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Long noncoding RNA UCA1 promotes multiple myeloma cell growth by targeting TGF-β

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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 signifi[illegible]ntly inhibited cell lines proliferation and pro[illegible] cell apoptosis. UCA1 could positively r[illegible]at[illegible] TGF-β in MM. Overexpression of TGF-β pa[illegible]ly reversed the effect of UCA1 knockdown.

CONCLUSIONS: UCA1 promotes MM cell li[illegible] proliferation by targeting TGF-β.

Key Words:
UCA1, TGF-β, Proliferation, [illegible]optosis, [illegible]iple myeloma.

Introduction

Multiple myel[illegible]MM), with a [illegible]average 5-year overall [illegible]viv[illegible]e, originates from the malignant t[illegible]ansformatio[illegible] plasma cells[1]. The incidence [illegible]MM varies a[illegible]ng to ethnicity, which i[illegible]ver in Asians than t[illegible]t in Caucasians[2]. Rece[illegible] the in[illegible]nce of MM is reported to increase som[illegible]sian co[illegible]ntries[3,4]. Therefore, it is urgen[illegible]stand t[illegible]echanisms underlying [illegible]pathog[illegible]s of [illegible]. Previous studies[5,6] show [illegible]olecul[illegible]ion aberrations play an important role in [illegible]regulation of cellular function incl[illegible] differentiation, proliferation, and apopt[illegible]oration of a novel functional module could provide a more effective therapeutic [illegible]t for MM.

noncoding RNAs (lncRNAs), although have [illegible]o protein-coding capacity, have an abnormal molecular expression in the biological processes of various di[illegible]. [illegible]NA X[illegible] was reported to be u[illegible]gulat[illegible] promo[illegible]e cell growth in nas[illegible]ryngeal [illegible]on[illegible] Cell proliferation an[illegible]stasis are en[illegible] in bladder cancer af[illegible]uc[illegible] CASC2 is k[illegible]ocked down[8]. LncRNAs [illegible]as also[illegible] to play a crucial part in the pr[illegible]s of MM d[illegible]pment. For instance, ln[illegible]ALAT1 funct[illegible]s as an oncogene in t[illegible]evelopment of MM through targeting LTBP3 [illegible]e[9]. LncRNA [illegible]EG3 promotes differentiation [illegible]M cells by t[illegible]eting BMP4[10]. A recent study d[illegible]ers that l[illegible]NA UCA1 was dysregulated in M[illegible]va L, Gromesova B, Kubaczkova V, Ka[illegible]L, Filipova J, Jarkovsky J, Brozova [illegible], Velichova R, Almasi M, Penka M, Bezdekova [illegible]M, Adam Z, Pour L, Krejci M, Kuglík P, [illegible]ajc[illegible], 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% CO_2 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-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 seed × 10^3 cells per well, CCK-8 reagent (Dojin Tokyo, Japan) was respectively a to the wel at 0, 24, 48, and 72 h accordin ructions and, then, the plate was inc d for at 37°C. OD (optical density) valu s exami g a microplate reader (Bio Ra cu

Colony Formati Assay

After culture FBS for 14 metha-nol and 0.1% al was utilized o fix and stain the c lls. Meanwh r comparison, the number c onies was ca d.

Cell optosi nalysis

in V C/7-A D Apoptosis Detection Kit I N BioT CH Co., Ltd, Nanjing, na) w d to ate the apoptosis rate of last a est software (BD Biosciences, n Diego A, USA) programmed Flow cyt (FACScan, BD Biosciences, San Die- was used to perform the compari- . The test was repeated at least 3 times.

rn Blot Assay

T 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 c 5% BSA. The antibodies that utilize GAPDH and TGF-β were purchas om Santa Cruz Biotechnology (Santa Cruz USA). And before analyzed by ECL kit (Th cientific Pierce, Thermo Fisher Scie ic, Wa MA, USA), the secondary anti s (Santa C technology, Santa Cruz , USA) as dilu 1:2,000 (v/v) in phos e-buff saline (PBS) and 0.1% Tween 20 i en.

Statistical A sis

Statistica ses were p ed by Statistical P Service So tions (SPSS) 17.0 (Chi go, IL,). Date was presented as me SD. Chi-s test, Student *t*-test an n-Meier metho were selected when a opriate. $p<0.05$ was considered statistically ificant.

Results

UCA1 Expression Was Increased Both Cell Lines and Tissues

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-

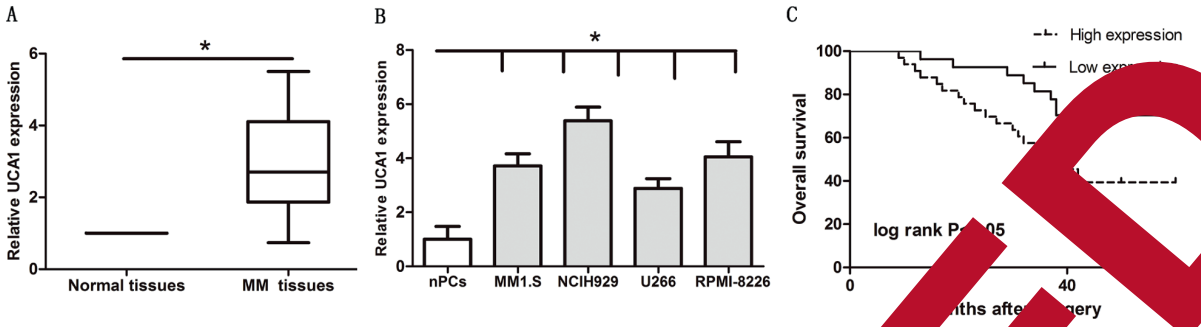
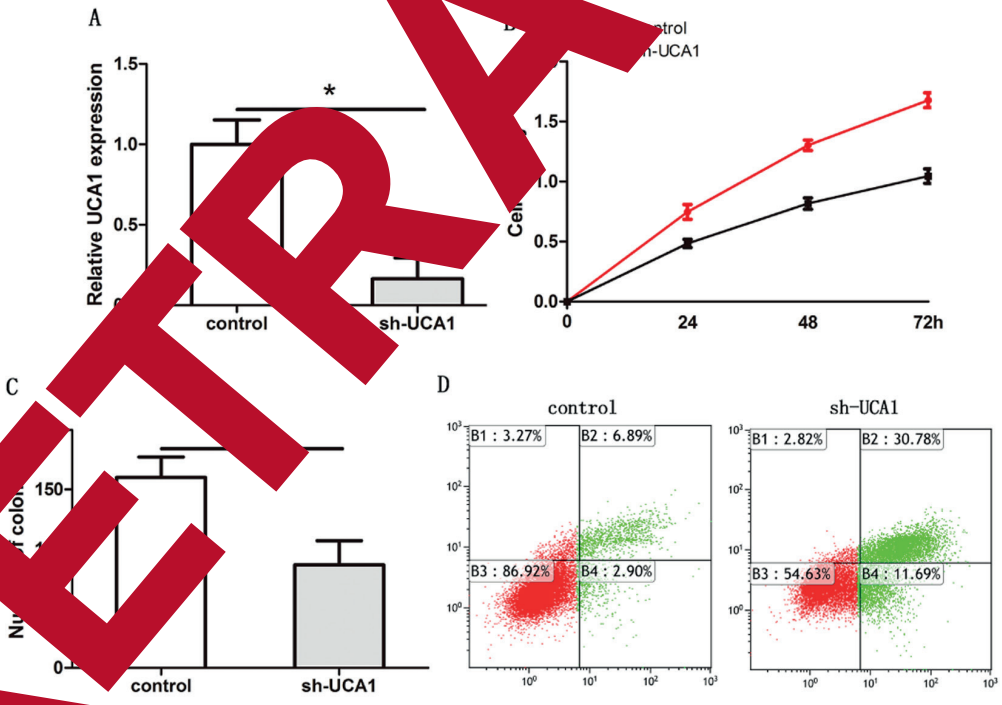


Figure 1. Expression levels of UCA1 were increased in MM tissues and cell lines, and ... associ... with poo... overall survival of MM patients. ***(A)*** UCA1 expression was significantly increased in the MM t...ssue... ...ith nor... ...ssues. ***(B)*** Expression levels of UCA1 relative to GAPDH were determined in the human MM ...lines ...rmal pl... cell line (nPCs) by RT-qPCR. ***(C)*** High level of UCA1 was associated with poor overall surv... MM pati... ...ta ...esented as the mean ± standard error of the mean. *$p<0.05$.

feration in cell lines at 24, 48, and 72 h (Figure 2B). Moreover, results of colony formation assay showed a significant decrease in colony numbers after downregulation of UCA1 in cell lines in 14 days (Figure 2C). These data suggested that downregulation of UCA1 also could suppress cell lines proliferation.

Dow...ulated Uc...Could Induce Cell Li...optosis

...ll apoptosis assay was performed to detect ...role of UCA... ...yed on MM cell lines apopto... Results show... hat after UCA1 was knocked d... the apopt... rate of these treated cells had a sig... ...ase (Figure 2D).



...ion of UCA1 decreased MM cell proliferation and induced cell apoptosis. ***(A)*** UCA1 expression in cancer ...s transduced with empty vector (control) or UCA1 virus (UCA1) was detected by RT-qPCR. GAPDH was used as an inter... ...ontrol. ***(B)*** The CCK8 assay showed that knockdown of UCA1 significantly decreased cell proliferation in MM cells. ***(C)*** ...formation assay demonstrated that oncogenic survival of cancer cells in the sh-UCA1 group was significantly increased ...ared with control group. ***(D)*** Cell apoptosis assay showed that the apoptosis rate was increased in the sh-UCA1 group compared with control group. The results represent the average of three independent experiments (mean ± standard error of the mean). *$p<0.05$, as compared with the control cells. *$p<0.05$.

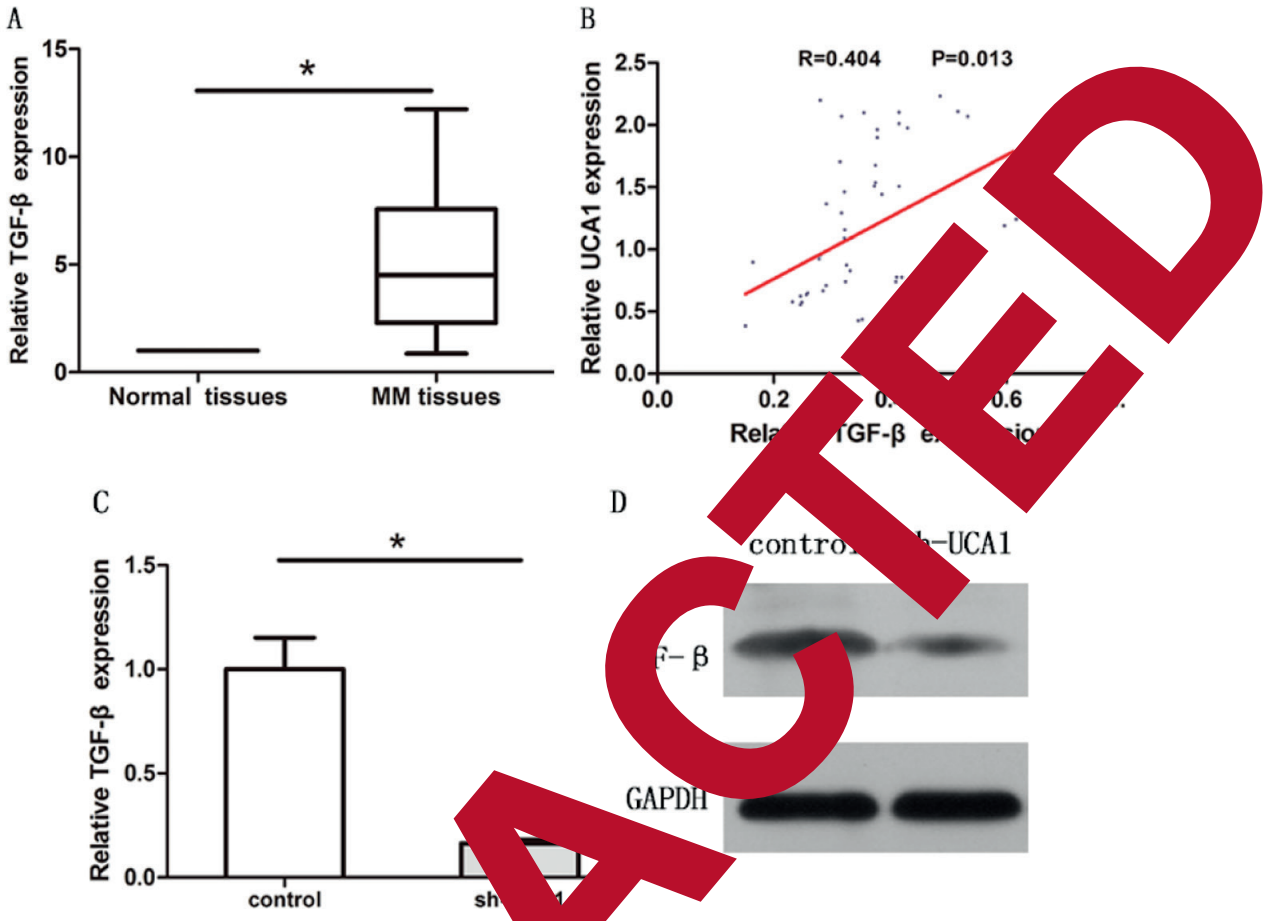


Figure 3. Interaction between UCA1 a[nd] TGF-β. ***(A)*** TG[F-β] is up-regulated in the MM tissues compared to the normal tissues. ***(B)*** The expression of TGF-β w[as] correlated [with] UCA1. ***(C)*** TGF-β expression was decreased in the sh-UCA1 group compared with control gro[up at] mRNA level. ***(D)*** TG[F-β] expression was decreased in the sh-UCA1 group compared with control group at protein le[vel]. The result[s] represent the average of three independent experiments Data are presented as the mean ± standard error of t[he] mean. *$p<0$[.05]

UCA1 Could Re[gu]late TGF-β [in] MM Cells

TGF-β may [act] as an oncogene [in] MM *via* suppressing a[po]ptosis, promoting a[n]esion in MM carcin[o]genesis. TG[F-β] may be a prognostic factor an[d] potential target [for] MM treatment. In our stu[dy], Real-time PCR assay was used to detect [the] expres[sion] level of TGF-β in both MM an[d] normal t[issue]s. As [co]mpared to the normal tissue[s], was u[p]gulated in the MM tis- (Fig[ure] A). F[urt]ermore, a positive corre- was [found] between UCA1 and TGF-β (Fig[ure] 3B). To [fur]ther confirm the mechanism of [TGF-β] and UCA1, we detected the mRNA a[nd] protein expression of TGF-β with down-re- ation of UCA1 expression. The results showed [that] down-regulation of UCA1 could decrease TGF-β e[xpress]ion (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 lncRNAs contribute to the progression of MM. Sun

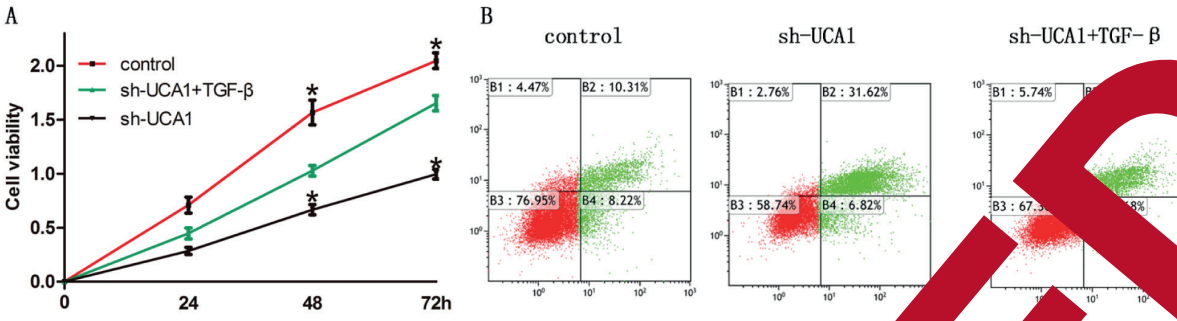


Figure 4. Overexpression of TGF-β expression could reverse the effect of UCA1 inhibition. CCK-8 assays showed that the inhibition effect was reversed when overexpressing TGF-β in cells transfected with UCA1 shRNA. Cell apoptosis assay showed that the promotion effect was reversed when overexpressing TGF-β in cells transfected with shRNA. The results represent the average of three independent experiments Data are presented as the mean ± standard error. *$p<0.05$.

et al[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[12]. LncRNA CCAT1 modulates HOXA1 expression and further promotes MM progression by sponging miR-181a-5p[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. UCA1 is up-regulated in esophageal cancer and promotes cell lines proliferation *via* SOX4. In patients with non-small cell lung cancer, down-regulated UCA1 indicates a better prognosis and acts as a ceRNA by targeting miR-3p[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 expression and mechanism of UCA1 in MM cell proliferation. Firstly, from the public samples of MM, we found the expression level of UCA1 up-regulation. Moreover, we discovered that UCA1 knockdown could suppress cell lines proliferation by performing colony formation assay and CCK-8 assay. Cell apoptosis assay, we discovered that knockdown of UCA1 could induce cell lines apoptosis.

TGF-β, important oncogene, has been related to the initiation of many cancers[17]. TGF-β is a biomarker in pancreatic cancer and can be used to predict patients' prognosis[18]. TGF-β activates PI3K/AKT/mTOR and emerges its function to promote cancer metastasis *in vitro*[19]. In breast cancer, TGF-β signaling was a fatal part in carcinogenesis and metastasis[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 dependent 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.

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