ADAM12 silencing promotes cellular apoptosis by activating autophagy in choriocarcinoma cells

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Received September 16, 2019; Accepted January 17, 2020

DOI: 10.3892/ijo.2020.5007

Abstract. ADAM metallopeptidase domain 12 (ADAM12) has been demonstrated to mediate cell proliferation and apoptosis resistance in several types of cancer cells. However, the effect of ADAM12 silencing on the proliferation and apoptosis of choriocarcinoma cells remains unknown. The present study revealed that ADAM12 silencing significantly inhibited cellular activity and proliferation in the human choriocarcinoma JEG3 cell line and increased the rate of apoptosis. In addition, ADAM12 silencing significantly increased the expression levels of the autophagy proteins microtubule-associated protein-light-chain 3 (LC3B) and autophagy related 5 (ATG5) and the fluorescence density of LC3B in JEG3 cells. However, the suppression of autophagy by 3-methyladenine could block ADAM12 silencing-induced cellular apoptosis. ADAM12 silencing reduced the levels of the inflammatory factors interleukin-1β, interferon-γ and TNF-α, and inactivated nuclear p65-NF-κB and p-mTOR in JEG3 cells. The downregulation of p-mTOR expression by ADAM12 silencing was rescued in 3-methyladenine-treated JEG3 cells, indicating that mTOR might participate in the autophagy-mediated pro-apoptotic effect of ADAM12 silencing. In conclusion, ADAM12 silencing promoted cellular apoptosis in human choriocarcinoma JEG3 cells, which might be associated with autophagy and the mTOR response. These findings indicate that ADAM12 silencing might be a potential novel therapeutic target for choriocarcinoma.

Introduction

Choriocarcinoma is a highly malignant tumour that develops from trophoblast cells and usually occurs in the uterus, and it can cause severe local damage and metastasize to other areas of the body (1). As the clinical presentation of choriocarcinoma may vary, diagnosis may be challenging and the prognosis of patients with choriocarcinoma is related to the clinical stage and trophoblastic activity (1,2). It is widely recognized that the regulatory process of trophoblast invasion may be associated with growth factors, chemokines, protein kinases and signaling pathways, and the changes in the regulation of these factors may lead to various pathological changes (3). Therefore, a deeper understanding of the mechanisms underlying cell proliferation and apoptosis in choriocarcinoma is required to develop novel treatment strategies and improve patient prognosis.

The disinterring and metalloprotease (ADAM) family consists of several type I transmembrane proteins that have been widely reported to be involved in various physiological functions, such as cell-binding and intracellular signalling, related to human tumour metastasis (4,5). Members of the ADAM family have two major structural regions, the de-integrin and the metal matrix protease regions, which degrade the extracellular matrix and control cell adhesion and movement by regulating cell adhesion and protease activity (5). Among the members of the ADAM family, ADAM metallopeptidase domain 12 (ADAM12) expression is highly associated with several types of epithelial cancer, including breast, skin, ovarian, stomach, lung, prostate and brain cancer (6-10). ADAM12 contributes to cell differentiation, tumour cell proliferation, migration and invasion (8,11-18) as well as apoptosis and endocrine resistance (19). Apoptosis is a well-known form of programmed cell death and is a highly regulated and controlled process. Autophagy allows the removal of unnecessary or dysfunctional cellular components and allows the orderly degradation and recycling of cellular components (20-22). Both apoptosis and autophagy are known to play roles in several diseases, including cancer (23-26). However, the specific role of ADAM12 silencing in the apoptosis and autophagy of choriocarcinoma cells, as well as the related mechanisms, has not yet been described. Therefore, the present study investigated the effects of ADAM12 silencing on the proliferation and apoptosis of the human choriocarcinoma JEG-3 cell line. Additionally, the potential mechanisms involved in autophagy and other signalling pathways were explored in JEG-3 cells following ADAM12 silencing.

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Key words: ADAM12, choriocarcinoma cell, proliferation, apoptosis, autophagy
Materials and methods

Cell culture and transfection. The human choriocarcinoma JEG-3 cell line was acquired from the Cell Bank of Type Culture Collection of the Chinese Academy of Sciences (27). The cells were cultured in DMEM medium (Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.) and maintained in an incubator containing 5% CO<sub>2</sub> at 37°C (28).

ADAM12-small interfering RNAs (ADAM12-siRNA; target 1, 5'-GCCTGATATCGCAATGTTCAAA-3'; target 2, 5'-CGCTGCAAATTACCGGTAAT-3'; and target 3, 5'-GCC AGATGAGAGCTAGAT-3') were synthesized by Shanghai GeneChem Co., Ltd. The siRNA targetted expression=(the grey value of measured protein value of internal parameter).

Cell proliferation. JEG-3 cells in DMEM were seeded in a 96-well plate (5x10<sup>3</sup> cells/well) and incubated for 24 h at 37°C. In addition, ADAM12-siRNA, ADAM12-siRNA-3MA and scramble-siRNA groups were included. At 24 h post-transfection, 10 µM 3-methyladenine (3MA; cat. no. M9281-100 mg; Sigma-Aldrich) was co-applied with siRNA transfection and used for blocking autophagy in JEG-3 cells, the ADAM12-siRNA-3MA group for 24 h.

Cell cycle analysis. JEG-3 cells were trypsinized to form a single cell suspension and washed 2-3 times with PBS. The number of cells was adjusted to 1x10<sup>6</sup> cells/ml. The cells were then resuspended in 1 ml pre-cooled PBS and centrifuged at 250 x g for 5 min at 4°C, and the supernatant was aspirated. The cells were gently resuspended with 20 µl PBS and incubated with 600 µl pre-cooled 100% ethanol (final concentration, 75%) overnight at 4°C. The fixed cells were centrifuged at 250 x g for 5 min at 4°C, and the supernatant was aspirated. Next, the cells were resuspended in 1 ml pre-cooled PBS, after which they were centrifuged at 250 x g for 5 min at 4°C and collected. This process was repeated 1-2 times to remove the ethanol. Next, the cells were incubated with 150 µl propidium iodide (Sigma-Aldrich; Merck KGaA) for 30 min at 4°C in the dark. The cells were subsequently analyzed using a CytoFLEX S flow cytometer (Beckman Coulter, Inc.) and CytExpert software (version 2.0; Beckman Coulter, Inc.). The percentage of cells in each stage of cell cycle was analyzed.

Flow cytometry detection of the apoptosis rate. The transfected and control JEG-3 cells were collected by trypsin digestion without EDTA, washed twice with PBS and centrifuged at 4°C for 5 min at 520 x g. Approximately 1x10<sup>6</sup> cells were collected. Then, 500 µl binding buffer was added to the cell suspension. The cells were incubated with 5 µl Annexin V-FITC and 5 µl propidium iodide (eBioscience™ Annexin V Apoptosis Detection Kit FITC; cat. no. 88-8005-72; Thermo Fisher Scientific, Inc.) for 5-15 min at room temperature in the dark. Apoptotic cells were detected within 1 h by a CytoFLEX S flow cytometer (Beckman Coulter, Inc.) and analysed using CytExpert software (version 2.0; Beckman Coulter, Inc.).

Western blotting. JEG-3 cells were treated with lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecylsulfate (SDS), 5 mM EDTA, 1 mM 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride, 5 µg/ml pepstatin, 5 µg/ml leupeptin, 5 µg/ml aprotinin and 10 mM 1,10-phenanthroline) for 30 min on ice. The samples were denatured by boiling in SDS sample buffer (SDS-PAGE Gel Preparation kit; CWBio). The protein concentration was determined using a BCA Protein Concentration Assay kit (CWBio), and 50 µg protein/lane were subjected to SDS-PAGE on 4-20% Tris-glycine gels and transferred onto polyvinylidene difluoride membranes. The membranes were dyed with 0.1% (w/v) ponceau S for 1-2 min at room temperature and washed. Then, the membranes were blocked with 5% milk in Tris-buffered saline and 0.1% Tween 20 (TBST) for 1 h at room temperature. The membranes were washed with TBST for 5 min, and incubated with primary antibodies (all used at a 1:1,000 dilution) against ADAM12 (cat. no. 14139-1-AP; ProteinTech Group, Inc.), autophagy related 5 (ATG5; cat. no. 10181-2-AP; ProteinTech Group, Inc.), microtubule-associated protein-light-chain 3 [LC3B, detected two bands LC3BI (upper bands) and LC3BII (lower bands); cat. no. 18725-1-AP; ProteinTech Group, Inc.], caspase-3 (detected pro- and cleaved caspase-3; cat. no. ab13847; Abcam), caspase-9 (detected pro- and cleaved caspase-9; cat. no. ab202068; Abcam), Bax (cat. no. 50599-2-lg; ProteinTech Group, Inc.), p53 (cat. no. 10442-1-AP; ProteinTech Group, Inc.), phosphorylated-mTOR (Ser2448; cat. no. ab109268; Abcam), mTOR (cat. no. ab2732; Abcam), p65-NF-κB (cat. no. 10745-1-AP; ProteinTech Group, Inc.); proliferating cell nuclear antigen (PCNA; cat. no. 10205-2-AP; ProteinTech Group, Inc.) and β-actin (cat. no. 60008-1-Ig; ProteinTech Group, Inc.) overnight at 4°C. The membranes were washed three times with TBST, 10 min/wash. Subsequently, the membranes were incubated with goat anti-rabbit IgG horseradish peroxidase-conjugated secondary antibodies (at 1:1,000 dilution; cat. no. SA00001-2; ProteinTech Group, Inc.) at room temperature for 1 h, and washed three times with TBST, 10 min/wash. The protein bands were visualized using the SuperSignal West Pico Chemiluminescent Substrate (EMD Millipore). ImageJ software (version 1.8.0; National Institutes of Health) was used to analyze the greyscale values, with β-actin or PCNA as the loading control. The calculation was performed as follows: The amount of relevant protein expression=(the grey value of measured protein/the grey value of internal parameter).
Immunofluorescence. JEG-3 cells were plated onto sterile coverslips and allowed to attach overnight at 4°C. JEG-3 cells were then washed with PBS and fixed with 4% paraformaldehyde in PBS (pH 7.4) for 20 min at room temperature. These cells were washed three times with PBS and permeabilized with 0.1% Triton X-100 in PBS for 5 min. The cells were then blocked with 1% bovine serum albumin (cat. no. V900933-100g; Sigma-Aldrich; Merck KGaA) and 22.52 mg/ml glycine in PBST (PBS + 0.1% Tween 20) for 30 min at 37°C to prevent non-specific binding of the antibodies. The cells were incubated with primary antibodies (diluted to 1:200 in 1% BSA in PBST) against LC3B (cat. no. 18725-1-AP; ProteinTech Group, Inc.) in a humidified incubator at 37°C for 1 h. After washing three times in PBS at 5 min for each wash, the cells were incubated for 1 h at 37°C in the dark with a fluorescein-conjugated secondary IgG antibody (1:200 in 1% BSA in PBS; Thermo Fisher Scientific, Inc.) in a humidified incubator at 37°C for 1 h. After washing three times with PBS for 5 min each in the dark, the cells were incubated with 0.1-1 µg/ml DAPI at room temperature for 1 min, washed with PBS and mounted with coverslips using a fluorescent mounting medium. The coverslip was sealed with nail polish to prevent drying and movement under the microscope. The slides were stored in the dark at -20 or 4°C. The slides were imaged using a Zeiss Axioplan-200 inverted fluorescence microscope (Carl Zeiss AG; magnification, x400). ImageJ software (version 1.8.0; National Institutes of Health) was used to analyse the immunofluorescence density (IFD). The formula used was as follows: IFD=(the fluorescence intensity of the region of interest/the area of the region of interest).

ELISA. A total of 50-100 µl of the prepared standard and samples of JEG-3 cells supernatant were added to an antibody-coated 96-well microplate [human interleukin 1β (IL-1β) ELISA kit; cat. no. CSB-E08053h; human interferon γ (IFNγ) ELISA kit; cat. no. CSB-E04577h; and human tumor necrosis factor α (TNFα) ELISA kit; cat. no. CSB-E04740h; all from CusaBio]. The plate was covered and incubated at room temperature for 2 h, and the solution was thoroughly decanted from the wells. The wells were washed with TBST four times using a squirt wash bottle or an automated 96-well plate washer. Next, 100 µl of diluted detection antibodies was added to the wells, and the plate was covered and incubated at room temperature for 1 h, after which the solution was thoroughly aspirated from the wells. After washing the wells four times, 100 µl of diluted HRP conjugate was added to each well and the plate was covered and incubated at room temperature for 30 min, after which the solution was thoroughly aspirated from the wells. After washing the wells four times, 100 µl of diluted TMB was added to each well, and the solution in the wells changed from blue to yellow.
The plate was evaluated within 30 min of stopping the reaction. The absorbance of each well was read at 450 and 550 nm with a microplate reader, and the 550 nm values were subtracted from the 450 nm values to correct for optical imperfections in the microplate. MasterPlex ReaderFit curve-fitting statistical software (version 2.0; Emerald Biotech Co., Ltd.) was used to plot a four-parameter logistic curve fit to the standards, and then the results for the test samples were calculated.

Statistical analysis. Data are presented as the means ± standard error of the mean of at least three independent experiments. Differences among experimental groups were statistically analysed by SPSS software (version 17.0; SPSS, Inc.) using the analysis of variance followed by the least significant difference or Dunnett’s post hoc tests, as applicable. P<0.05 was considered to indicate a statistically significant difference.

Results

Transfection and ADAM12 silencing in JEG-3 cells. In the present study, untransfected JEG3 cells were observed using an optical microscope (magnification, x200; Fig. 1A). JEG-3 cells were transfected with three different targets of ADAM12-siRNA or scrambled-siRNA. Western blotting revealed that at 48 h post-transfection, ADAM12 expression was decreased in cells transfected with ADAM12-siRNA (target 1 and 3) but not in cells transfected with the si-NC or ADAM12-siRNA target 2, compared with the BC cells. ADAM12-siRNA target 3 exhibited the best transfection efficiency compared with the other two targets (P<0.05; Fig. 1B and C). Therefore, this siRNA was selected for subsequent experimentation.

ADAM12 silencing decreases cell proliferation and increases apoptosis in JEG-3 cells. The CCK-8 assay showed that cell proliferation was significantly decreased in the si-ADAM12 group compared with the si-NC group (P<0.05; Fig. 2A). Additionally, flow cytometry analysis revealed that the rate of apoptosis in the si-ADAM12 group was significantly increased compared with the si-NC group (P<0.05; Figs. 2C and S1A). Together, these findings confirmed that cell apoptosis was increased after ADAM12 silencing.

The levels of apoptosis-associated proteins were measured by western blotting following ADAM12-siRNA transfection (Fig. 3A). The results revealed that the levels of caspase-3 (Fig. 3G-L) and caspase-9 were not changed (P>0.05; Fig. 3J-L), but the levels of Bax and p53 levels were significantly increased in the si-ADAM12 group compared with the si-NC group (Fig. 3C and E; P<0.05).

ADAM12 silencing increases autophagy in JEG-3 cells. The expression levels of the autophagy-associated proteins LC3B

Figure 2. JEG-3 cell proliferation and apoptosis after ADAM12 silencing. (A) The CCK-8 assay showed that cell proliferation in the si-ADAM12 group was decreased compared with the BC and si-NC groups. (B) The CCK-8 assay showed that cell proliferation in the si-ADAM12 + 3MA group was slightly decreased compared with the si-ADAM12 group. (C) Flow cytometry showed that the apoptosis rate was increased in the si-ADAM12 group compared with the blank control and si-NC groups. (D) Flow cytometry test showed that the apoptosis rate was decreased in the si-ADAM12 + 3MA group compared with the si-ADAM12 group. *P<0.05, as indicated. ADAM12, ADAM metallopeptidase domain 12; CCK-8, Cell Counting Kit-8; siRNA, small interfering RNA; BC, blank control; NC, negative control; 3MA, 3-methyladenine; OD, optical density; n.s., not significant.
and ATG5 in JEG-3 cells were detected by western blotting after ADAM12 silencing. The results showed that the levels of LC3B1 and LC3BII in the si-ADAM12 cells were higher than those in the control cells (P<0.05; Fig. 3D and F). Furthermore, the level of ATG5 expression was increased in si-ADAM12 cells compared with the si-NC cells (P<0.05; Fig. 3B).

In addition, the expression of the autophagy protein LC3B was investigated using immunofluorescence analysis. The IFD of LC3B in the si-ADAM12 group was significantly increased compared with the BC and si-NC groups (P<0.05; Fig. 4A and B).

Autophagy mediates the effect of ADAM12 silencing on cell proliferation and apoptosis in JEG-3 cells. To investigate whether cell apoptosis was mediated by autophagy in JEG-3 cells after ADAM12 silencing, the ADAM12-siRNA transfected JEG-3 cells were treated with an autophagy inhibitor 3-methyladenine (3MA). The cell cycle of the 3MA-treated JEG-3 cells after ADAM12 silencing was analyzed.

Figure 3. Western blotting was used to investigate the expression levels of apoptosis- and autophagy-associated proteins after ADAM12 silencing in JEG-3 cells. (A) Western blotting bands of autophagy-associated proteins (ATG5 and LC3B) and apoptosis-associated proteins (caspase 3, caspase 9, Bax and p53) in the BC, si-NC and si-ADAM12 groups. (B) Quantification of western blotting showed that the level of ATG5 expression was increased in the si-ADAM12 group compared with the BC and si-NC groups. (C) Quantification of western blotting showed that the level of Bax expression was increased in the si-ADAM12 group compared with the BC and si-NC groups. (D) Quantification of western blotting showed that the level of LC3B1 expression was increased in the si-ADAM12 group compared with the BC and si-NC groups. (E) Quantification of western blotting showed that the level of p53 expression was increased in the si-ADAM12 group compared with the BC and si-NC groups. (F) Quantification of western blotting showed that the level of LC3BII expression was increased in the si-ADAM12 group compared with the BC and si-NC groups.
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(Figs. 5 and S2), and the results showed that the number of cells in the si-ADAM12 group was reduced in the S and G2 phases (P<0.05; Fig. 5C-E) but increased in the G1 phase, compared with the si-NC group (P>0.05; Fig. 5B). However, no differences were observed in all the cell cycle phases (G1, S and G2) between the si-ADAM12 + 3MA and si-ADAM12 groups (P>0.05; Fig. 5B-E).

The CCK-8 assay showed that the cell proliferation in the si-ADAM12 + 3MA group was decreased compared with the si-ADAM12 and si-NC groups (Fig. 2B). Flow cytometry was to analyse the apoptosis rate and demonstrated that the rate of apoptosis in the si-ADAM12 group was significantly increased (P<0.05; Figs. 2C, D and S1A) compared with the BC and si-NC groups. No differences were observed in all the cell cycle phases (G1, S and G2) between the si-ADAM12 + 3MA and si-ADAM12 groups (P>0.05; Fig. 5B-E).

The CCK-8 assay showed that the cell proliferation in the si-ADAM12 + 3MA group was decreased compared with the si-ADAM12 and si-NC groups (Fig. 2B). Flow cytometry was to analyse the apoptosis rate and demonstrated that the rate of apoptosis in the si-ADAM12 group was significantly increased (P<0.05; Figs. 2C, D and S1A) compared with the BC and si-NC groups. No differences were observed in all the cell cycle phases (G1, S and G2) between the si-ADAM12 + 3MA and si-ADAM12 groups (P>0.05; Fig. 5B-E).

ADAM12 silencing decreases the inflammatory response in JEG-3 cells.

ELISA results showed that IL-1β (Fig. 7A),...
IFN-γ (Fig. 7B) and TNF-α (Fig. 7C) levels were significantly reduced in the si-ADAM12 group compared with the BC and si-NC groups ($P<0.05$).

**Autophagy fails to impact the effect of ADAM12 silencing on the inflammatory response in JEG-3 cells.** IL-1β (Fig. 7D), IFN-γ (Fig. 7E) and TNF-α (Fig. 7F) were significantly reduced in the si-ADAM12 + 3MA group compared with the si-NC group ($P<0.05$), while IL-1β (Fig. 7D) and TNF-α concentrations (Fig. 7F) exhibited little difference between the si-ADAM12 + 3MA and si-ADAM12 groups ($P>0.05$). In addition, the level of IFN-γ was significantly decreased in the si-ADAM12 + 3MA group compared with the si-ADAM12 group ($P<0.05$; Fig. 7E).

**NF-κB and mTOR signalling.** To investigate the potential mechanism by which ADAM12 silencing-mediated apoptosis in human choriocarcinoma cells, the levels of p65-NF-κB (p65; Fig. 6A and B), mTOR, and p-mTOR were measured in JEG-3 cells (Fig. 6A and F). ADAM12 silencing significantly reduced p65 expression in the si-ADAM12 group compared with the si-NC group ($P<0.05$; Fig. 6B), but the level of p65 protein was similar in the si-ADAM12 + 3MA and si-ADAM12 groups ($P>0.05$; Fig. 6B). Furthermore, mTOR expression levels did...
not differ among the si-NC, si-ADAM12 and si-ADAM12 + 3MA groups (Fig. 6D), while p-mTOR expression was down-regulated in the si-ADAM12-treated cells, compared with the si-ADAM12 + 3MA group, and this effect was significantly rescued in the si-ADAM12 + 3MA group (P<0.05; Fig. 6F).

Discussion

The prognosis of choriocarcinoma is related to clinical stage and trophoblastic activity (1,2). Treatment of choriocarcinoma may include chemotherapy, radiation therapy or combination therapy (29,30). Cancer-targeted therapies based on specific genes or functional proteins are receiving increasing attention (31,32). ADAM12 is a metalloprotein with cell adhesion and hydrolytic activities and has been widely reported to mediate tumour cell proliferation (16) and apoptosis resistance (9). The present study focused on the effect of ADAM12 silencing on cell apoptosis and autophagy in choriocarcinoma cells. Multiple direct and indirect interactions have been described suggesting mechanistic overlap and interaction between the apoptosis machinery and autophagy proteins (23-25). Although it was originally identified as a cell survival mechanism, autophagy has highly context-specific effects in mediating cell death (33,34). In specific contexts,
cell death can also be led by autophagy (35-41). Studies in mammalian and other model systems show that autophagy can paradoxically have pro-apoptosis or pro-survival functions, depending on the context (23,42,43). It has recently been shown that there are interplays between autophagy-dependent apoptosis and other types of cell death including apoptosis and necrosis (26).

The present study revealed that ADAM12 silencing promoted cell apoptosis by activating autophagy in human choriocarcinoma JEG-3 cells. Furthermore, ADAM12 silencing decreased cell proliferation and increased the rate of apoptosis. The levels of the apoptotic proteins Bax and p53 expression were upregulated by ADAM12 silencing in JEG-3 cells but cleaved caspase-3 levels were unchanged. Therefore, it was speculated that ADAM12 silencing-induced apoptosis may occur through a caspase-independent manner, as there are caspase-independent apoptotic pathways, such as the apoptosis-inducing factor-mediated pathway (44) and TNF receptor superfamily member 1A-mediated apoptosis (45).

It was reported that the levels and activity of the apoptotic proteins p53 and Bcl-2 can be regulated through diverse mechanisms (46-48). The present study showed that ADAM12
silencing enhanced cell apoptosis but that after the inhibition of autophagy, the rate of cell apoptosis did not change, which suggested that autophagy indeed mediated the pro-apoptotic effect of ADAM12 silencing. The autophagy-related protein ATG5 is a key protein that is involved in the extension of the phagophore membrane in autophagy vesicles and forms a complex with autophagy related 12 and autophagy related 16 like 1 (49). These complexes are required for the conjugation of LC3-I to phosphatidylethanolamine to form LC3-II (50-54). Accordingly, the present study provided evidence for the upregulation of autophagy by ADAM12 silencing by detecting autophagy markers, such as ATG5, LC3I and LC3II proteins. The results showed that ADAM12 silencing upregulated the expression levels of these autophagy proteins in JEG-3 cells.

In the cellular network of signal integration, autophagy and immunomodulation are interdependent (53-56). Autophagy is involved in the induction and suppression of inflammation and vice versa (55,57,58). The proinflammatory cytokine TNFα has been shown to stimulate autophagy, and autophagy also contributes to the secretion of this cytokine (59-61). However, autophagy is regulated in its response to inflammation, such as participating in the regulation of inflammasome activation (62) and the clearance of protein complexes, such as inflammasomes, through proteasomal degradation (63). In the present study, ADAM12 silencing not only increased autophagy but also exerted an anti-inflammatory effect in JEG-3 cells, but this anti-inflammatory effect of ADAM12 silencing was not blocked when autophagy was inhibited in JEG-3 cells. At the molecular level, autophagy plays a context-dependent pro-survival or pro-death role by regulating different signalling pathways, such as p53, Bcl-2, and mTOR in cancer (23,42,43,64). The p53 gene is one of the target genes of the transcription factor NF-κB (65), and the Bcl-2 gene is transcriptionally regulated by NF-κB and directly links the TNF-α/NF-κB signalling pathways (66,67). NF-κB targets inflammation not only directly by increasing the production of inflammatory cytokines, chemokines and adhesion molecules, but it also regulates cell proliferation, apoptosis, morphogenesis and differentiation (68-70). In the present study, ADAM12 silencing reduced the expression level of nuclear p65-NF-κB in JEG-3 cells. However, ADAM12 silencing did not change the expression level of p65-NF-κB in JEG-3 cells, even after autophagy was inhibited.

mTOR is a serine/threonine protein kinase and a key factor serving as the convergence point for several upstream stimuli and pathways to regulate cell growth, cell proliferation, cell motility, cell survival, protein synthesis, translation and autophagy (71-76). Growth factors, glucose and amino acids also activate mTOR and suppress autophagy, whereas nutrient deprivation will suppress mTOR, leading to the
activation of autophagy (77-80). mTOR and autophagy are closely associated, and defects in signalling through either pathway are known to drive the onset of a range of human diseases, such as cancer and neurodegenerative diseases (80-82). In the present study, ADAM12 silencing increased the expression level of autophagy proteins in JEG-3 cells and significantly reduced the level of phosphorylated mTOR in JEG-3 cells, which is responsible for the activation of the mTOR signalling pathway. However, the downregulation of phosphorylated-mTOR expression was rescued after the suppression of autophagy in JEG-3 cells, and the pro-apoptotic effect of ADAM12 silencing was blocked after the suppression of autophagy. These data confirmed that i) there might be a relationship between the impeded nuclear enrichment of p65-NF-κB by ADAM12 silencing and the anti-inflammatory process of ADAM12 silencing; and ii) ADAM12 silencing activated autophagy via the regulation of the mTOR signalling pathway.

The present study only investigated one choriocarcinoma cell line, and further studies are warranted to assess other choriocarcinoma cell lines and to validate the results obtained. In conclusion, the present study revealed that ADAM12 may...
serve as a potential anticancer agent due to its effects on proliferation and apoptosis of choriocarcinoma cells.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Hunan Provincial Natural Science Foundation of China (grant no. 2016JJ2169).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors’ contributions

ZHT designed the experiments. LW, ZHT, YZ, NKK, HNL and YZ performed the experiments and analyzed the data. LW and ZHT wrote the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Autophagy shapes β and 

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