ATF3 promotes migration and M1/M2 polarization of macrophages by activating tenascin-C via Wnt/β-catenin pathway

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Abstract. There are different polarization states of macrophages, including the classically activated M1 phenotype and the alternatively activated M2 phenotype. These have different functions in the inflammation process. Activating transcription factor 3 (ATF3) is a key transcriptional regulator that inhibits the inflammatory response. However, the effects of ATF3 on migration and anti-inflammatory control mechanisms of macrophages have not been thoroughly investigated. The present study investigated the effect of ATF3 on macrophage migration and M1/M2 polarization. Results revealed that overexpression of ATF3 promoted macrophage migration and the expression of the M2 phenotype markers [cluster of differentiation (CD) 163, mannose receptor C type 1, arginase 1 and peroxisome proliferator-activated receptor γ] and inhibited expression of the M1 phenotype markers (monocyte chemoattractant protein-1, inducible nitric oxide synthase, CD16 and tumor necrosis factor-α), whereas knockdown of ATF3 resulted in a contrary effect. In addition, the wingless-type MMTV integration site family member (Wnt)/β-catenin signaling pathway was activated and the expression level of tenascin (TNC) was significantly upregulated by overexpression of ATF3. Additionally, inhibition of Wnt/β-catenin signaling significantly attenuated the upregulatory effect of ATF3 on TNC. Finally, the effect of ATF3 on macrophage migration and markers of the M1 or M2 state was investigated using TNC-specific siRNA. In conclusion, the results of the present study suggested that ATF3 promotes macrophage migration and reverses M1-polarized macrophages to the M2 phenotype by upregulation of TNC via the Wnt/β-catenin signaling pathway.

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Abbreviations: ATF3, activating transcription factor 3; TNC, tenascin; IFN-γ, interferon-γ; TNF-α, tumor necrosis factor-α; ATF/CREB, activating transcription factor/cAMP responsive element binding protein; TLR4, Toll-like receptor 4

Key words: activating transcription factor 3, macrophage, M1/M2 polarization, tenascin-C

Introduction

Macrophages are essential components of innate immunity that serve a role in inflammation and host defense via production of pro- or anti-inflammatory mediators in response to various stimuli (1). Macrophages respond to stimulation in a polarized manner. The differentiation of macrophages to the classic activation (M1) phenotype is triggered by interferon (IFN)-γ, bacterial lipopolysaccharide (LPS), interleukin (IL)-1β, or tumor necrosis factor α (TNF-α), whereas IL-3 or IL-13 stimulate macrophage differentiation of the alternative activation phenotype (M2) (2-5). The M1 phenotype is characterized by the expression of high levels of pro-inflammatory cytokines. Conversely, M2 macrophages express an anti-inflammatory functional profile and are associated with wound repair and angiogenesis (6). Therefore, inflammatory associated diseases may result from a sustained pro-inflammatory reaction and failure of anti-inflammatory control mechanisms of macrophages.

Activating transcription factor 3 (ATF3), a member of the mammalian activating transcription factor/cAMP responsive element binding protein (ATF/CREB) family of transcription factors, is induced in a variety of stressed tissues, including mechanically and toxin-injured liver tissue, blood-deprived heart tissue and injured peripheral nerves (7,8). The transcriptional target of ATF3 varies in different cell types and conditions, which therefore leads to diverse effects on cell survival, proliferation and death (9). In neurons, ATF3 is frequently reported to be a novel neuronal marker of nerve injury, and induction of ATF3 expression enhances nerve regeneration (10,11). In cardiac myocytes, ATF3 is a novel cytoprotective factor in doxorubicin-induced apoptosis (12). Additionally, it is reported that ATF3 protects renal cells and b-cells against oxidative stress-induced cell death and apoptosis (13). Therefore, ATF3 is a protective factor in numerous tissues.

Conversely, ATF3 is an inducible transcriptional repressor in innate immune cells, which regulates the magnitude and duration of inducible pro-inflammatory gene expression. Recently, it was revealed that ATF3 is an important transcriptional regulator that inhibits the inflammatory response by modulating the expression of cytokines and chemokines and

demonstrated that ATF3 is a negative regulator of Toll-like receptor 4 (TLR4) signaling in macrophages (14). Activation of TLR4 by LPS induces the expression of ATF3, which subsequently inhibits the expression of various inflammatory genes induced by TLR signaling, including IL-6, IL-12β, and TNF-α. Additionally, ATF3 may modulate the expression levels of IFN-γ in macrophages by controlling basal and inducible levels of IFN-β, and the expression of genes downstream of IFN (15). Therefore, ATF3 is able to negatively regulate transcription of pro-inflammatory cytokines in macrophages. Understanding the exact role of ATF3 in macrophages in the context of inflammation is of primary concern, and may lead to the design of beneficial therapeutics for inflammatory associated diseases. However, the effects of ATF3 on recruitment and anti-inflammatory control mechanisms of macrophages remain to be investigated.

The present study investigated whether ATF3 exerted anti-inflammatory activities by modulating M1/M2 differentiation of macrophages. Macrophage migration and markers of M1/M2 macrophages were tested following overexpression of ATF3. Subsequently, the underlying mechanism of how overexpression of ATF3 modulates macrophage migration and M1/M2 polarization was investigated.

Materials and methods

Cell culture. Mouse macrophage RAW 264.7 cells (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's modified Eagle's medium (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA) supplemented with 10% fetal bovine serum, 100 U/ml streptomycin and 100 U/ml penicillin (Gibco; Thermo Fisher Scientific, Inc.) in a humidified atmosphere at 37˚C and 5% CO_2. Wntpalmitoyltransferase inhibitor (IWP-2,*N*-(6-M ethyl-2-benzothiazolyl)-2-[(3,4,6,7-tetrahydro-4-oxo-3-phenylthieno[3,2-*d*]pyrimidin-2-yl)thio]-acetamide) was purchased from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) and 30 μM was used to treat the cells.

Plasmids and siRNA transfection. For overexpression of ATF3, ATF3 cDNA generated from reverse transcription-quantitative PCR (RT-qPCR; The primers used for the cloning were: 5'-AAAAAGCTTATGATGCTTCAACATCCAGG-3' and 5'-TTTGAATTCTTAGCTCTGCAATGTTCCTT-3') was subcloned into a pcDNA™3.1 plasmid (Invitrogen; Thermo Fisher Scientific, Inc.) between Hind3 and EcoR I sites to express ATF3 in abundance in *E. coli* DH5α cells to generate an ATF3 expression plasmid. The third generation of cells ($5x10^5$) were seeded into a 24-well plate and transfected with the pcDNA-*ATF3* or the empty vector negative control plasmid, psDNA3.1(-), using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.), according to the manufacturer's protocol at 37˚C. After incubation for 48 h, cells were harvested and ATF3 expression was determined. To knockdown ATF3 and tenascin (TNC), The third generation of cells ($5x10^5$) were seeded into a 24-well plate and *ATF3* siRNA, its negative control (sham), or TNC siRNA and its negative control siRNA (scramble) were transfected at 37˚C using Lipofectamine 2000 (Invitrogen; Thermo Fisher Scientific, Inc.; 0.1 μM siRNA with 20 μl Lipofectamine). Following transfection for 48 h, the transfection medium was exchanged for normal medium and the cells were used in the subsequent experiments or harvested for ATF3 and TNC expression measurement.

Migration assays. Migration assays were performed using 8 μm pore size filters within 24-well transwell cell culture chambers with polycarbonate filters (Corning Life Sciences, Glendale, Arizona, USA) as previously described (16). A total of $5x10^5$ cells were transfected with pcDNA-*ATF3* or the psDNA3.1(-), and subsequently seeded into the upper chamber of the transwell. Transwells were either uncoated (5 μm pore size) or coated with Matrigel™ (BD Biosciences, Franklin Lakes, NJ, USA) diluted 1:50 (8 μm pore size). As a chemoattractant, monocyte chemoattractant protein-1 (MCP-1; 100 ng/ml; R&D Systems, Inc., Minneapolis, MN, USA) was present in the lower wells. After incubation for 18 h at 37˚C in 5% CO_2, cells in the lower chambers that passed through the filter were counted under a Carl Zeiss Primo Vert microscope (Carl Zeiss AG, Oberkochen, Germany).

Western blotting. Total protein was lysed using radioimmunoprecipitation assay (RIPA) buffer (Beyotime Institute of Biotechnology, Nantong, China). After determining the protein concentration with a Bicinchoninic acid assay kit (Beyotime Institute of Biotechnology, Haimen, China), ~30 μg of proteins were loaded onto 10% gels and subjected to SDS-PAGE, prior to transfer onto polyvinylidene difluoride membranes (Invitrogen; Thermo Fisher Scientific, Inc.). Subsequently, membranes were blocked for 1 h in a blocking solution (5% skimmed milk, 0.05% Tween 20) at 37˚C and probed with the following primary antibodies: Mouse anti-β-catenin (2698; 1:1,000), rabbit anti-c-myc 9402; 1:1,000) and anti-cyclin D1 (2292; 1:1,000; all from Cell Signaling Technology, Inc., Danvers, MA, USA), and mouse anti-ATF3 (sc81189; 1:500), rabbit anti-TNC (sc20932; 1:1,000), and mouse anti-β-actin (sc130300; 1:1,000; all from Santa Cruz Biotechnology, Inc., Dallas, TX, USA) at 4˚C overnight. Horseradish peroxidase-conjugated rabbit anti-mouse (sc358917; 1:3,000; Santa Cruz Biotechnology) or goat anti-rabbit (RPN4301; 1:5,000; GE Healthcare Life Sciences, Chalfont, UK) secondary antibodies were added to the membranes for 1 h at room temperature. Protein bands were detected using the Enhanced Chemiluminescence substrate detection system (Amersham Biosciences Corporation, Piscataway, USA). The intensities of the resulting bands were quantified using Carestream Molecular Imaging software version 5.0.2.30 (Carestream Health, Woodbridge, CT, USA) on a Gel Logic 2000 imaging system (Kodak, Rochester, NY, USA).

Reverse transcription-quantitative polymerase chain reaction (RT-qPCR). Reverse transcription PCR was performed on an Applied Biosystems® 7500 fast sequence detection system (Applied Biosystems; Thermo Fisher Scientific, Inc.). Briefly, total RNA was extracted using TRIzol® reagent (Invitrogen; Thermo Fisher Scientific, Inc.) and reverse transcribed using the MMLV Reverse Transcriptase kit (Takara Biotechnology Co., Ltd., Dalian, China) according to the manufacturer's protocol. qPCR was performed using SYBR Green reagent (Qiagen, Inc., Valencia, CA, USA). Cycling conditions were as follows: An initial predenaturation step at 95˚C for 5 min,

Table I. Primers used.

Gene	Primer sequences
MCP-1	F: 5'-TCAGCCAGATGCAGTTAACGC-3'
	R: 5'-TGGATGCATTAGCTTCAGATTTACG-3'
CD16	F: 5'-GACAGTGTGACTCTGAAG-3'
	R: 5'-GCACCTGTACTCTCCAC-3'
iNOS	F: 5'-CCCTTCCGAATTTCTGGCAGCAGC-3'
	R: 5'-GGCTGTCAGAGCCTCGTGGCTTTGG-3'
TNF-19α	F: 5'-TTGACCTCAGCGCTGAGTTG-3'
	R: 5'-CCTGTAGCCCACGTCGTAGC-3'
Arg-1	F: 5'-CAGAAGAATGGAAGAGTCAG-3'
	R: 5'-CAGATATGCAGGGAGTCACC-3'
CD163	F: 5'-ATGGGTGGACACAGAATGGTT-3'
	R: 5'-CAGGAGCGTTAGTGACAGCAG-3'
Mrc-1	F: 5'-TCTTTTACGAGAAGTTGGGGTCAG-3'
	F: 5'-ATCATTCCGTTCACAGAGGG-3'
PPARγ	R: 5'-GGAGATCTCCAGTGATATCGACCA-3'
	F: 5'-ACGGCTTCTACGGATCGAAACT-3'
TNC	R: 5'-GTTTGGAGACCGCAGAGAAGAA-3'
	F: 5'-TGTCCCCATATCTGCCCATCA-3'
GAPDH	R: 5'-AGGTCGGTGTGAACGGATTTC-3'
	F: 5'-TGTAGACCATGTAGTTGAGGTCA-3'

MCP-1, monocyte chemoattractant protein 1; CD, cluster of differentiation iNOs, inducible nitric oxide synthase; TNF-α, tumor necrosis factor-α; Arg-1, arginase 1; Mrc-1, mannose receptor C type 1; PPARγ, peroxisome proliferator-activated receptor γ; TNC, tenascin.

followed by 40 cycles of denaturation at 95˚C for 15 sec, annealing at 58˚C for 30 sec and extension at 72˚C for 20 sec. The experiment was performed three times. The relative expression levels of the target genes were calculated using the $2^{-\Delta\Delta Cq}$ method (17) and normalized to GAPDH. Forward and reverse sequences of the primers used for all target genes and GAPDH are listed in Table I.

Statistical analysis. Data are expressed as the mean ± standard deviation. Comparisons between two groups were analyzed by unpaired Student's t-test. Experiments were repeated three times. $P<0.05$ was considered to indicate a statistically significant difference analyzed using SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA).

Results

Overexpression of ATF3 promotes the migration of macrophages. To examine whether ATF3 regulates macrophage migration, the pcDNA-*ATF3* plasmid and *ATF3* siRNA were utilized to overexpress and knockdown the ATF3 protein, respectively (Fig. 1A). Cell viability was tested using an MTT assay and the ATF3 siRNA had no effect on cellular viability (data not show).

Subsequently, migration of macrophages was evaluated in the presence of chemotaxis-inducing agent MCP-1. Results demonstrated that overexpression of ATF3 significantly promoted the migration of macrophages compared with the empty vector [pcDNA3.1(-)], whereas knockdown of ATF3 significantly reduced migration compared with the sham group and the difference between the control and sham group were not significant (Fig. 1B).

Overexpression of ATF3 promotes macrophage differentiation of the M2 phenotype. To determine the function of ATF3 in the process of macrophage polarization, markers of the M1 phenotype [MCP-1, inducible nitric oxide synthase (iNOS), cluster of differentiation (CD) 16 and TNF-α] and the M2 phenotype [CD163, mannose receptor C type 1 (Mrc-1), arginase 1 (Arg-1) and peroxisome proliferator-activated receptor γ (PPARγ)] were measured. Results revealed that the mRNA expression levels of M1-associated genes encoding MCP-1, iNOS, CD16 and TNF-α, were reduced following transfection with pcDNA-*ATF3* compared with the empty vector control group, and were enhanced following transfection with *ATF3* siRNA compared with the sham group ($P<0.05$; Fig. 2A). In addition, overexpression of *ATF3* in RAW 264.7 cells enhanced the expression levels of the genes encoding CD163, Mrc-1, Arg-1 and PPARγ compared with the empty vector control group, and these levels were then reduced by knockdown of *ATF3* compared with the sham group ($P<0.05$; Fig. 2B). These results suggested that *ATF3* promotes polarization of M2 in macrophages.

Overexpression of ATF3 activates the Wnt/β-catenin signaling pathway. β-catenin, encoded by the *CTNNB1* gene, is a transcriptional co-activator and serves a role in the inflammatory response (18,19). To explore the mechanism of ATF3 regulation of macrophage migration and M2 polarization, the effect of ATF3 on Wnt/β-catenin signaling was investigated. As demonstrated in Fig. 3, overexpression of ATF3 resulted in enhanced expression levels of β-catenin and its target genes cyclin D1 and c-myc compared with cells transfected with the empty vector control. This suggested that overexpression of ATF3 induced the activation of the Wnt/β-catenin signaling pathway in macrophages.

TNC is activated by ATF3 via the Wnt/β-catenin signaling pathway. The gene encoding TNC is a canonical Wnt target (20), and serves a role in macrophage behavior and function (21,22). Therefore, the association between ATF3 and TNC was investigated. Results revealed that overexpression of AFT3 significantly upregulated the mRNA expression levels of *TNC* (Fig. 4A) and the TNC protein (Fig. 4B) compared with the empty vector control, and this effect was partially inhibited by IWP-2, an inhibitor of Wnt/β-catenin signaling, which suggested that ATF3 activates TNC via the Wnt/β-catenin signaling pathway.

ATF3 regulates the migration and polarization of M2 macrophages by upregulating TNC expression levels. To further investigate the role of TNC in ATF3-mediated macrophage migration and polarization, cells were transfected with pcDNA-*ATF3* and knockdown of TNC using *TNC* siRNA (Fig. 5A), prior to determining cell viability and the expression levels of M2-associated genes. Results revealed that transfection of pcDNA-*ATF3* significantly promoted macrophage

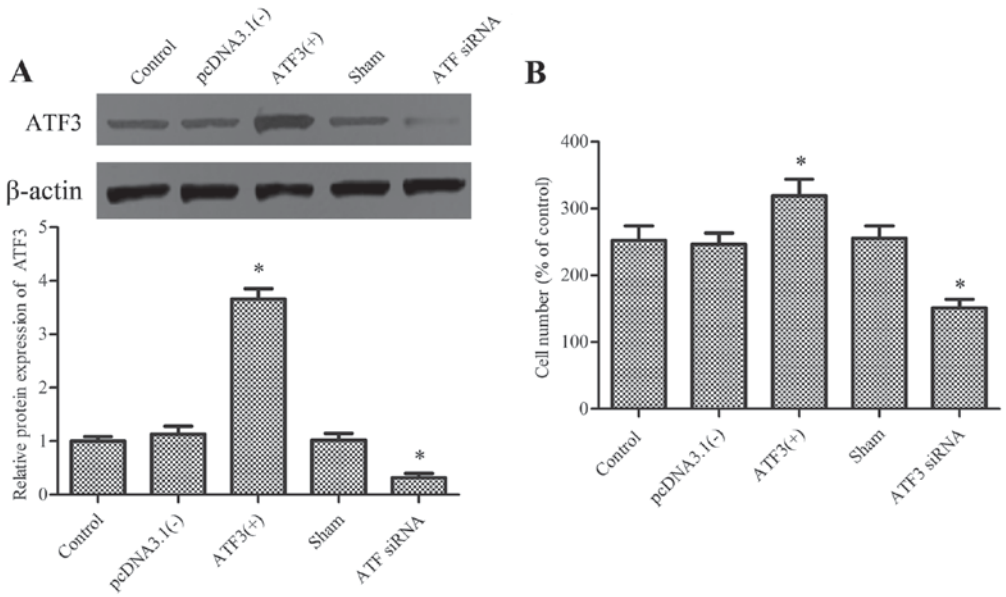

Figure 1. Effect of ATF3 overexpression on macrophage migration. (A) RAW 264.7 cells transfected with pcDNA3.1(-), pcDNA-*ATF3* (ATF3 (+)), *ATF3* siRNA and negative control siRNA (sham) for 48 h. Cells without transfect vector were defined as the control group. The protein expression levels of ATF3 were measured by western blotting and densitometric analysis. (B) RAW 264.7 cells were transfected with pcDNA3.1(-), pcDNA-*ATF3* [ATF3 (+)], *ATF3* siRNA and negative control siRNA (sham), and migration assays were performed using 8 μm pore size filters within 24-well transwell cell culture chambers with polycarbonate filters. Data are expressed as the mean ± standard deviation of the three independent experiments. *P<0.05 vs. sham or pcDNA3.1(-). ATF3, activating transcription factor 3; siRNA, short interfering RNA; pcDNA3.1(-), empty vector.

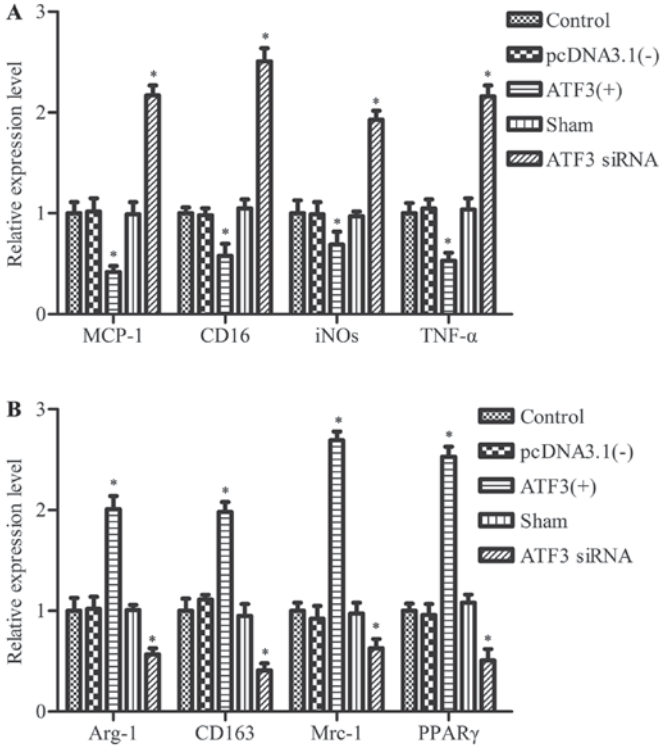

Figure 2. Effect of ATF3 overexpression on the characterization of M1 or M2 macrophage polarization. RAW 264.7 cells transfected with pcDNA3.1 (-), pcDNA-*ATF3* (ATF3 (+)), *ATF3* siRNA or negative control siRNA (sham) for 48 h. mRNA expression levels of markers of the (A) M1 state and (B) M2 state were determined using reverse transcription-quantitative polymerase chain reaction. Data are expressed as the mean ± standard deviation of the three independent experiments. *P<0.05 vs. sham or pcDNA3.1 (-). ATF3, activating transcription factor 3; siRNA, short interfering RNA; pcDNA3.1(-), empty vector; MCP-1, monocyte chemoattractant protein-1; iNOS inducible nitric oxide synthase; CD, cluster of differentiation; TNF-α, tumor necrosis factor; Mrc-1, mannose receptor C type 1; Arg-1, arginase 1; PPARγ, peroxisome proliferator-activated receptor γ.

migration compared with the empty vector control, whereas transfection with *TNC* siRNA reduced the migration induced by pcDNA-*ATF3* compared with the scrambled control group (Fig. 5B). In addition, the expression levels of genes associated with the M1 phenotype that were downregulated by pcDNA-*ATF3* were enhanced by transfection with *TNC* siRNA (Fig. 5C), whereas M2 gene expression levels that were upregulated by pcDNA-*ATF3* were inhibited by *TNC* siRNA (Fig. 5D).

These results suggested that ATF3 regulates macrophage migration and M2 polarization, in part, by upregulation of TNC.

Discussion

Macrophages are primary producers of pro-inflammatory mediators and the migration of macrophages from the circulation into injured tissues serves a crucial role in wound healing. Macrophage polarization is closely associated with homeostatic tissue remodeling, resolution of inflammation, remodeling and tissue repair (23). ATF3 is a transcriptional modulator, induced by LPS and the TLR-dependent injury response, that negatively regulates numerous pro-inflammatory cytokines and chemokines in macrophages (24). Previous reviews have reported that ATF3 modulates the expression levels of a number of inflammatory genes (25). Therefore, the present study investigated the effect of ATF3 on macrophage migration and polarization.

Migration of macrophages serves a role in the onset and course of inflammation. Chen *et al* (26) revealed that the epithelium-derived exosomal ATF3 inhibited the expression of monocyte chemoattractant protein 1 and macrophage migration, and Zmuda *et al* (27) suggested that ATF3 knockout islets

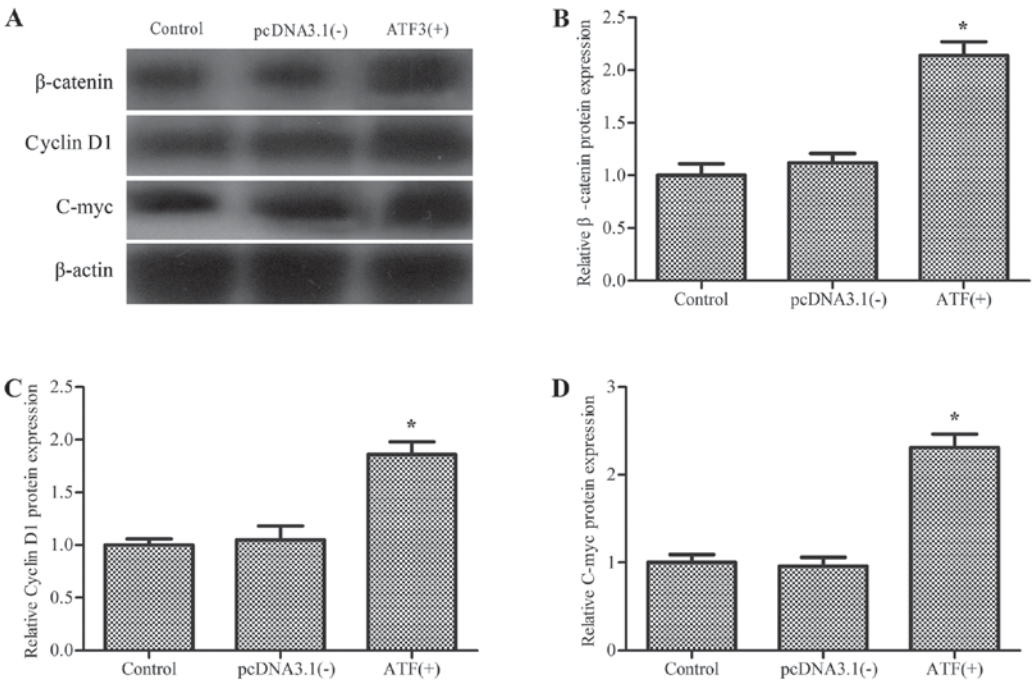

Figure 3. Effect of ATF3 overexpression on the Wnt/β-catenin signaling pathway. (A) RAW 264.7 cells were transfected with pcDNA3.1(-) or pcDNA-*ATF3* (ATF3 (+)) for 48 h. (A) Representative western blots demonstrating the expression levels of β-catenin, cyclin D1 and c-myc in all groups. The histograms demonstrate the quantification of (B) β-catenin, (C) cyclin D1 and (D) c-myc. Data are expressed as the mean ± standard deviation of the three independent experiments. *P<0.05 vs. pcDNA3.1(-). ATF3, activating transcription factor 3; pcDNA3.1(-), empty vector.

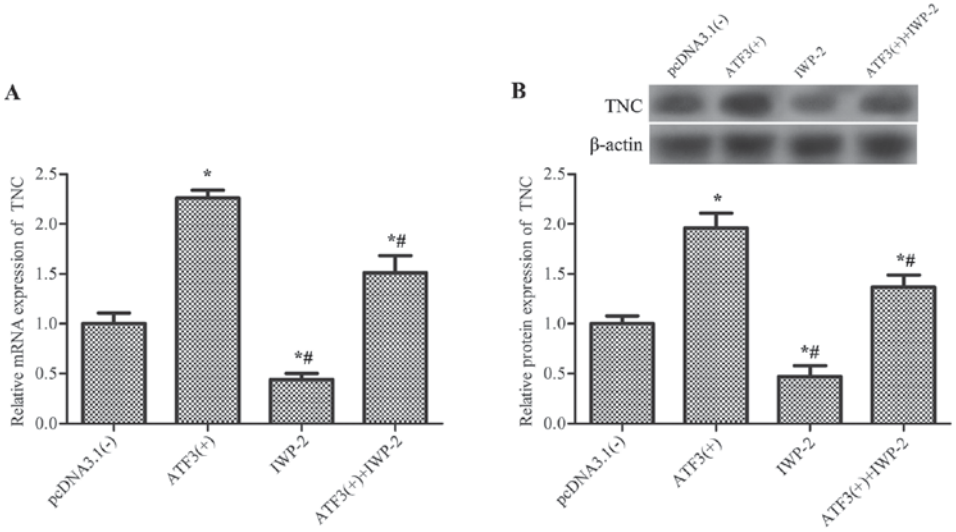

Figure 4. Effect of ATF3 overexpression on TNC expression. RAW 264.7 cells were treated with pcDNA-*ATF3* (ATF3 (+)), control, Wnt/β-catenin inhibitor IWP-2 alone or ATF3(+) +IWP-2. (A) *TNC* mRNA expression levels were measured by reverse transcription-quantitative polymerase chain reaction and (B) TNC protein expression levels were determined using western blot and densitometric analysis. Data are expressed as the mean ± standard deviation of the three independent experiments. *P<0.05 vs. pcDNA3.1(-) and #P<0.05 vs. ATF3(+). ATF3, activating transcription factor 3; TNC, tenascin; IWP-2, *N*-(6-Methyl-2-benzothiazolyl)-2-[(3,4,6,7-tetrahydro-4-oxo-3-phenylthieno[3,2-*d*]pyrimidin-2-yl)thio]-acetamide.

inhibited macrophage recruitment *in vivo*. The present study revealed that overexpression of ATF3 promoted M2 marker expression and suppressed the expression levels of M1-associated markers. This suggested that ATF3 may reverse M1-polarized macrophages to M2 phenotypes, and that the ATF3-mediated anti-inflammatory function is closely associated with macrophage phenotype. It is evident that ATF3 serves an important role in injury in numerous tissues, and it was revealed that ATF3 may protect against acute kidney and lung injury (28,29). M2 macrophages exhibit immunoregulatory functions including defense against infection, promotion of angiogenesis and wound healing (30). The results of the present study suggested that ATF3 may be a protective regulator for injured tissues by promoting the polarization of M2 macrophages.

ATF3 serves a role in the cellular adaptive-response network in response to signals perturbing homeostasis (25). Previous data has suggested that ATF3 activates the Wnt/β-catenin signaling pathway in human breast cancer

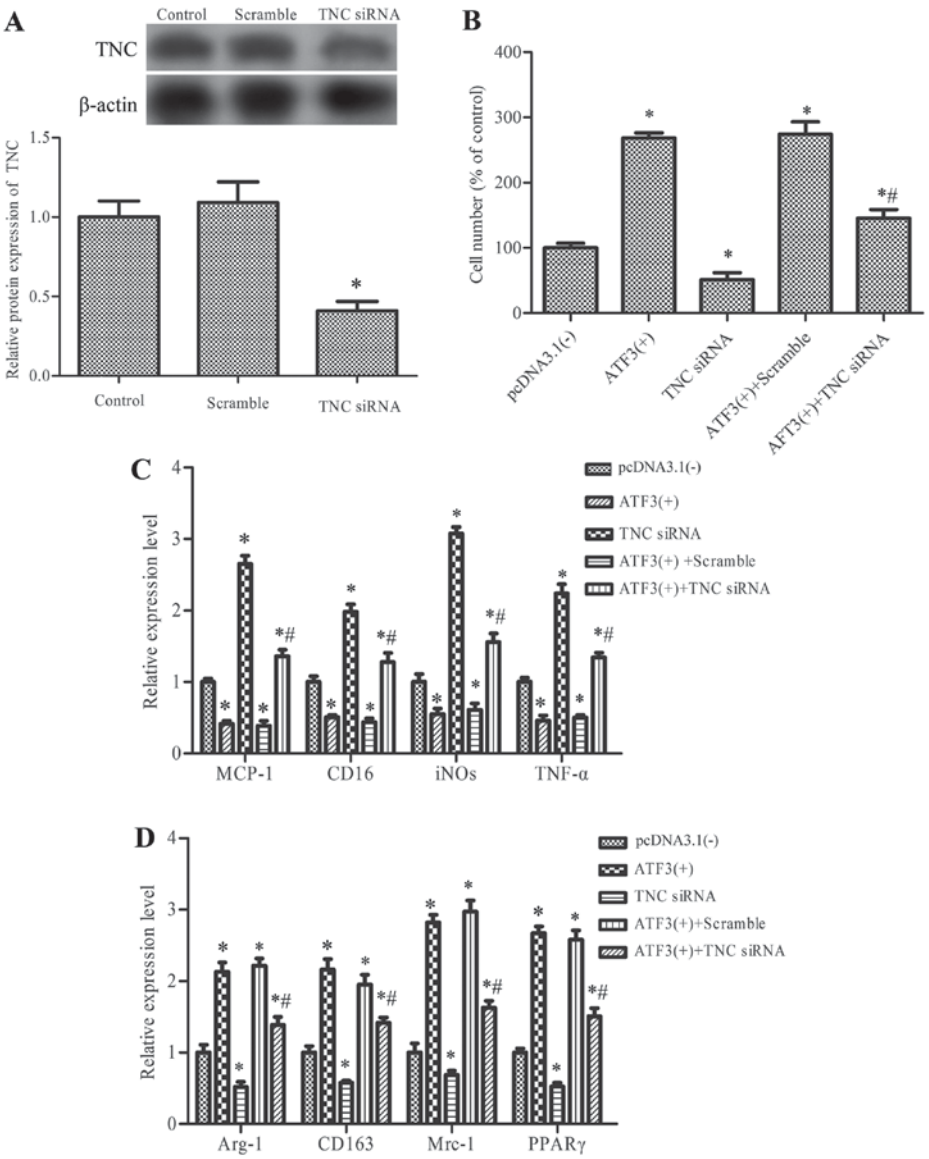

Figure 5. The role of TNC in the regulation of ATF3 on macrophage migration and M1/M2 polarization. (A) RAW 264.7 cells were transfected with *TNC* siRNA or negative control siRNA (scramble). Cells without transfect vector were defined as the control group. TNC protein expression levels were determined using western blot and densitometric analysis. Cells were treated with pcDNA-*ATF* (ATF3 (+)), *TNC* siRNA, ATF3(+) +scramble or ATF3(+) +*TNC* siRNA. Cells without transfect vector were defined as the control group. (B) macrophage migration, (C) mRNA expression of markers of M1 state and (D) mRNA expression of markers of the M2 state, were measured. Data are expressed as the mean ± standard deviation of the three independent experiments. *P<0.05 vs. pcDNA3.1(-) and $^{\#}$P<0.05 vs. ATF3(+) + scramble. ATF3, activating transcription factor 3; TNC, tenascin; siRNA, short interfering RNA; MCP-1, monocyte chemoattractant protein-1; iNOS, inducible nitric oxide synthase; CD, cluster of differentiation; TNF-α, tumor necrosis factor; Mrc-1, mannose receptor C type 1, Arg-1; arginase 1; PPARγ, peroxisome proliferator-activated receptor γ.

cells (31), which concurs with the results of the present study, as overexpression of ATF3 activated the Wnt/β-catenin pathway in macrophages. Various components of the Wnt/β-catenin signaling pathway are involved in the inflammatory response, including in inflammatory conditions in humans and in the LPS-treated macrophage cell line (32). Wnt upregulates the expression levels of TNC (20), which is a large hexameric extracellular matrix glycoprotein that is highly expressed during embryonic development, cancer invasion and wound healing. It has been reported that TNC may be expressed in macrophages and regulates their behavior and function (21,22). TNC has been suggested to act as pro-inflammatory modulator in various diseases (33,34) and accelerates macrophage migration (35). The results of the present study demonstrated that overexpression of ATF3 upregulated TNC expression levels via the Wnt/β-catenin signaling pathway. In addition, the present study demonstrated that TNC was an effector for ATF3 in modulating macrophage migration and M2 polarization.

In conclusion, the present study revealed that overexpression of ATF3 in macrophage cells promoted their migration to chemotaxis-inducing agent MCP-1, and influenced the M1/M2 phenotype. These results emphasize that ATF3 expression levels affect macrophages, partially by upregulating TNC via the Wnt/β-catenin signaling pathway. The present study may provide an insight into the positive regulation of ATF3 on macrophage migration, and tissue regeneration via modulation of the M2 macrophage.

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