Int J Clin Exp Med 2018;11(8):7917-7926
www.ijcem.com /ISSN:1940-5901/IJCEM0077639

Original Article

Autophagy attenuates TNF-α-induced proliferation inhibition in the A375 melanoma cell line

Zhaoyan Liu, Jianying Gu, Chuanyuan Wei, Lu Lu, Nanhang Lu, Yong Zhang, Zihao Feng, Yanwen Yang, Fazhi Qi

Department of Plastic Surgery, Zhongshan Hospital, Fudan University, Shanghai, P.R. China

Received April 9, 2018; Accepted May 16, 2018; Epub August 15, 2018; Published August 30, 2018

Abstract: *Objective:* Autophagy plays multiple context-dependent roles in melanomagenesis and treatment resistance, and it has recently been reported that autophagy may be regulated by TNF-α, an important immunoinflammatory cytokine that mediates cell proliferation, differentiation and death. However, the role and mechanism of TNF-α in autophagy remainunclear. In this study we investigated the role and mechanism of TNF-α in autophagy in A375 cells. *Methods:* CCK-8 assays, colony formation assays and Cell-IQ Alive Image Monitoring System were used to investigate the viability, proliferation and migration of A375 cells induced by TNF-α, and Western blotting was used to explore the involvement of the main signals in this process; JNK, p38, ERK and P65. We used western blotting to detecte the expression of autophagy-associated proteins that induced by TNF-α. *Results:* TNF-α inhibited cell proliferation but not migration in A375 cells, and this effect was mediated by the p38/NF-κB signaling pathway. The expression of autophagy-associated proteins in cells treated with TNF-α was down-regulated compared to the control groups, and p38 activation was significantly up-regulated during this process. After all these studies, we showed the protective role of autophagy in TNF-α-induced proliferation inhibition in the A375 melanoma cell line. *Conclusion:* These data show that TNF-α-induced proliferation inhibition in the A375 melanoma cell line, and autophagy plays a protective role in this process.

Keywords: TNF-α, autophagy, proliferation, A375

Introduction

Malignant melanoma has the highest mortality rate of cutaneous tumors originating from normal melanocytes, accounts for 75% of all skin cancer-related mortality and is the most common cause of cancer-related death among young people [1]. Although early tumors can be cured by surgical resection and the average 5-year survival rate reaches 99% [2], once distal and systemic metastasis occurs, the 5-year survival rate falls to 10% or less [3]. Over the past 20 years, melanoma morbidity has increased to more than that of any other cancers worldwide, affecting 21.9 and 55.4 people per 10000 people in the USA and Australia, respectively [4]. Therefore, reliable biomarkers and treatment strategies are urgently needed to prevent premature loss of life.

Many immunoinflammatory cytokines, synthesized and secreted by tumor and stromal components, play important roles in rapid progression and metastasis [5]. Of these cytokines, TNF-α has anti-tumor activity in various tumor cell lines. For example, TNF-α was used as a combination therapy in a clinical drug trial and produced encouraging therapeutic effects [6]. Then, a large number of clinical trials were conducted to confirm the effectiveness of TNF-α in soft tissue sarcomas and metastatic melanoma [7]. However, a large amount of data have confirmed that TNF-α functions as a tumor-promoting factor [8]. Indeed, TNF-α is critical for early tumor progression [9]; mediates the proliferation, differentiation and death of multiple cells [10, 11]; and may even provide new solutions for targeted immunotherapy. Currently, TNF-α seems to be the most fascinating and intensely studied cytokine over the past three decades and has an intricate role in melanomagenesis [12].

Studies have shown that autophagy is a physiological cellular process which degrades and recycles cytoplasmic proteins and organelles in

melanoma cells [13-15], thereby maintaining metabolic needs and renewing certain organelles. Indeed, autophagy was found to be associated with metabolic stress in tumor regions, where it supports tumor cell survival. For this reason, autophagy is deemed a promoter of apoptosis under certain circumstances [13, 14]. Other studies have reported that oncogenic transformation and tumor development are associated with apoptotic pathway deficiencies. Now, a growing body of evidence suggests that autophagy may be equally important for tumor inhibition and promotion, depending on the tumor microenvironment. Along with increasing research on autophagy in cancer, autophagy defects have been shown to promote cancer, which shed light on how stimulating autophagy might be an approach for cancer prevention. Consistently, several autophagy-regulating proteins, including LC3 and Beclin-1, that have been used in many experiments to measure autophagic turnover were revealed to be reliable biomarkers for the presence of autophagy in tumors [15]. In addition, the proteins ATG3, ATG5, ATG7, ATG12 and ATG16L1 are also necessary for autophagy and have been proposed as potential biomarkers.

Both TNF-α and autophagy are related to tumor development and cancer therapy, while the relationship between these factors still remains largely unknown in melanoma. In this study, the effect and mechanism that TNF-α plays on autophagy were studied in A375 cells, and TNF-α-induced proliferation inhibition was investigated.

Material and methods

Cell culture and reagents

The melanoma A375 cell line was purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China); was cultured in DMEM medium (Gibco, NY, USA) supplemented with 10% FBS (Gibco, NY, USA), 100 μg/ml streptomycin and 100 U/ml penicillin; and was maintained in an incubator at 37°C with 5% CO_2. All the experiments used cells in good condition and at a suitable density. The NF-κB pathway inhibitor PDTC (10 nM), p38 pathway inhibitor SB203580 (10 μM), and autophagy inducer rapamycin [16, 17] (V900930, 20 nM) were all purchased from Sigma (St Louis; USA) and used at the recommended concentrations.

Western blot

Cells were lysed in RIPA buffer (Beyotime, Shanghai, China) supplemented with phosphatase and protease inhibitors for 30 min and then centrifuged (4°C, 12000 r/min) for 15 min. BCA reagent (Beyotime, Shanghai, China) was used for detecting the total protein concentration. Then, total protein (15 μg) was separated on SDS-PAGE (5% to 10%) gels and transferred onto polyvinylidene fluoride membranes. After blocking with 5% bovine serum albumin for 1 h, membranes were incubated with the following antibodies overnight at 4°C: anti-human polyclonal Beclin-1 (1:1000, ab62-557, Abcam, Cambridge, UK), anti-human polyclonal LC3 (1:1000, ab48394, Abcam, Cambridge, UK), anti-human monoclonal ATG3 (1:1000, ab108251, Abcam, Cambridge, UK), anti-human monoclonal ATG5 (1:1000, ab108237, Abcam, Cambridge, UK), anti-human monoclonal ATG7 (1:1000, ab52472, Abcam, Cambridge, UK), anti-human monoclonal ATG12 (1:1000, ab109491, Abcam, Cambridge, UK), anti-human monoclonal ATG16L1 (1:1000, ab187671, Abcam, Cambridge, UK) and anti-human polyclonal β-actin (1:3000, ab8227, Abcam, Cambridge, UK). Membranes were then exposed to secondary antibody for 60 min. Bands were incubated with a DAB kit and analyzed with an imaging system. Band densities in every sample were quantified using Adobe Photoshop CS6 (Adobe, San Jose, CA, USA).

CCK-8, colony formation assay

Cells were seeded with complete medium containing a concentration gradient of TNF-α (0, 1, 10, 50 ng/ml), and 1000 cells were in each well of a 96-well. At each time point (0, 1, 2, 3, 4, 5, 6 d), 10 μl of CCK-8 reagent (Yeasen, Shanghai, China) was dissolved in culture medium and then added into 6 replicate wells. Cells were incubated for 3 h in 5% CO_2 at 37°C, and the absorbance at 450 nm was measured in each individual well. The data obtained were graphed on a line chart using GraphPad Prism 6 (Version 6.0; GraphPad Software; San Diego, CA, USA).

For the colony formation assay, cells were digested into a single cell suspension and seeded into 6-cm culture dishes, which each contained 1000 cells. To each dish, appropriate complete medium was added, and then, the cells were cultured in an incubator for two

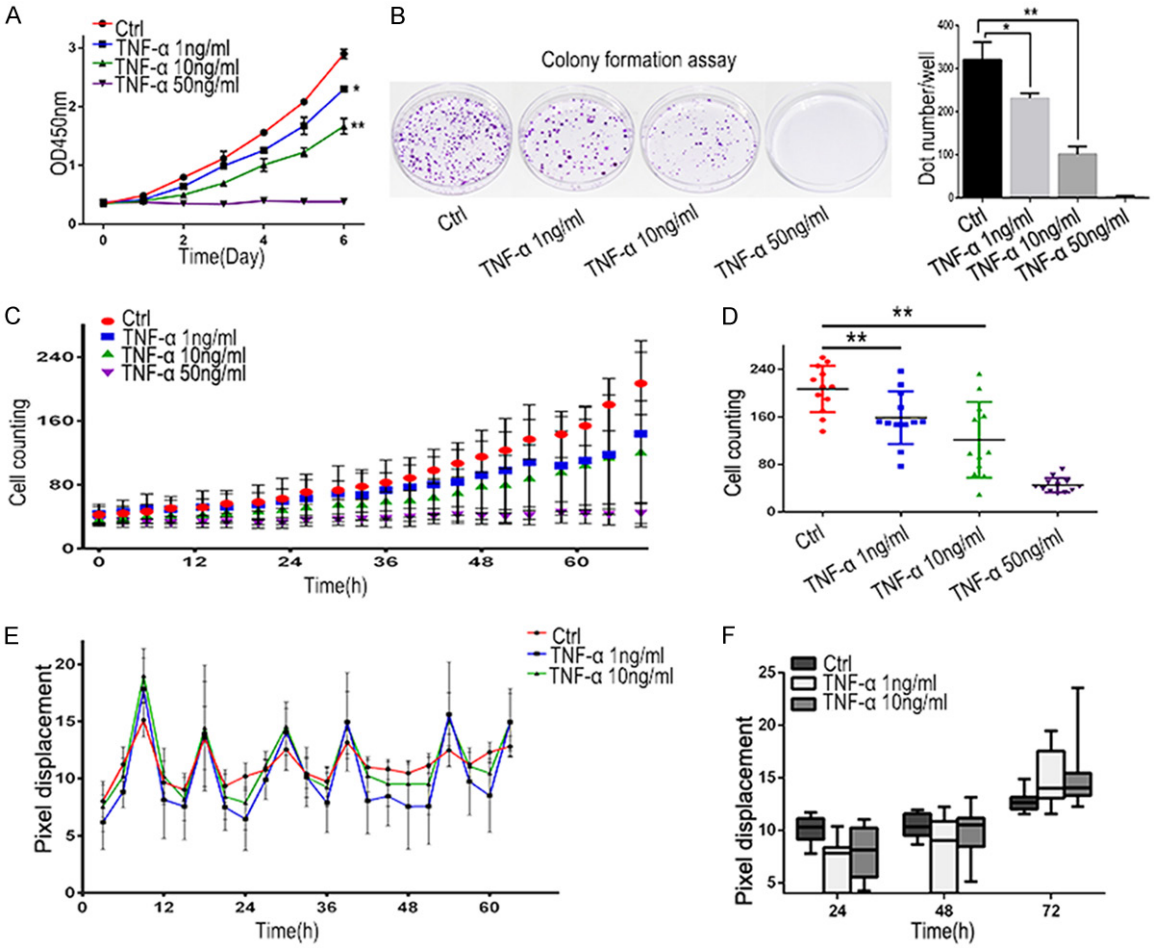

Figure 1. TNF-α inhibits the proliferative ability but not the migration of A375 cells. (A and B) Cell proliferative ability was significantly inhibited by TNF-α in a concentration-dependent manner, as determined through CCK-8 and colony formation assays; (C) The proliferation inhibition induced by TNF-α was further confirmed by the Cell-IQ Alive Image Monitoring System, and (D) Cell counting was performed after 72 h; (E) Pixel displacement was measured in groups with or without TNF-α in real-time through the Cell-IQ Alive Image Monitoring System; (F) The specific migration distance is shown at three time points. Values are the means ± S.D. of at least three independent experiments. *P<0.05, **P<0.01.

weeks. After that, cell colonies were washed with phosphate-buffered saline, fixed with 4% paraformaldehyde and stained with 0.4% crystal violet for 15 min. The number of colonies containing more than 10 cells was counted manually, and the numbers from duplicate wells were averaged.

Live measurement of cell bio-behaviors

Cell bio-behaviors, including total cell number, cell movement and cell morphology, were supervised by a real-time cell monitoring system using a Cell-IQ cell culturing platform (Chip-Man Technologies, Tampere, Finland) equipped with a phase-contrast microscope (Nikon CFI Achromat phase contrast objective with 10× magnification, Japan) and camera (Nikon, Japan). The instrument was controlled by Imagen software (Chip-Man Technologies). Images were captured at 5 min intervals for 72 h. Analysis was carried out with freely distributed image software (Cell-IQ Imagen v2.9.5c, McMaster Biophotonics Facility, Hamilton, ON, Canada) using the Manual Tracking plug-in created by Fabrice Cordelieres (Institut Curie, Orsay, France). The Cell-IQ system uses machine vision technology to monitor and record time-lapse data and can also analyze and quantify cell functions and morphological parameters. The distance moved for each individual cell in the image field was measured. Each group contained 6-12 replicate image sites.

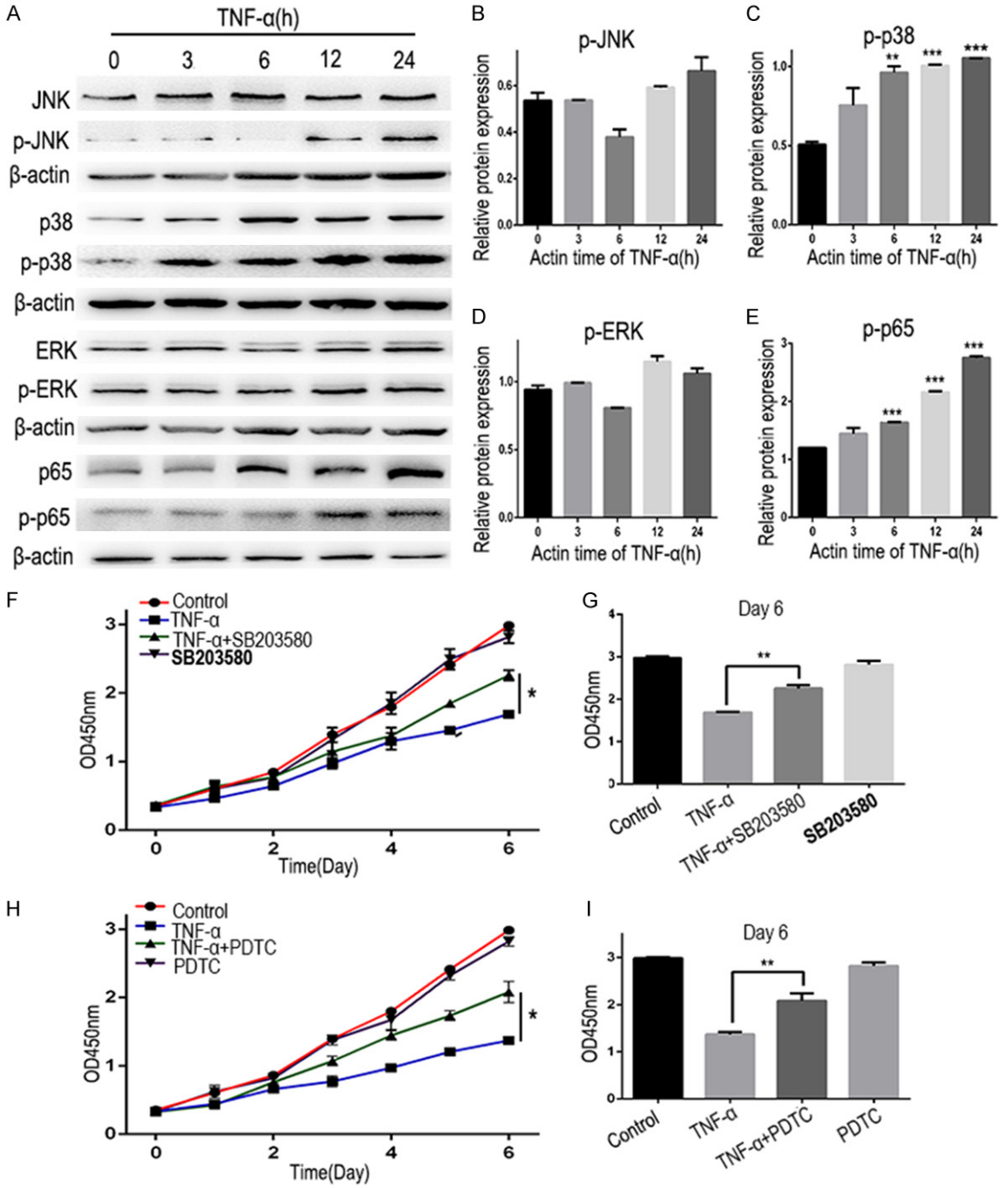

Figure 2. TNF-α inhibits proliferation through the p38/NF-κB pathway. (A) Western blotting was used to detect JNK, p38, ERK and p65 protein expression following treatment with a TNF-α concentration gradient; (B-E) Quantification of protein expression shown in (A) and normalized to β-actin expression; (F, G) CCK-8 assays were performed to examine the proliferative ability following TNF-α, TNF-α/SB203580, SB203580, and blank control treatments; after 6 days, the 450 nm OD value was then detected among the groups to determine differences; (H, I) CCK-8 assays were performed to examine the proliferative ability after TNF-α, TNF-α/PDTC, PDTC, and blank control treatment; after 6 days, the 450 nm OD value was detected among the groups to identify differences. Values are the means ± S.D. of at least three independent experiments. *P<0.05, **P<0.01, ***P<0.001.

Statistical analysis

All results presented here were proven in at least three separate experiments. Data were shown as means ± S.D., and t-test analyses with SPSS software (version 20.0, Chicago, USA) was performed for each experimental groups (induced by TNF-α in different concen-

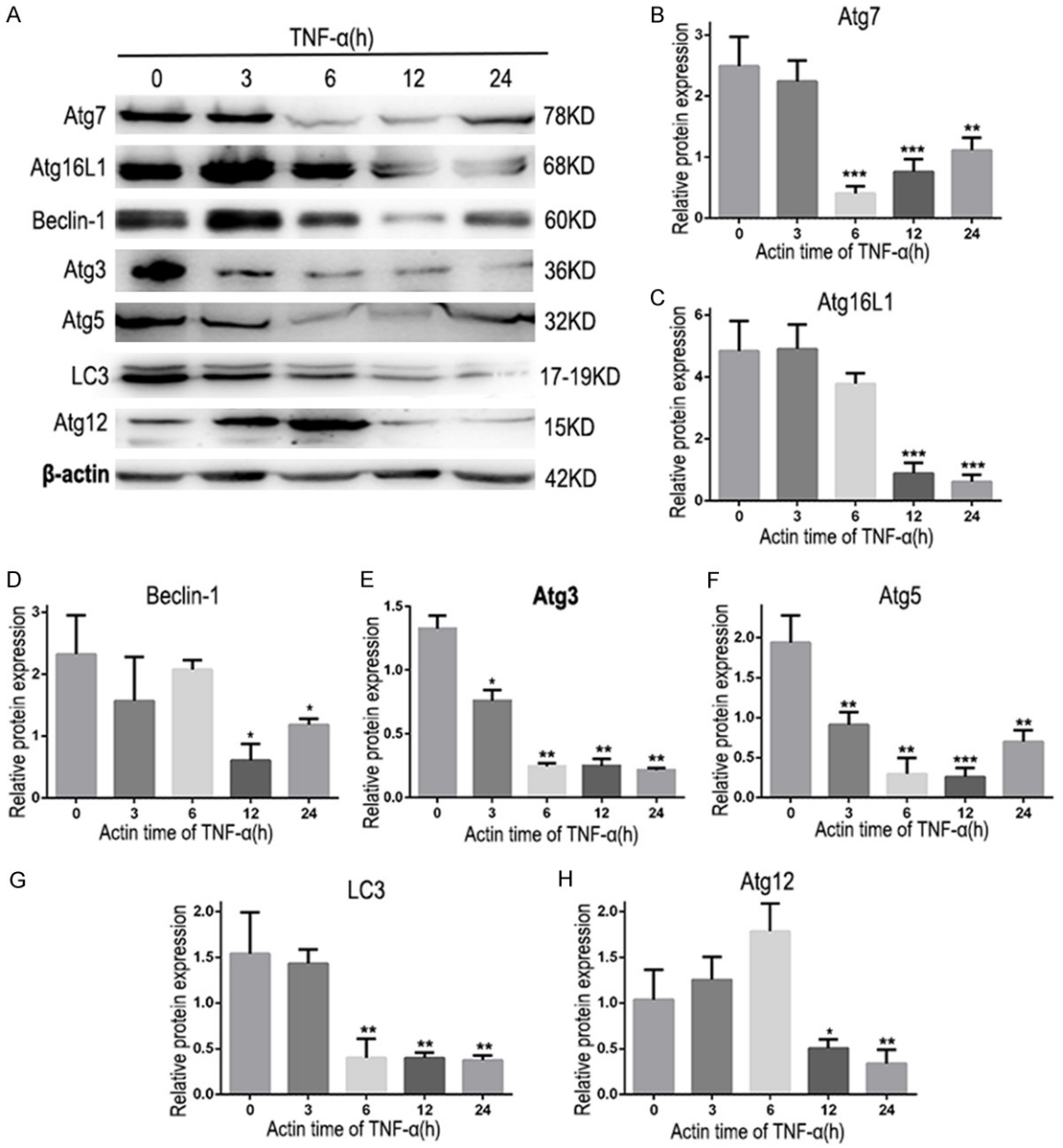

Figure 3. TNF-α inhibits the process of autophagy. (A) Western blotting was used to detect the expression of autophagy-associated proteins following a temporal gradient of TNF-α exposure, and β-actin was used as the internal reference; (B-H) Quantification of the protein expression shown in (A) and normalized to β-actin expression. Values are the means ± S.D. of at least three independent experiment. *P<0.05, **P<0.01, ***P<0.001.

trations) and control groups. Statistical significance was defined as $p<0.05$.

Results

TNF-α inhibits the proliferation but not migration of A375 cells

TNF-α plays an intricate role in tumor progression. According to the characteristics of abnormal proliferation and early lymphatic and hematogenous metastasis in malignant melanoma, CCK-8 and clone formation assays were first performed in the A375 cell line with a concentration gradient of TNF-α (**Figure 1A**, **1B**). We found that proliferation capacity was significantly decreased with increasing TNF-α concentrations. To further confirm this result, we performed another proliferative assay with the Cell-IQ Alive Image Monitoring System (**Figure 1C**, **1D**), which can provide further insight into cell activity, growth rate and behavior and real time kinetic data on morphological changes. In

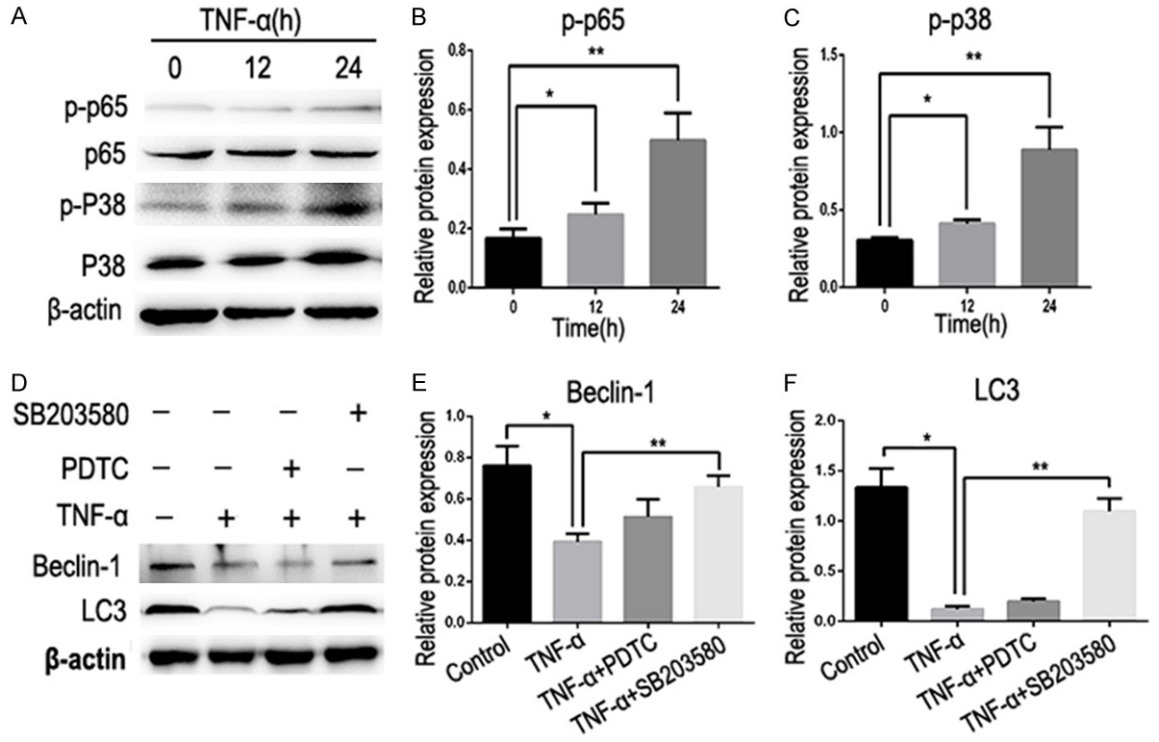

Figure 4. TNF-α induces autophagy inhibition through the p38 pathway. (A) Western blotting was used to detect the expression of p-p38 and p-p65 following a temporal gradient of TNF-α exposure, and β-actin was used as the internal reference; (B, C) Quantification of the protein expression shown in (A) and normalized to β-actin expression; (D) The expression of Beclin-1 and LC3 was detected after treatment with TNF-α alone or combined with p38 and NF-κB pathway inhibitors, and β-actin was used as the internal reference; (E, F) Quantification of the protein expression shown in (A) and normalized to β-actin expression. Values are the means ± S.D. of at least three independent experiments. *P<0.05, **P<0.01.

addition, the results were consistent with the CCK-8 and clone formation assays. Using the above experiments, we found that the anti-proliferative effect was significant when the TNF-α concentration was 10 ng/ml, and almost all cells died at 50 ng/ml TNF-α. Therefore, we chose 10 ng/ml as a suitable concentration for follow-up experiments. Second, the function of TNF-α in cell migration was detected through the Cell-IQ Alive Image Monitoring System (**Figure 1E**, **1F**), and there was no obvious difference in cell migration among the different TNF-α concentrations. Then, we recorded the migration distance at three different time points and marked the migration trajectory: again, no substantial differences were found.

TNF-α inhibited proliferation through the p38/NF-κB pathway

The above data showed that TNF-α significantly suppressed cell proliferation in a dose-dependent manner. To reveal the possible mechanism and determine the main responsible signaling molecule involved in the TNF-α pathway, we first examined the levels of p38, JNK, ERK1/2 and NF-κB and their active protein forms at different time points (a temporal gradient: 0, 3, 6, 12, 24 h) and found that the p-p38 and p-p65 protein levels were significantly upregulated compared to the those in the control group, while no significant changes were identified regarding p-JNK and p-ERK protein expression (**Figure 2A-E**). Then, we inhibited the p38 pathway by employing the specific inhibitor SB203580. Through the CCK-8 assay growth curve (**Figure 2F**), we found that there was no difference between the TNF-α and SB203580 groups, while there was a significant difference between the TNF-α and TNF-α/SB203580 groups. In addition, we compared the optical density (OD) values at 450 nm on day 6 among the different treatment groups (**Figure 2G**), and the OD value was substantially higher in the TNF-α/SB203580 group than in the TNF-α group. Similarly, we evaluated the NF-κB pathway with the specific inhibitor PDTC, and the result was similar (**Figure 2H**, **2I**). These data indicate that TNF-α inhibited A375 cell proliferation possibly

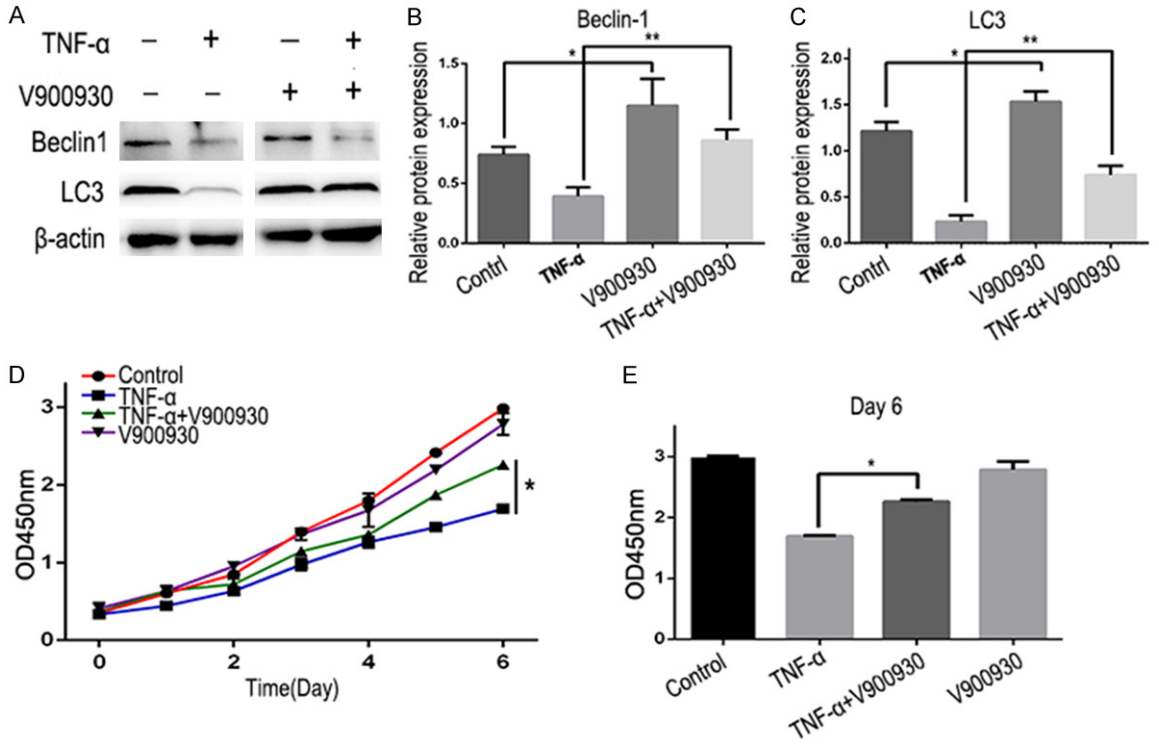

Figure 5. Autophagy attenuates the inhibited proliferation induced by TNF-α. (A) Western blotting was used to examine the expression of autophagy-associated proteins following treatment with TNF-α alone, rapamycin alone or TNF-α and rapamycin combined, and β-actin was used as the internal reference; (B, C) Quantification of the protein expression shown in (A) and normalized to β-actin expression; (D) CCK-8 assays were used to detect the proliferative ability following treatment with TNF-α alone, rapamycin alone or TNF-α and rapamycin combined; (E) Quantification of the 450 nm OD values shown in (A) after 6 days. Values are the means ± S.D. of at least three independent experiments. *P<0.05, **P<0.01.

though p38/NF-κB signaling pathway activation.

TNF-α inhibits the process of autophagy

Here, the relationship between TNF-α and autophagy was investigated by determining LC3, Beclin-1, ATG3, ATG5, ATG7, ATG12 and ATG16-L1 protein levels. According to Western blot analysis (**Figure 3A-H**), we found that Beclin 1, Atg3, and atg7 in the TNF-α group was temporarily elevated compared to the control group at 1 hour, 3 hours and 6 hours. After 12 hours, Becline1, LC3, Atg7, Atg16L1, Atg3, Atg5 and Atg12 protein expression significant decreased in the TNF-α group compared to the control group. All these data suggest that the expression of autophagic protein decreases in the long-term effects of TNF-α.

TNF-α inhibited autophagy through the p38 pathway

TNF-α plays an inhibitory effect on the process of autophagy. To further understand the underlying mechanism, we detected the protein expression of Beclin-1 and LC3, which are widely used to determine the presence of autophagy. Since the p38 and NF-κB pathways were activated after the TNF-α stimulation (**Figure 4A-C**), we examined Beclin-1 and LC3 expression after cells were treated with both TNF-α and a p38 or NF-κB pathway inhibitor (**Figure 4D-F**). We found that LC3 and Beclin-1 protein levels were substantially higher in the TNF-α/SB203580 group compared with the TNF-α group. These data indicated that TNF-α inhibits the process of autophagy possibly through activation of the p38 signaling pathway.

Autophagy attenuates the proliferation inhibition induced by TNF-α

Autophagy plays a crucial role in the progression of melanoma, including cell proliferative ability. We speculated that autophagy may play a role in TNF-α-induced proliferation. To test this hypothesis, we used the autophagy inducer V900930 (namely, Rapamycin) and verified the effect through Western blotting (**Figure**

5A-C). Beclin-1 and LC3 expression was substantially increased in the V900930-treated group. Then, we examined cell viability through CCK-8 assays (**Figure 5D, 5E**), and there was no obvious difference between the control and V900930-treated groups, which indicated that V900930 does not influence A375 cell proliferation. However, after TNF-α treatment, V900930 significantly weakened the proliferation inhibition compared with the inhibition observed in the TNF-α alone group, which suggests that in response to TNF-α, autophagy protects against cell proliferation inhibition.

Discussion

TNF-α is one of the central factors in inflammatory responses and activates a series of downstream transduction proteins by binding to the receptor TNFR1, ultimately activating multiple signaling pathways that lead to various biological effects, such as inflammatory responses, apoptosis, proliferation, angiogenesis, tumor invasion, and tumor metastasis [18]. In general, the NF-κB and MAPK pathways are the main downstream signals activated by TNF-α binding to TNFR1 [19]. Another important pathway is the binding of TNF-α to TNF receptor I-associated death domain protein, which recruits caspase-8 and ultimately leads to cell apoptosis.

Autophagy can maintain cell energy supply levels by effectively using cell "waste" to prevent cell damage; autophagy deficiencies in cells (in non-apoptotic environments) can lead to increased genetic instability, chronic cell death and inflammatory responses, eventually contributing to tumor development. Although it is very difficult to track tumor cell autophagy in human tissues, studies have shown that autophagy favors cell survival in tumor microenvironments. Evidence supporting an up-regulated autophagy level in melanoma has been confirmed by the immunohistochemical detection of LC3-II in melanoma tissues [20-22], as well as by electron microscopy studies displaying features typical of active autophagy [23]. Therefore, autophagy regulation may be an option to explore for early melanoma therapy, and many scholars have begun to explore the use of autophagy as a new drug target in melanoma treatment. For example, Rangwala and his colleagues reported that treatment with hydroxychloroquine and dose-intense temozolomide in patients with advanced solid tumors and melanoma is associated with autophagy modulation [24], and Marino et al. showed that autophagy induction following esomeprazole treatment delayed melanoma cell death [25]. As an ancient, cellular catabolic pathway, autophagy is worth continuously and intensively studying in relation to the melanoma field and is expected to become an alternative target in melanoma.

Here, we revealed the protective role of TNF-α in melanoma cell autophagy, the main findings are as follows: First, proliferative ability was largely inhibited by TNF-α, as determined through CCK-8 assays, colony formation assays, and Cell-IQ Alive Image Monitoring System, and the inhibitory effect was substantially increased as the dose increased. Cell growth almost stopped when the concentration reached 50 ng/ml. Furthermore, we found that TNF-α inhibited proliferation through the p38/NF-κB signaling pathway. Second, the role TNF-α plays in autophagy was evaluated, and the presence of autophagy was detected by the specific biomarkers Beclin-1, LC3, ATG3, ATG5, ATG7, ATG12 and ATG16L1. Following the temporal gradient of TNF-α exposure, the expression of autophagy-associated proteins was gradually decreased, and the data were statistically significant. Then, the p38 and NF-κB signal pathways were inhibited by specific inhibitors, and autophagy-associated proteins were significantly up-regulated in the SB203580/TNF-α group compared to the group with only TNF-α added, which indicates that autophagy was inhibited by TNF-α through the p38 signaling pathway. Importantly, the autophagy inducer V900930 significantly weakened the ability of TNF-α to inhibit proliferation, which suggests that autophagy plays a protective role against the inhibition of cell proliferation. In conclusion, our work demonstrates that TNF-α inhibits the proliferative ability of A375 cells through the p38/NF-κB signaling pathway, and TNF-α inhibited autophagy via the p38 signaling pathway but not the NF-κB pathway. Thus, we have demonstrated that autophagy plays a protective role in TNF-α-induced proliferation inhibition.

Acknowledgements

The authors would like to thank the Pathology Department of Zhongshan Hospital for providing tumour samples.

Disclosure of conflict of interest

None.

Abbreviations

TNF-α, tumor necrosis factor-alpha; CCK-8, cell counting kit-8; ATG, autophagy related gene; EMT, epithelial-mesenchymal transition; LC3, microtubule-associated light chain 3; FBS, fetal bovine serum; BCA, bicinchoninic acid; SPSS, Statistics Package for Social Science; NF-κB, nuclear factor kappa B; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase.

Address correspondence to: Jianying Gu, Department of Plastic Surgery, Zhongshan Hospital, Fudan University, 180 Feng Lin Road, Shanghai 200032, P.R. China. Tel: +86-21-60268029; Fax: +86-21-64037269; E-mail: zhongshanhospital@163.com

References

[1] Cichorek M, Wachulska M, Stasiewicz A and Tyminska A. Skin melanocytes: biology and development. Postepy Dermatol Alergol 2013; 30: 30-41.

[2] Gray-Schopfer V, Wellbrock C and Marais R. Melanoma biology and new targeted therapy. Nature 2007; 445: 851-857.

[3] Tsao H, Chin L, Garraway LA and Fisher DE. Melanoma: from mutations to medicine. Genes Dev 2012; 26: 1131-1155.

[4] Rat C, Hild S, Gaultier A, Khammari A, Bonnaud-Antignac A, Quereux G, Dreno B and Nguyen JM. Anxiety, locus of control and sociodemographic factors associated with adherence to an annual clinical skin monitoring: a cross-sectional survey among 1000 high-risk French patients involved in a pilot-targeted screening programme for melanoma. BMJ Open 2017; 7: e016071.

[5] Carswell EA, Old LJ, Kassel RL, Green S, Fiore N and Williamson B. An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci U S A 1975; 72: 3666-3670.

[6] O'Malley WE, Achinstein B and Shear MJ. Journal of the National Cancer Institute, Vol. 29, 1962: Action of bacterial polysaccharide on tumors. II. Damage of sarcoma 37 by serum of mice treated with Serratia marcescens polysaccharide, and induced tolerance. Nutr Rev 1988; 46: 389-391.

[7] van Horssen R, Ten Hagen TL and Eggermont AM. TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility. Oncologist 2006; 11: 397-408.

[8] Eggermont AM, de Wilt JH and ten Hagen TL. Current uses of isolated limb perfusion in the clinic and a model system for new strategies. Lancet Oncol 2003; 4: 429-437.

[9] Mueller MM. Inflammation in epithelial skin tumours: old stories and new ideas. Eur J Cancer 2006; 42: 735-744.

[10] Yoshizumi M, Abe J, Tsuchiya K, Berk BC and Tamaki T. Stress and vascular responses: atheroprotective effect of laminar fluid shear stress in endothelial cells: possible role of mitogen-activated protein kinases. J Pharmacol Sci 2003; 91: 172-176.

[11] Wang X and Lin Y. Tumor necrosis factor and cancer, buddies or foes? Acta Pharmacol Sin 2008; 29: 1275-1288.

[12] Aggarwal BB, Gupta SC and Kim JH. Historical perspectives on tumor necrosis factor and its superfamily: 25 years later, a golden journey. Blood 2012; 119: 651-665.

[13] Levine B and Yuan J. Autophagy in cell death: an innocent convict? J Clin Invest 2005; 115: 2679-2688.

[14] Martinet W, Agostinis P, Vanhoecke B, Dewaele M and De Meyer GR. Autophagy in disease: a double-edged sword with therapeutic potential. Clin Sci (Lond) 2009; 116: 697-712.

[15] Tang DY, Ellis RA and Lovat PE. Prognostic impact of autophagy biomarkers for cutaneous melanoma. Front Oncol 2016; 6: 236.

[16] Li R, Zhang L, Shi Q, Guo Y, Zhang W and Zhou B. A protective role of autophagy in TDCIPP-induced developmental neurotoxicity in zebrafish larvae. Aquat Toxicol 2018; 199: 46-54.

[17] Li Q, Li L, Fei X, Zhang Y, Qi C, Hua S, Gong F and Fang M. Inhibition of autophagy with 3-methyladenine is protective in a lethal model of murine endotoxemia and polymicrobial sepsis. Innate Immun 2018; 1753425918771170.

[18] Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. Nat Rev Immunol 2003; 3: 745-756.

[19] Wajant H, Pfizenmaier K and Scheurich P. Tumor necrosis factor signaling. Cell Death Differ 2003; 10: 45-65.

[20] Hersey P and Zhang XD. Adaptation to ER stress as a driver of malignancy and resistance to therapy in human melanoma. Pigment Cell Melanoma Res 2008; 21: 358-367.

[21] Lazova R, Klump V and Pawelek J. Autophagy in cutaneous malignant melanoma. J Cutan Pathol 2010; 37: 256-268.

[22] Lazova R, Camp RL, Klump V, Siddiqui SF, Amaravadi RK and Pawelek JM. Punctate LC3B expression is a common feature of solid tumors and associated with proliferation, metastasis, and poor outcome. Clin Cancer Res 2012; 18: 370-379.

[23] Ma XH, Piao S, Wang D, McAfee QW, Nathanson KL, Lum JJ, Li LZ and Amaravadi RK. Measurements of tumor cell autophagy predict invasiveness, resistance to chemotherapy, and survival in melanoma. Clin Cancer Res 2011; 17: 3478-3489.

[24] Rangwala R, Leone R, Chang YC, Fecher LA, Schuchter LM, Kramer A, Tan KS, Heitjan DF, Rodgers G, Gallagher M, Piao S, Troxel AB, Evans TL, DeMichele AM, Nathanson KL, O'Dwyer PJ, Kaiser J, Pontiggia L, Davis LE and Amaravadi RK. Phase I trial of hydroxychloroquine with dose-intense temozolomide in patients with advanced solid tumors and melanoma. Autophagy 2014; 10: 1369-1379.

[25] Marino ML, Pellegrini P, Di Lernia G, Djavaheri-Mergny M, Brnjic S, Zhang X, Hagg M, Linder S, Fais S, Codogno P and De Milito A. Autophagy is a protective mechanism for human melanoma cells under acidic stress. J Biol Chem 2012; 287: 30664-30676.