Abstract. – OBJECTIVE: To investigate the role of long noncoding RNA (lncRNA) UCA1 in the multiple myeloma (MM) development.

PATIENTS AND METHODS: In samples of MM, the expression of UCA1 and TGF-β was investigated using real-time PCR. UCA1 lentiviral small hairpin RNA (shRNA) was transfected in MM cell lines. CCK-8 and colony formation assay were used to detect cell lines proliferation. The cell apoptosis assay was conducted to detect cell apoptosis. Western blot was utilized to detect the protein level of TGF-β.

RESULTS: The expression level of UCA1 increased in MM samples and cell lines, and its high expression was associated with poor MM prognosis. Downregulation of UCA1 significantly inhibited cell lines proliferation and promoted cell apoptosis. UCA1 could positively regulate TGF-β in MM. Overexpression of TGF-β partially reversed the effect of UCA1 knockdown.

CONCLUSIONS: UCA1 promotes MM cell lines proliferation by targeting TGF-β.

Key Words: UCA1, TGF-β, Proliferation, Apoptosis, Multiple myeloma.

Introduction

Multiple myeloma (MM), with a poor average 5-year overall survival rate, originates from the malignant transformation of plasma cells. The incidence of MM varies according to ethnicity, which is lower in Asians than that in Caucasians. Recently, the incidence of MM is reported to increase in some Asian countries. Therefore, it is urgent to understand the mechanisms underlying the pathogenesis of MM. Previous studies show that molecular expression aberrations play an important role in the regulation of cellular function including differentiation, proliferation, and apoptosis. The exploration of a novel functional molecule could provide a more effective therapeutic target for MM.

Long noncoding RNAs (lncRNAs), although have no protein-coding capacity, have an abnormal molecular expression in the biological processes of various diseases. LncRNA XIST was reported to be upregulated and promote the cell growth in nasopharyngeal carcinoma. Cell proliferation and metastasis are enhanced in bladder cancer after lncRNA CASC2 is knocked down. LncRNAs was also found to play a crucial part in the progress of MM development. For instance, lncRNA MALAT1 functions as an oncogene in the development of MM through targeting LTBP3 gene. LncRNA MEG3 promotes differentiation of MM cells by targeting BMP4. A recent study discovers that lncRNA UCA1 was dysregulated in MM (Sedlarikova L, Gromesova B, Kubaczkova V, Radova L, Filipova J, Jarkovsky J, Brozova L, Velichova R, Almasi M, Penka M, Bezděkova R, Stork M, Adam Z, Pour L, Krejci M, Kuglik P, Hajek R, Sevcikova S. Deregulated expression of long non-coding RNA UCA1 in multiple myeloma. Eur J Haematol 2017; 99: 223-233). However, it remains unclear how UCA1 plays its underlying role in MM development.

Patients and Methods

Clinical Sample

60 MM samples were collected from the MM patients, and 15 healthy control samples were also collected in our hospital. This study conforms to requirements of the Ethics Committee of the Affiliated Hospital of Weifang Medical University. The patients had provided the written informed consent.

Cell Transfection

Supplemented with 10% fetal bovine serum (FBS – Life Technologies, Carlsbad, CA, USA), 1640 Medium (Life Technologies, Carlsbad, CA, USA) was utilized to cultivate multiple myeloma cell lines (MM1.S, NCIH929, U266, and RPMI-8226) and a normal plasma cell line (nPCs) in 5% CO2 at 37°C. After synthesized, UCA1, which has been targeted by lentiviral small hairpin RNA,
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was cloned into the pLenti-EF1a-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA). UCA1 shRNA (sh-UCA1) and the empty vector (control) were packaged by 293T cells. Then, MM cells were transfected with sh-UCA1 and empty vector (control). After synthesized, lentivirus targeting TGF-β was cloned into the pLenti-EF1a-E-EGFP-F2A-Puro vector (Biosettia Inc., San Diego, CA, USA) according to the manufacturer’s instructions. Also, 293T cells were used to package TGF-β virus (TGF-β) and the empty vector (EV). Then, MM cells were transfected with TGF-β virus (TGF-β) and empty vector (EV).

**RNA Isolation and Real-time PCR**

The total RNA was separated by TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed to cDNAs via reverse Transcription Kit (TaKaRa Biotechnology Co., Ltd., Dalian, China). The expression level of UCA1 and TGF-β were detected by SYBR Green Real-time PCR. GAPDH was used for normalization. And ABI PRISM7500 system (Applied Biosystems, Foster City, CA, USA) was used to perform Real-time PCR assay.

**Cell Counting Kit-8 Assay**

In a 96-well plate, cell lines were seeded 4 × 10^3 cells per well, CCK-8 reagent (Dojindo, Tokyo, Japan) was respectively added to the wells at 0, 24, 48, and 72 h according to the instructions and, then, the plate was incubated for 2 h at 37°C. OD (optical density) value was examined using a microplate reader (Bio-Rad, Hercules, CA, USA).

**Colony Formation Assay**

After cultured with FBS for 14 days, methanol and 0.1% crystal violet was utilized to fix and stain the cells. Meanwhile, for comparison, the number of colonies was calculated.

**Cell Apoptosis Analysis**

Annexin V-APC/7-AAD Apoptosis Detection Kit II (KeyGEN BioTESSCH Co., Ltd, Nanjing, China) was used to estimate the apoptosis rate of osteoblast and Cell Quest software (BD Biosciences, San Diego, CA, USA) programmed Flow cytometry (FACScan, BD Biosciences, San Diego, CA, USA) was used to perform the comparison. The test was repeated at least 3 times.

**Western Blot Assay**

The protein collected from the cell lines was examined using bicinchoninic acid assay (BCA) Protein Assay Kit (Beyotime, Shanghai, China). The polyvinylidene difluoride (PVDF) membranes were blocked in 0.1% Tween 20 containing 5% BSA. The antibodies that utilized to against GAPDH and TGF-β were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). And before analyzed by ECL kit (Thermo Scientific Pierce, Thermo Fisher Scientific, Waltham, MA, USA), the secondary antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA) was diluted to 1:2,000 (v/v) in phosphate-buffered saline (PBS) and 0.1% Tween 20 for 1 h, and then.

**Statistical Analysis**

Statistical analyses were performed by Statistical Product and Service Solutions (SPSS) 17.0 (Chicago, IL, USA). Date was presented as mean ± SD. Chi-square test, Student t-test and Kaplan-Meier method were selected when appropriate. p<0.05 was considered statistically significant.

**Results**

**UCA1 Expression Was Increased Both in MM Cell Lines and Tissues**

First, Real-time PCR assay was used to detect UCA1 expression in the MM tissues and normal tissues. High-expression of UCA1 was detected in the MM tissues as compared with the normal samples (Figure 1A). Moreover, the expression of UCA1 was also up-regulated in MM cell lines when compared to a normal cell line (Figure 1B). Furthermore, Kaplan-Meier analysis and log-rank test was utilized to evaluate the interrelation within UCA1 expression and MM patients’ prognosis. We found out that patients with higher expression level of UCA1 had poorer overall survival rate than the ones with low expression (Figure 1C). Collectively, high-expressed UCA1 plays a crucial role in the development of MM.

**Downregulated UCA1 Could Suppress Cell Lines Proliferation**

Then, MM cell NCIH929 was transfected with UCA1 shRNA and empty vector (control). The efficacy was detected by Real-time PCR assay (Figure 2A). CCK-8 assay and colony formation assay were performed to explore the effect of UCA1 on the proliferation of MM cell lines. Results of CCK-8 assay showed that after downregulation of UCA1, there was a significant decrease in proli-
Downregulated UCA1 Could Induce Cell Lines Apoptosis

Cell apoptosis assay was performed to detect the role of UCA1 played on MM cell lines apoptosis. Results showed that after UCA1 was knocked down, the apoptosis rate of these treated cells had a significant increase (Figure 2D).

Figure 1. Expression levels of UCA1 were increased in MM tissues and cell lines, and were associated with poor overall survival of MM patients. (A) UCA1 expression was significantly increased in the MM tissues compared with normal tissues. (B) Expression levels of UCA1 relative to GAPDH were determined in the human MM cell lines and a normal plasma cell line (nPCs) by RT-qPCR. (C) High level of UCA1 was associated with poor overall survival of MM patients. Data are presented as the mean ± standard error of the mean. *p<0.05.
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UCA1 Could Regulate TGF-β in MM Cells

TGF-β may act as an oncogene in MM via suppressing apoptosis and promoting adhesion in MM carcinogenesis. TGF-β may be a prognostic factor and potential target for MM treatment. In our study, Real-time PCR assay was used to detect the expression level of TGF-β in both MM and normal tissues. As compared to the normal tissues, TGF-β was up-regulated in the MM tissues (Figure 3A). Furthermore, a positive correlation was observed between UCA1 and TGF-β (Figure 3B). To further confirm the mechanism of TGF-β and UCA1, we detected the mRNA and protein expression of TGF-β with down-regulation of UCA1 expression. The results showed down-regulation of UCA1 could decrease TGF-β expression (Figure 3C and Figure 3D). Based on the above findings, we illustrated that UCA1 could positively regulate TGF-β.

Overexpression of TGF-β Expression Could Reverse the Effect of UCA1 Inhibition

To explore whether overexpression of TGF-β could influence the cell line proliferation of UCA1 shRNA, the CCK-8 assays showed that the inhibition effect was reversed when overexpressing TGF-β in cells transfected with UCA1 shRNA (Figure 4A). The cell apoptosis assay showed that the promotion effect was reversed when overexpressing TGF-β in cells transfected with UCA1 shRNA (Figure 4B). In sum, this finding indicated that the potential role of UCA1 in MM development depends on regulating its target gene TGF-β.

Discussion

The previous studies had reported that IncRNAs contribute to the progression of MM. Sun
et al\textsuperscript{11} reported that downregulated lncRNA H19 mediates NF-kappa B pathway and suppresses MM cell growth. LncRNA MALAT-1 functions as an oncogene in MM to inhibit cell apoptosis in MM\textsuperscript{12}. LncRNA CCAT1 modulates HOXA1 expression and further promotes MM progression by sponging miR-181a-5p\textsuperscript{13}. UCA1 has been found to influence the molecular biology of different cancers. UCA1 targets miR-16 to induce imatinib resistance in chronic myeloid leukemia cells\textsuperscript{14}. UCA1 is up-regulated in esophageal cancer and promotes cell lines proliferation via SOX4\textsuperscript{15}. In patients with non-small cell lung cancer, down-regulated UCA1 indicates a better prognosis and acts as a ceRNA by targeting miR-193a-3p\textsuperscript{16}. A recent report reveals that UCA1 is up-regulated in MM, but its function in MM remains unknown.

In our study, we determined the molecular mechanism of UCA1 in MM cell lines proliferation. Firstly, from the clinic samples with MM, we found the expression level of UCA1 was up-regulation. Moreover, we discovered that UCA1 knockdown could suppress cell lines proliferation by performing colony formation assay and CCK-8 assay. By cell apoptosis assay, we discovered that knockdown of UCA1 could induce cell lines apoptosis.

TGF-β, an important oncogene, has been reported to function in many cancers\textsuperscript{17}. TGF-β acts as biomarkers in pancreatic cancer and can be used to predict patients’ prognosis\textsuperscript{18}. TGF-β activates PI3K/AKT/mTOR and emerges its function on prostate cancer metastasis \textit{in vitro}\textsuperscript{19}. In breast cancer, TGF-β signaling was a fatal part in carcinogenesis and metastasis\textsuperscript{20}. In our research, TGF-β expression was increased in MM samples, and had a positive association with UCA1. To further investigate whether the regulatory role of UCA1 on cell lines proliferation and apoptosis was depended on TGF-β, we performed a rescue experiment, and found that UCA1 could positively regulate TGF-β.

**Conclusions**

Taken together, we identified UCA1 is generally high-expressed in MM samples and cell lines. Meanwhile, we found that the expression level of UCA1 was usually related to TGF-β. Mechanistically, we suggested that UCA1 promoted MM cell proliferation and inhibited MM cells apoptosis by suppressing TGF-β. In future, UCA1 may be a therapeutic target in MM.

**Conflict of Interest**

The Authors declare that they have no conflict of interest.

**References**


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