcriptome sequencing analysis results indicated that two long non-coding RNAs, *RP11-5407.3* and *MALAT1*, were significantly different between groups, suggesting that RUNX3 may affect their expression level (Fig. 6). These findings indi-
cate that the expression of RUNX3 may affect the expression of other genes, which may also be associated with cervical cancer in vitro.

Discussion

Cervical cancer has been confirmed to be associated with HPV infection; furthermore, 0.7% of women initially found to be infected with high-risk HPV will develop invasive carcinomas within 3 years (2,3). In recent years, immunological mechanisms and genetic factors have been demonstrated to play critical roles in cervical cancer (4,27-30). A previous study demonstrated that RUNX3 is likely a potential gene involved in cervical cancer susceptibility, and was associated with the type of HPV infection and cervical intraepithelial neoplasia progression (22). In the present study, three types of cervical cancer cell lines were selected: SiHa, HeLa and C33A, infected by HPV-16, HPV-18 or not infected, respectively. It was recently reported that RUNX3 may play a key role in the development and progression of cervical cancer (12). It was also observed in the present study that RUNX3 may inhibit cervical cancer cell proliferation, particularly in the SiHa cell lines, indicating its potential function as a tumor suppressor; inconsistencies in these results may be due to the different cell lines. In addition, the expression levels of RUNX3 were lower in the three cell lines, which were almost the same level in the control groups with or without any intervention. The molecular mechanisms and signaling pathways underlying the role of RUNX3 in cervical cancer remain elusive. The aim of the present study was to elucidate the potential effects of RUNX3 on cervical cancer by transcriptome sequencing, which may help improve the current therapy options and prognosis of patients with cervical cancer.

It was previously demonstrated that the tumorigenicity of human gastric cancer cell lines was significantly associated with the level of RUNX3 in nude mice, which suggested that suppression of RUNX3 function was directly associated with the occurrence and progression of human gastric cancer (9). In a previous study on ovarian cancer, the researchers observed that the overexpression of RUNX3 in A2780s cells rendered them more resistant to carboplatin, whereas the sensitivity of
A2780cp cells to carboplatin increased significantly following inhibition of RUNX3 (31). In the present study, the upregulated expression of RUNX3 consistently inhibited the proliferation and promoted the apoptosis of cervical cancer cells, particularly in the SiHa cell line. The results were similar to those of a recent study on cervical cancer, which reported that upregulated expression of RUNX3 inhibited the proliferation, migration and invasion of cervical cancer cells (12). According to these results, it may be concluded that RUNX3 acts as a tumor suppressor gene in cervical cancer (12).

As the mechanisms of action of RUNX3 have not yet been fully elucidated, the molecular mechanisms and signaling pathways of RUNX3 have become a focus in cancer research. It is generally recognized that the transcription factor RUNX3, which is a key effector of the TGF-β signaling pathway, acts on the TGF-β receptor, thereby promoting cell proliferation and apoptosis through the TGF-β signal transduction pathway (7,10,32-34). This may explain its wide involvement in tumorigenesis (35). However, several other signaling pathways are affected by RUNX3. The Wnt signaling pathway was confirmed to be associated with RUNX3 in intestinal tumorigenesis (36) through the formation of a ternary complex with β-catenin/TCF4 and attenuation of Wnt signaling activity, whereas the inactivation of RUNX3 may promote intestinal adenoma formation (36). In addition, the mitochondria-mediated pathway has been demonstrated to be associated with RUNX3, inducing apoptosis in gastric cancer cells (37). In a study on hepatocellular carcinoma (HCC), the researchers reported that RUNX3 suppressed Notch signaling in HCC SMMC7721 cells (38). In the present study, the TNF and FoxO signaling pathways were demonstrated to be associated with RUNX3.

TNF is a member of the TNF superfamily of cytokines, which mediates cell processes such as differentiation, inflam-
Figure 7. mRNA expression levels of different genes were partly consistent with the results of transcriptome sequencing in cervical cancer cells, which were all confirmed in SiHa, HeLa and C33A cell lines. *P<0.05. ORF, RUNX3 overexpression cells; RUNX3, Runt-related transcription factor 3; shRNA, short hairpin RNA.

Figure 8. Signaling pathways potentially involved in RUNX3-overexpressing cells with Kyoto Encyclopedia of Genes and Genomes enrichment analysis. The size of the point indicates the number of differentially expressed genes in the pathway, and the color of the point corresponds to a different q value range. RUNX3, Runt-related transcription factor 3; q value, P-adjusted value.
mation, proliferation and apoptosis (39), and has double the effect in cancer cells. More specifically, it has been reported that TNF is associated with cervical cancer (40). In addition, the TNF-α/TNFRI1/NIK-xB pathway is potentially implicated in tongue cancer and lung metastasis (41). The NF-xB signaling axis (defined by the interactions of NF-xB dimers, IkB regulators and IKK complexes) is responsive to external stimuli and signal received (42). FoxO is a subfamily member of the forkhead transcription factor family. FoxO has been revealed as a key determinant of cell fate, and to play an important functional role as a tumor suppressor in different types of cancer (43,44). During apoptosis, FoxO is involved in mitochondria-dependent and -independent processes that trigger the expression of death receptor ligands, such as Fas ligand, TNF apoptosis ligand, Bcl XL, bNIP3 and Bim (43,44). The most important pathway associated with FoxO is the PI3K/AKT pathway. The PI3K/AKT pathway is also dysregulated and activated in a wide variety of cancers, such as breast, thyroid and cervical cancers (43).

In conclusion, the present study demonstrated that RUNX3 may affect the expression levels of RPII-5407.3 and MALAT1. According to previous reports, MALAT1 may participate in tumor formation, such as lung, prostate and ovarian cancer (50-53). It was recently reported that overexpression of MALAT1 could sponge miR-429 and regulate cervical cancer pathogenesis in vivo and in vitro (54), while the associations between MALAT1 and RUNX3 remain unclear. However, the role of RPII-5407.3 has not yet been fully elucidated, and the association between RPII-5407.3 and RUNX3 remains unknown (55,56). These results indicate that RUNX3 may affect the expression of IncRNAs, which may be associated with cervical cancer in vitro. The specific mechanisms of action and role of RUNX3 in cervical cancer will be further investigated in future studies.

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Availability of data and materials

All data generated or analyzed during the present study are included in this published article.

Authors' contributions

XY, ZZ and YS contributed to analysis and manuscript preparation. QG and MX performed the data analyses and wrote the manuscript. BZ, YW, LZ and HL helped perform the analysis with constructive discussions.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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