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Research article

TUG1 Regulates Osteoblast Apoptosis of Periprosthetic Osteolysis in Joint Replacement by Binding BMP-7

Bin Wu[a#], Yang Liu[b#*]

[a]*Department of Orthopaedics, TongLiao City Hospital, Tongliao, Neimenggu 028050, China.*

[b]*Department of Orthopedics, Affiliated Hospital of inner Mongolia University for the nationalities, No 1742, HuoLinHe Street, Tongliao, Neimenggu 028050, China.*

Abstract

Background: Artificial joint replacement (AJR) is the primary chosen for the patients with femoral neck fracture. Periprosthetic osteolysis is the main factor that affected the success of joint replacement. However, the potential mechanism still needs further identification.

Methods: The clinical tissues were obtained from the surgical operation. Real-time PCR was performed to determine the expression of TUG1 and BMP-7, western blot was performed to determine BMP-7 protein expression. Cell apoptosis was determined using Flow cytometry analysis. RNA pull-down was performed to determine the interaction between TUG1 and BMP-7. In vivo experiments were performed to verify the role of TUG1.

Results: Overexpressed TUG1 and down-regulated BMP-7 was observed in both clinical periprosthetic wear tissues and CoCrMo (CoPs). Knockdown of TUG1 significantly inhibited osteoblast apoptosis. TUG1 targets BMP-7 to regulate its expression. Knockdown of TUG1 inhibited osteoblast apoptosis by BMP-7. In vivo experiments verified that knockdown of TUG1 promoted the osteoblast apoptosis.

Conclusion: TUG1 regulated osteoblast apoptosis of periprosthetic osteolysis in joint replacement by targeting the expression of BMP-7.

Keywords: periprosthetic osteolysis; TUG1; BMP-7; osteoblast apoptosis

INTRODUCTION

Artificial joint replacement (AJR) is widely used in the treatment of end-stage arthritis, rheumatoid arthritis, and femoral neck fracture, and significantly decreased joint pain and restore joint function [1]. It has been reported that aseptic loosening and periprosthetic osteolysis is the major complication in AJR, and could cause long-term failure of joint replacement [2]. Previous studies have reported that the inflammatory mediators and cell apoptosis are involved in the failure of AJR [3,4]. In the present study, the role of wear particles on AJR was explored, and the potential mechanism was presented.

Long non-coding RNA (lncRNA) is a set of non-coding RNA with the length of more than 200 nt, and plays an important role in various physiology processes, such as cell apoptosis, epigenetic modification and inflammation. Mounting studies have reported that lncRNA mediated the mechanism of AJR. For example, lncRNA DANCR mediated osteoblast differentiation in regulating FOXO1 in total hip arthroplasty [5]. lncRNA PRNCR1 regulated osteogenic differentiation and contributed to osteolysis after hip replacement in regulating CXCR4 [6]. Taurine-upregulated gene 1 (TUG1) is a 7.1-kb lncRNA, and located at the chromosome 22q12 [7]. TUG1 was first identified as a transcript, and recently was shown as a negative prognostic factor in various diseases, such as osteosarcoma [8] and urothelial carcinoma of bladder [9]. In the present study, we examined TUG1 expression in osteolysis of AJR to determine the role of AJR in osteolysis.

Bone morphogenetic proteins (BMPs), belongs to the transforming growth factor (TGF) superfamily, and served as the regulators in osteoblast differentiation from multipotent stem cells [10]. BMP-7 is the member of BMPs, and was primarily recognized as the osteogenesis factor, and plays an important role in cell growth, proliferation, and apoptosis, as well as the physiology process of tumor formation [11]. It has been reported

#These authors contributed equally.

*Corresponding author: Yang Liu

Mailing address: Department of Orthopedics, Affiliated Hospital of Inner Mongolia University for the nationalities, No 1742, HuoLinHe Street, TongLiao, Neimenggu 028000,China.

E-mail: Liuyang0919@126.com

TEL: +86-0475-8267850

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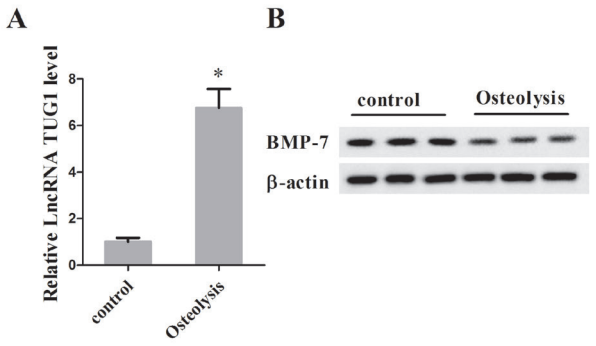

Figure 1. The expression of TUG1 and BMP-7 in clinical tissues. The clinical osteolysis and the control tissues were obtained. A: real-time PCR was performed to determine TUG1 expression; B: western blot was used to determine the level of BMP-7 protein. *p<0.05 vs. control.

that miR-542-3p could significantly suppressed the proliferation and differentiation of osteoblast via inhibiting BMP-7 [12]. The potential role of BMP-7 in osteolysis of AJR was still unclear.

In the present study, we attempt to explore the potential role of TUG1 and BMP-7 in osteolysis of AJR. We found that TUG1 was increased, while BMP-7 was decreased in clinical osteolysis tissues and, we then performed in vitro experiments to explore the potential mechanism of osteolysis. The present study suggested that TUG1 is a novel marker of osteolysis and will be helpful for further clinical therapy in osteolysis of AJR.

MATERIALS AND METHODS

Patients and materials collection

This study enrolled patients who were received operation in our hospital between 2015.1 and 2017.12. The periprosthetic tissues were obtained from 20 controls without total joint arthroplasty and 20 patients with total joint arthroplasty, among which, the 20 patients were reoperated on for aseptic loosening of total hip arthroplasty. The study was approved by TongLiao City Hospital, and all participants were signed the informed consent.

Cell culture

CoCrMo metal particles (CoPs), with a diameter of 1.67 ± 1.18 μm, were purchased from Sandvik (Stockholm, Sweden). CoPs were washed in 75% ethanol solution at room temperature for 36 h and suspended in phosphate buffered saline (PBS) for the following study. The mice osteoblast cell line MC3T3-E1 was purchased from American Type Culture Collection (ATCC). The cells were cultured in Alpha Minimum Essential Medium with ribonucleosides, deoxyribonucleosides, 2 mM L-glutamine and 1 mM sodium pyruvate as well as 10% fetal bovine serum, but without ascorbic acid at 37 °C for 24 h.

The CoPs (200μg/ml) were pretreated with ultrasound for 20 min and then co-cultured with Osteoblast cell line MC3T3-E1 for 20 h.

Real-time PCR

Total RNA were isolated from cells or tissues using TRIZOL reagent (Invitrogen). The quantified RNA was reversed transcripted into cDNA using the BeyoRT™ Ⅱ cDNA Kit (Beyotime, China) according to the manufacturer's instruction. cDNA samples were used as template for real-time PCR. The reactions were carried out using the StepOnePlus Real-Time PCR system in an ABI illumina instrument. The relative expression level of mRNA was calculated using $2^{-\Delta\Delta Ct}$ method.

Western blot

Cells or tissues were lysed using lysis buffer, and the proteins were isolated after centrifuged at 13,000 rpm for 20 min at 4 °C. The BCA method was used to quantify the protein. After the SDS-PAGE, the separated protein was transferred to the PVDF and incubated using the primary antibodies at 4 °C for 24 h. The membrane was then incubated using the second antibody at room temperature for 1 h. The protein bands were visualized using ECL chemiluminescence.

Cell transfection

Cells were seeded in six-well plates and transfected with siRNA or negative control by Lipofectamine 2000 (Invitrogen) when grew to reach about 70% conflu-

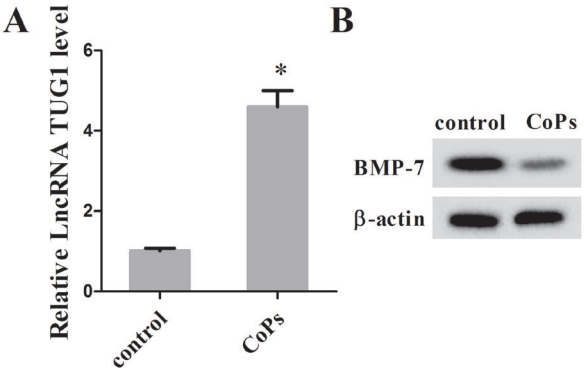

Figure 2. The expression of TUG1 and BMP-7 in CoPs-stimulated osteoblast cell line MC3T3-E1. The MC3T3-E1 cells were stimulated using CoPs to mimic osteolysis; cells were divided into two groups: control and CoPs. A: A: real-time PCR was performed to determine TUG1 expression; B: western blot was used to determine the level of BMP-7. *p<0.05 vs. control.levels. *P<0.01 vs. control.

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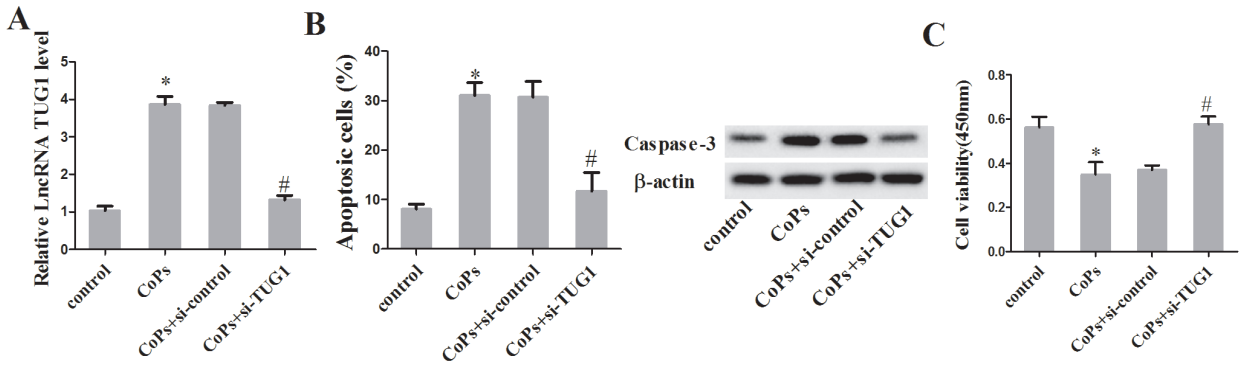

Figure 3. Role of TUG1 on osteoblast apoptosis. MC3T3-E1 cells were divided into control, CoPs, CoPs+si-control and CoPs+si-TUG1 groups. A: the expression of TUG1 was determined using real-time PCR; B: cell apoptosis was measure using Flow cytometry; C: cell proliferation was detected using MTT assay. *P<0.05 vs. control; #P<0.05 vs. CoPs+si-control.

ence. The lentiviral particles were purchased from GenePharma Co., Ltd. The transfection efficiencies were examined by qRT-PCR.

RNA pull-down

For testing the interaction between lncRNA TUG1 and BMP-7, RNA pull-down was performed using Pierce Magnetic RNA–Protein Pull-Down Kit (Termofsher, CA) in accordance with the instructions from the manufacturer. Briefy, the total RNA was extracted from cells; the magnetic beads were incubated with Probes for biotin-labeled TUG1. The relative expression levels were analyzed by real-time PCR.

Flow cytometry analysis

Cell apoptosis was determined using flow cytometry assay. Briefly, Cells were stained with annexin V and propidium iodide (PI) using annexin V-FITC/PI apoptosis detection kits (Beyotime, Shanghai, China). After incubation for 15 min, the cells were examined by flow cytometry (FACScan; BD Biosciences).

Cell proliferation assay

Cells were plated in 96-well plates (2 × 10^5 cells/mL, 100 μL/well). After 48 h, cell proliferation and viability were examined using the MTT assay. All experiments were performed in triplicate.

In vivo mouse model construction

A total of 16 C57BL/J6 mice were purchased from Experimental Animal Center of Chinese academy of sciences, Shanghai, China. The mice were anesthetized with and fixed in the prone position Ketamine (70 mg/kg). The skin was disinfected by scrubbing three times topically with 0.5% iodophors. Then, a 1.0 cm×1.0 cm area of periosteum was exposed by making a 10 mm

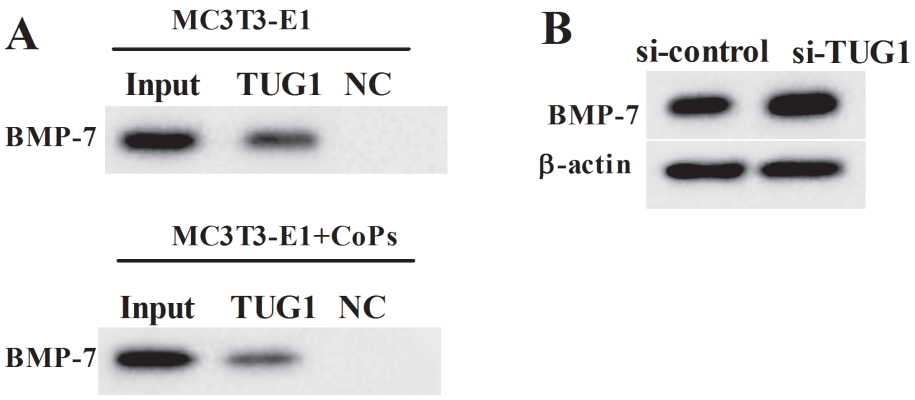

Figure 4. TUG1 targets BMP-7 to regulate its expression. A: RNA pull-down was performed to determine the interaction between TUG1 and BMP-7. B: CoPs were divided into si-control and si-TUG1 groups, western blot was performed to determine the expression of BMP-7.

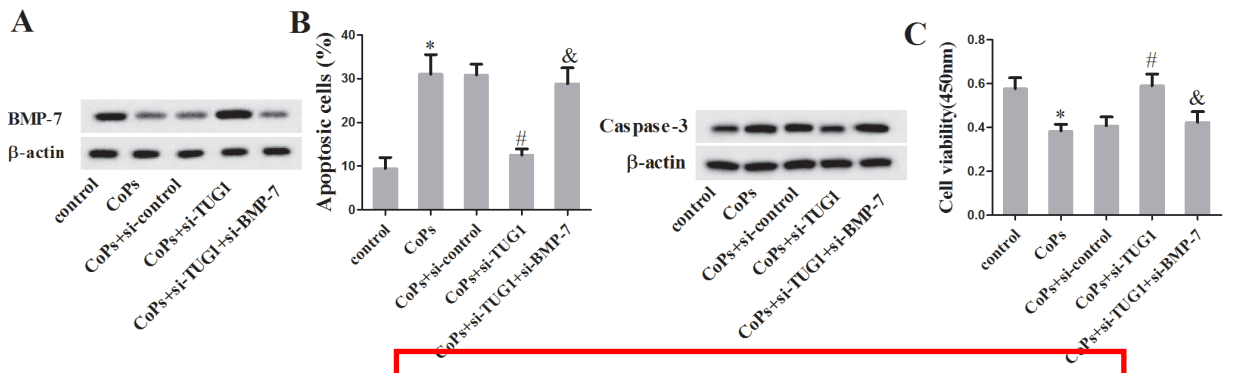

Figure 5. Role of TUG1 and BMP-7 on osteoblast apoptosis. MC3T3-E1 cells were divided into control, CoPs, CoPs+si-control, CoPs+si-TUG1 and CoPs+si-TUG1+si-BMP-7 groups. A: western blot was used to determine BMP-7 protein level; B: Flow cytometry assay was used to determine cell apoptosis; C: MTT assay was used to detect cell proliferation. *P<0.05 vs. control; #P<0.05 vs. CoPs+si-control; &P<0.05 vs. CoPs+si-TUG1.

midline sagittal incision over the calvarium that was anterior to the line connecting both external ears. The control animals received no particles (sham group) and the experimental animals received 30 ml of the CoCr-Mo particle powder suspension. The incision was closed using 4-0 nylon thread.

Bone mineral density determination

The bones that isolated from the osteolysis mice were washed using PBS. Bone mineral density was measured using a micro-CT scanner (u80, Scanco Medical AG, Switzerland) according to the manufacturer's instructors [3]. Data were analyzed by Advance Bone Analysis Software (GE Health Care Co).

Statistical analysis

All data were presented as means±SD. Statistical analysis was performed using Student's t-test or variance (ANOVA) analysis. Differences between experimental groups were considered significant for p values <0.05. All experiments were repeated for three independent times.

RESULTS

The increased TUG1 and decreased BMP-7 was observed in periprosthetic wear tissues

To determine the expression level of lncRNA TUG1 and BMP-7 in periprosthetic wear tissues, the real-time PCR and western blot were carried out. As is presented in Figure 1, the TUG1 (Figure 1A) was significantly increased, while BMP-7 (Figure 1B) was notably decreased in periprosthetic wear tissues rather than the normal tissues.

The expression pattern of TUG1 and BMP-7 in vitro

To examine the expression of TUG1 and BMP-7 in MC3T3-E1 that co-cultured with CoPs, the CoPs were first stimulated using ultrasound and then co-cultured with MC3T3-E1. The results indicated that the TUG1 (Figure 2A) was increased, while BMP-7 (Figure 2B) was decreased in comparing with control.

Knockdown of TUG1 suppressed cell apoptosis of osteoblasts

To explore whether the expression of TUG1 affect cell apoptosis of osteoblasts, the co-cultured MC3T3-T1 were then transfected with si-TUG1. We found that MC3T3-T1 cells stimulated by CoPs promoted the expression of TUG1, while cells then transfected with si-TUG1 significantly decreased its expression (Figure 3A). Additionally, CoPs stimulation induced cell apoptosis, promoted the expression of apoptosis-related protein Caspase-3 (Figure 3B), but decreased cell viability (Figure 3C), however, cells transfected with si-TUG1 significantly abolished the effect of CoPs.

TUG1 targets BMP-7 to regulate its expression

To determine the interaction between TUG1 and BMP-7, RNA pull-down was performed. Results revealed that TUG1 pulled down BMP-7 in the compounds in both MC3T3-E1 and CoPs stimulated MC3T3-E1 (Figure 4A). MC3T3-T1 cells transfected with si-TUG1 significantly promoted the expression of BMP-7 (Figure 4B).

Knockdown of TUG1 suppressed osteoblast cell apoptosis by promoting the expression of BMP-7

To determine the potential mechanism of TUG1 and

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BMP-7 in cell apoptosis, MC3T3-E1were stimulated by CoPs, and then divided into: control, CoPs, CoPs+-si-control, CoPs+si-TUG1 and CoPs+si-TUG1+si-BMP-7. We found that CoPs stimulation inhibited the expression of BMP-7, then si-TUG1 transfection promoted the expression of BMP-7, however, cells co-transfected with si-TUG1 and si-BMP-7 significantly abolished the effect of si-TUG1 (Figure 5A). Then we determined cell apoptosis and viability, we found that CoPs stimulation promoted cell apoptosis and Caspase-3 expression but decreased cell viability, then si-TUG1 reversed the effect of CoPs, however, si-BMP-7 abolished the effect of si-TUG1 (Figure 5B and 5C).

To verify the role of TUG1 in vivo

The osteolytic mouse model was separated into two groups, one group received the injection of si-control, and the other received the injection of si-TUG1. After 14 days, the bone mineral density was detected. The results revealed that TUG1 knockdown significantly promoted bone mineral density in comparing with si-control (Figure 6A). Real-time PCR revealed that si-TUG1 injection significantly decreased the expression of TUG1 (Figure 6B). Western blot analysis showed that si-TUG1 injection promoted the expression of BMP-7 (Figure 6C).

DISCUSSION

Recently, the mechanism of osteolysis has been reported by various studies. It has been reported that wear debris could induce aseptic loosing and osteolysis [13-15]. Thus, in the present study, the clinical patients of AJR with or without aseptic loosing were enrolled for studying the mechanism of osteolysis. Our clinical study identified that TUG1 was increased, while BMP-7 was decreased in wear particles of AJR in comparing with the normal control, indicating that TUG1 and BMP-7 might serve an important role in AJR.

It has been reported that many lncRNAs have been reported to be involved in the osteolysis. For example, lncRNA KCNQ1OT1 was identified to promote osteogenic differentiation to relieve osteolysis via activating Wnt/β-cateinin [16]. lncRNA TSIX promoted osteoblast apoptosis in particle-induced osteolysis [3]. In the present study, lncRNA TUG1 was served as the important marker to study its role in osteolysis of AJR. The results revealed that the overexpressed TUG1 is along with the occurrence of osteolysis.

LncRNA is reported to mediate physiology processes via regulating the target proteins. For example, lncRNA DGCR5 targets PRDM5 to regulate neuronal apoptosis in acute spinal cord injury [17]. lncRNA THOR mediated the progression of retinoblastoma through regulating c-myc and IGF2BP1 [18]. In addition, lncRNA TUG1 has been reported to target YAP to regulate cell proliferation and migration of renal cell carcinoma [19]. In the present study, our result revealed that TUG1 mediated the osteolysis by regulating BMP-7, indicating that TUG1 targets BMP-7 to regulate the osteolysis in AJP.

It has been reported that the signaling protein BMP-7 showed a significant role in the development of mammalian organs such as the kidney and the eye [20]. BMP-7 could regulate receptor-regulated Smads (Smad1, Smad5, and Smad8) and inhibitory Smads (Smad6 and Smad7) by binding to the type I and II receptors in a complex wound-healing signaling network [21]. In addi-

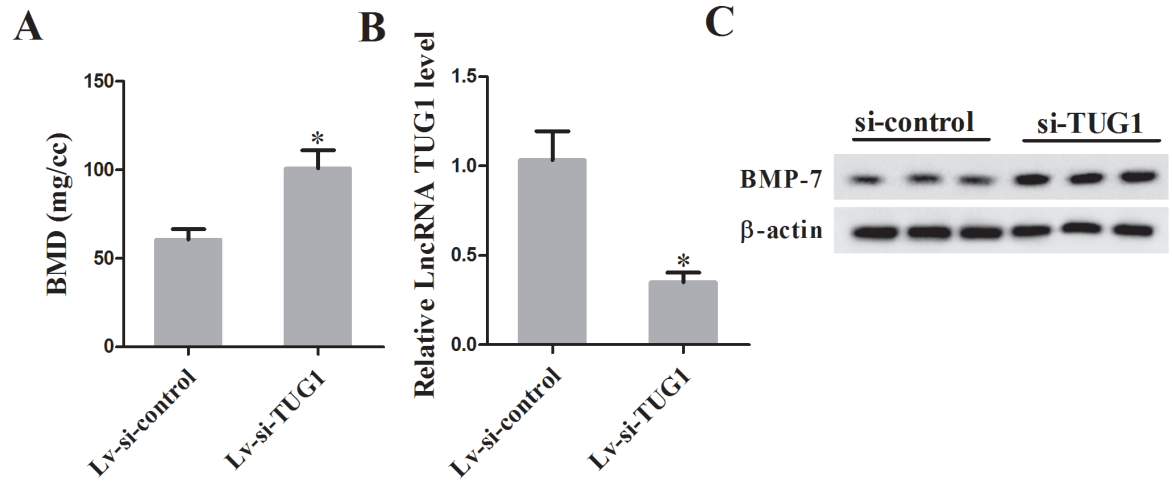

Figure 6. To verify the role of TUG1 in mice osteolytic model. A total of 16 mice were divided into Lv-si-control and Lv-si-TUG1 (n=8 in each group). A: the bone mineral density was determined. B: the expression of TUG1 was determined using real-time PCR. C: the expression of BMP-7 was detected using western blot. *P <0.05 vs. si-control.

tion, BMP-7 was identified to regulate cell apoptosis of keratocyte [21], mouse metanephric mesenchymal cells (29247399), nucleus pulposus cells [22]. In the present study, BMP-7 was identified to regulate cell apoptosis of osteoblast, with the potential mechanism of knockdown of TUG1 suppressed osteoblast apoptosis by upregulating BMP-7.

Taken together, in the present study, we found that the wear debris in aseptic loosing of AJR showed increased TUG1 and decreased BMP-7 in clinical sample. The in vitro study identified that TUG1 regulated the expression of BMP-7, and BMP-7 mediated osteoblast apoptosis. Thus, the present study indicated that lncRNA TUG1 regulated osteoblast apoptosis by regulating BMP-7 and further regulate osteolysis in AJR. The present study provided great help for further clinical therapy of osteolysis in AJR and further study should verify the results in vivo.

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