Involvement of β-catenin in matrine-induced autophagy and apoptosis in WB-F344 cells

BU-SHAN XIE[2,3], XING-XING HE[2], ZHENG-LIN AI[3] and SHU-KUN YAO[1]

[1]Department of Gastroenterology, China-Japan Friendship Hospital, Beijing 100029;
[2]Department of Gastroenterology, The First Affiliated Hospital of Nanchang University, Nanchang 330006;
[3]Graduate School, Peking Union Medical College and Chinese Academy of Medical Sciences, Beijing 100730, P.R. China

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Abstract. Matrine, one of the main components extracted from *Sophora flavescens*, has exhibited pharmacological effects on the differentiation in rat liver oval cells. However, its function and mechanism have not yet been fully elucidated. To further investigate them, an *in vitro* model was established using a rat liver oval cell line called WB-F344 and treated with matrine. Initially, a significant increase in the number of monodansylcadaverine-positive cells and in the levels of microtubule-associated protein 1A/1B-light chain 3 (LC3)-II, which is a specific marker for detecting autophagy, was observed in matrine-treated cells. This indicated that autophagy was stimulated by matrine, which was further confirmed by transmission electron microscopy. Additionally, the apoptotic oval cells were easily detected under matrine treatment using an Annexin-V-fluorescein isothiocyanate/propidium iodide assay, indicating that autophagy and apoptosis were synchronously induced by matrine. A decrease in B-cell lymphoma (Bcl-2) mRNA expression, but an increase in Bcl2-associated X protein (Bax) mRNA expression were observed in matrine-treated cells, which led to an upregulation of the Bax/Bcl-2 ratio, a molecular marker for determining the extent of apoptosis. Next, the molecular mechanism of matrine-induced autophagy and apoptosis was analyzed in WB-F344 cells. β-catenin degradation was downregulated by matrine and rapamycin, a foregone chemical agonist of autophagy, whereas it was upregulated by 3-methyladenine, a specific inhibitor of autophagy. Additionally, β-catenin activation induced an increase in LC3-II levels and reversed the Bax/Bcl-2 mRNA ratio under matrine treatment, whereas inhibition of β-catenin by RNA interference induced a decrease of the LC3-II amount and of the Bax/Bcl-2 mRNA ratio. Finally, matrine treatment attenuated p53; however, with little or no change in LC3-II levels, but a decrease in β-catenin levels occurred in WB-F344 cells upon treatment with pifithrin-α, a chemical inhibitor of p53, revealing that p53, interfering with β-catenin, may not be involved in matrine-induced autophagy in WB-F344 cells. These results demonstrate that β-catenin is involved in matrine-induced autophagy and apoptosis in WB-F344 cells, while β-catenin is negatively regulated by autophagy and positively by p53, indicating that β-catenin may be involved in the crosstalk between autophagy and apoptosis in WB-F344 cells.

Correspondence to: Professor Shu-Kun Yao, Department of Gastroenterology, China-Japan Friendship Hospital, Ministry of Health, 2 East Yinghua Road, He Pingli, Chao Yang, Beijing 100029, P.R. China
E-mail: yaoshukun6@yahoo.com.cn

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Introduction

Matrine is one of the main active components of the traditional Chinese medical plant *Sophora flavescence* (1). Matrine has been widely employed in the treatment of chronic viral hepatitis in China (2) and also exhibits antitumor effects by inhibiting proliferation and inducing apoptosis or differentiation *in vitro* (3-5). Previously, autophagic cell death, referred to as type II programmed cell death, has been demonstrated to be induced by matrine in C6 glioma cells (6).

WB-F344 cells, derived from a single cloned epithelial cell isolated from rat liver, can be regarded as the cultured analogue of liver precursor cells. These epithelial cells share certain phenotypic and functional characteristics with hepatocytes and bile duct cells. When they were implanted into a rat liver, they gained morphological and functional properties of hepatocytes (7,8). WB-F344 cells also have the potential to differentiate into biliary cells *in vitro* and develop cholangiocarcinomas when transplanted with chemically transformed WB-F344 cells into the liver of syngeneic rats *in vivo* (9,10).

The majority of studies of liver oval cells focuses on hepatocytic differentiation induced by matrine *in vitro*. However, its mechanism of liver stem cells under drug treatment remains to be elucidated. In the present study, apoptosis was stimulated by matrine in WB-F344 cells and a concomitant increase in autophagy was detected. Additionally, β-catenin has a role in the matrine-induced autophagy and apoptosis, while β-catenin is negatively regulated by autophagy as a feedback mechanism.

Materials and methods

Materials. 3-methyladenine (3-MA), rapamycin, dimethylsulfoxide (DMSO), lithium chloride (LiCl), pifithrin-α (PFT-α) and antibodies against microtubule-associated protein 1 light chain 3 (LC3) were purchased from Sigma (St. Louis, MO, USA). Antibodies against p70S6 (Thr389), phosphorylated (p)-Akt (Thr473), p-extracellular signal-regulated kinases (ERK)1/2 (Thy202/Tyr204) and p-c-Jun N-terminal kinases (JNK) (T183/Y185) were from Cell Signaling Technology, Inc. (Beverly, MA, USA), β-catenin, beclin-1, β-actin and p53 antibody from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Matrine, which was dissolved in Dulbecco's modified Eagle medium (DMEM; Invitrogen, Carlsbad, CA, USA) was added to the cells with a final concentration of 0.2-4 mg/ml matrine that was a gift from Baiyunshan Mingxing Pharmaceutical Co., Ltd. (Guangzhou, China) and with a purity >98%. Constitutively, active β-catenin was a gift from Dr Yanming Li (Peking University School of Medicine, Beijing, China).

Cell lines and cell culture. WB-F344 cells were purchased by the Institute of Materia Medica (IMM), Chinese Academy of Medical Sciences and Peking Union Medical College (all in Beijing, China). The cells were cultivated in a 25 cm^3 flask with DMEM, supplemented with 15% (v/v) fetal bovine serum, 100 KIU/l penicillin and 100 mg/l streptomycin (all from Invitrogen). These were kept at 37˚C in a humidified atmosphere containing 5% CO_2. The flasks were subcultured every two days with a split ratio of 1:2.

Cell viability assay. The cells were trypsinized and cultured in 96-well flat bottom microtiter plates. Following matrine treatment, 100 μl MTT (Sigma; 5 mg/ml/well) was added and incubated for 4 h. The crystals were dissolved in 150 μl DMSO. The absorbance of the solution was measured spectrophotometrically at 570 nm using a microtiter plate reader (Becton-Dickinson, Franklin Lanes, NJ, USA).

Confocal microscopy. The cells were incubated for 1 h with 0.05 mM monodansylcadaverine (MDC; Sigma). After 1 h incubation at 37˚C, the cells were fixed in 4% paraformaldehyde for 15 min and immediately analyzed using a scanning confocal microscope (Olympus, Tokyo, Japan). The percentages of distribution of MDC dots were counted in five non-overlapping fields and the statistical data were obtained from three repeated experiments.

Electron microscopy. The trypsinized cells were fixed with ice-cold glutaraldehyde [(Sigma) 3% in 0.1 M cacodylate buffer (pH 7.4)] for 30 min. The cells were post-fixed in OsO_4 (Sigma) and embedded in Epon (Sigma); 0.1-mm thin sections were stained with uranyl acetate/lead citrate (Fluka, St. Louis, MO, USA). The observation was performed on a JEM-1230 electron microscope (Jeol, Tokyo, Japan).

Flow cytometric analysis. The cells were treated with the indicated concentrations of chemotherapeutic agent, then cells were collected and incubated with Annexin V-fluorescein isothiocyanate (FITC) (Beijing Biosea Biotechnology Co., Ltd., Beijing, China) for 30 min at 4˚C and in the dark, then incubated with propidium iodide (PI) (Beijing Biosea Biotechnology Co., Ltd.) for 5 min. Analysis was immediately performed by flow cytometry (FACSAria, Becton-Dickinson, Franklin Lakes, NJ, USA).

The cells were cultured in six-well tissue culture plates and received different treatments. They were then trypsinized and fixed in 70% ethanol at 4˚C overnight. Then cells were collected and resuspended in staining buffer (50 μg/ml PI in phosphate-buffered saline) for 5 min in the dark at room temperature and then analyzed by flow cytometry.

Small interfering (si)RNA transfection. The cells were seeded at 40% confluence per well in six-well plates overnight and transfected with β-catenin siRNA or control siRNA duplex (Santa Cruz Biotechnology, Inc.) using Lipofectamine 2000 (Invitrogen Life Technologies, Carlsbad, CA, USA). The successful targeted knockdown was confirmed by western blot analysis.

Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative *PCR (qPCR)*. Total RNA was purified using TRIzol agent (Invitrogen Life Technologies) according to the manufacturer's instructions. The total RNA was reverse-transcribed using cDNA reverse transcription kits (Invitrogen Life Technologies). Hot-start PCR was then performed. The PCR results were verified by varying the number of PCR cycles for each cDNA and set of primers. The target gene primer pairs were as follows: Bax forward, 5'-CCAAGAAGCTGAGCGAGTGTC-3' and reverse, 3'-TGAGGACTCCAGCCACAAAGA-5'; Bcl-2 forward, 5'-CCGGGAGATCGTGATGAAGT-3' and reverse, 3'-ATCCCAGCCTCCGTTATCCT-5'; p53 forward, 5'-GGCCTCTGTCATCTTCCG-3' and reverse, 3'-CCGTCACCATCAGAGCAAC-5'; GAPDH forward, 5'-ATCGGACGCCTGGTTACC-3' and reverse, 3'-GACTGTGCCGTTGAACTTGC-5'. The amplified products were separated on 1.5-2% agarose gels and visualized under ultraviolet transillumination.

qPCR was performed using the ABI Prism 7700 Sequence Detection system (Applied Biosystems, Grand Island, NY, USA). For each PCR run, the samples were incubated in a 96-well plate at 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. The data were analyzed using the ΔΔCt method according to the manufacturer's instructions. The data were normalized to the housekeeping gene GAPDH. Changes in the gene expression were illustrated as a fold increase/decrease, as compared with the control. The experiments were repeated thrice.

Western blot analysis. The cell lysates were separated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto nitrocellulose membranes (Millipore Co., Bedford, MA, USA). The membranes were incubated with the primary antibodies overnight at 4˚C, then the secondary antibodies at room temperature for 2 h. The antibodies used included LC3, p70S6 (Thr389), Akt and p-Akt (Thr473), ERK1/2 and p-ERK1/2 (Thy202/Tyr204), JNK and p-JNK (T183/Y185), β-catenin, beclin-1 and p53. β-actin antibody was used as a control. The protein bands were observed on

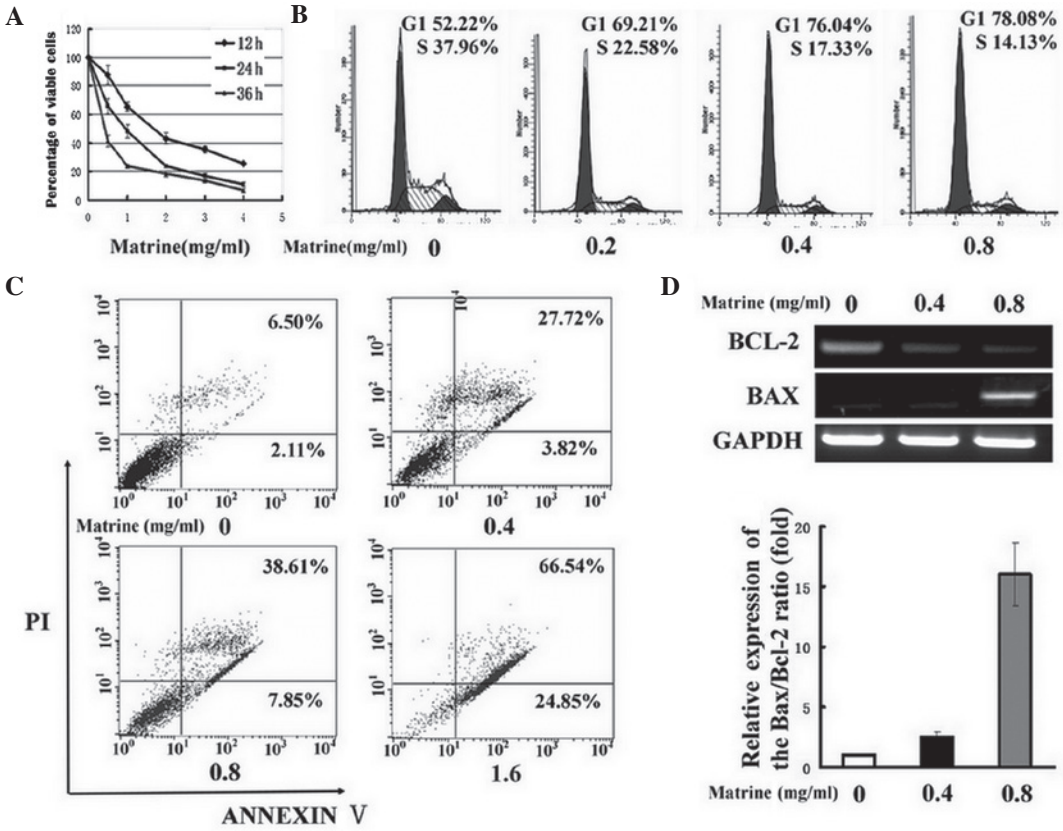

Figure 1. Matrine blocks oval cell cycle progression and induces apoptosis. (A) The effects of matrine at different concentrations (0-4 mg/ml) on the regression of WB-F344 cells for 12, 24 or 36 h was examined by the MTT assay. (B) The cells were incubated with matrine at concentrations of 0.2 (0.2), 0.4 (0.4) and 0.8 mg/ml (0.8) for 24 h, and accumulation of the G1/G0-phase was detected by flow cytometry. The untreated cells (0) were used as a control. (C) Apoptotic oval cells were stimulated by matrine at concentrations of 0.4 (0.4), 0.8 (0.8) and 1.6 mg/ml (1.6) for 24 h as detected by the Annexin V/PI assay. Using this assay, the viable (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), late apoptotic or necrotic cells (Annexin V^+/PI^+) were classified. (D) Bax and Bcl-2 mRNA levels were measured in WB-F344 cells following exposure to matrine at 0.4 (0.4) and 0.8 mg/ml (0.8) by RT-PCR and then qPCR, and compared with that of untreated cells (0). GAPDH was used as an internal control. The ΔΔCt value of the Bax/Bcl-2 ratio in untreated cells was normalized to 1 as a control. PI, propidium iodide; Bcl2, B-cell lymphoma 2; Bax, Bcl2-associated X protein; mRNA, messenger RNA; RT-PCR, real-time polymerase chain reaction; qPCR, quantitative PCR.

X-ray film or using an enhanced chemiluminescence system (Pierce Biotechnology, Rockford, IL, USA).

Statistical analysis. Statistical analysis was performed using SPSS 17 statistics software (SPSS, Inc., Chicago, IL, USA). The data are expressed as the mean ± standard deviation with the number of individual experiments described in the Figure legends. $P<0.05$ was used to indicate a statistically significant difference.

Results

Matrine inhibits cell cycle and induces apoptosis of WB-F344 cells. The effect of matrine treatment on WB-F344 cells was examined with an MTT assay. The cell viability was inhibited by matrine (0-4 mg/ml) in a dose- and time-dependent manner. The IC_{50} for matrine of WB-F344 cells for 24 h was 0.84 mg/ml (Fig. 1A). Based on these results, 0.8 mg/ml matrine for 24 h was used for further experiments. The cell cycle and apoptosis were further analyzed by flow cytometric analysis. An evident decline in the percentage of the S-phase cells, but a significant elevation tendency of the G0/G1-phase was observed in rat oval cells following exposure to matrine (Fig. 1B). Additionally, the Annexin-V-FITC/PI assay revealed that matrine induced a significant increase in the number of apoptotic oval cells (Fig. 1C). These results indicated that matrine blocked the oval cell cycle progression at the G0/G1 phase and induced apoptosis. Next, the mRNA expression of Bcl-2 and Bax, the key regulators of cell proliferation and apoptosis, were examined. As shown in Fig. 1D, RT-PCR and qPCR assays indicated a decrease in Bcl-2 mRNA expression, but an increase of Bax mRNA expression in matrine-treated cells in a dose-dependent manner, leading to an upregulation of the Bax/Bcl-2 ratio in comparison with untreated cells.

Matrine induces autophagy in WB-F344 cells, which inhibits apoptosis. The present study tested whether matrine induced autophagy in WB-F344 cells. The changes of autophagosome morphology were initially visualized with MDC staining, proposed as a tracer for autophagic vacuoles (11). MDC-stained punctuate structures, indicative of the formation of autophagosomes, increased in the cytoplasm of WB-F344 cells following matrine treatment (Fig. 2A). The cumulative autophagic process was further confirmed by transmission electron microscopy (Fig. 2B). Next, immunoblotting analysis indicated that the ratio of LC3-II/β-actin, a specific molecular marker for autophagy detection (12), was significantly upregulated; however, the levels of ribosomal S6 protein kinase

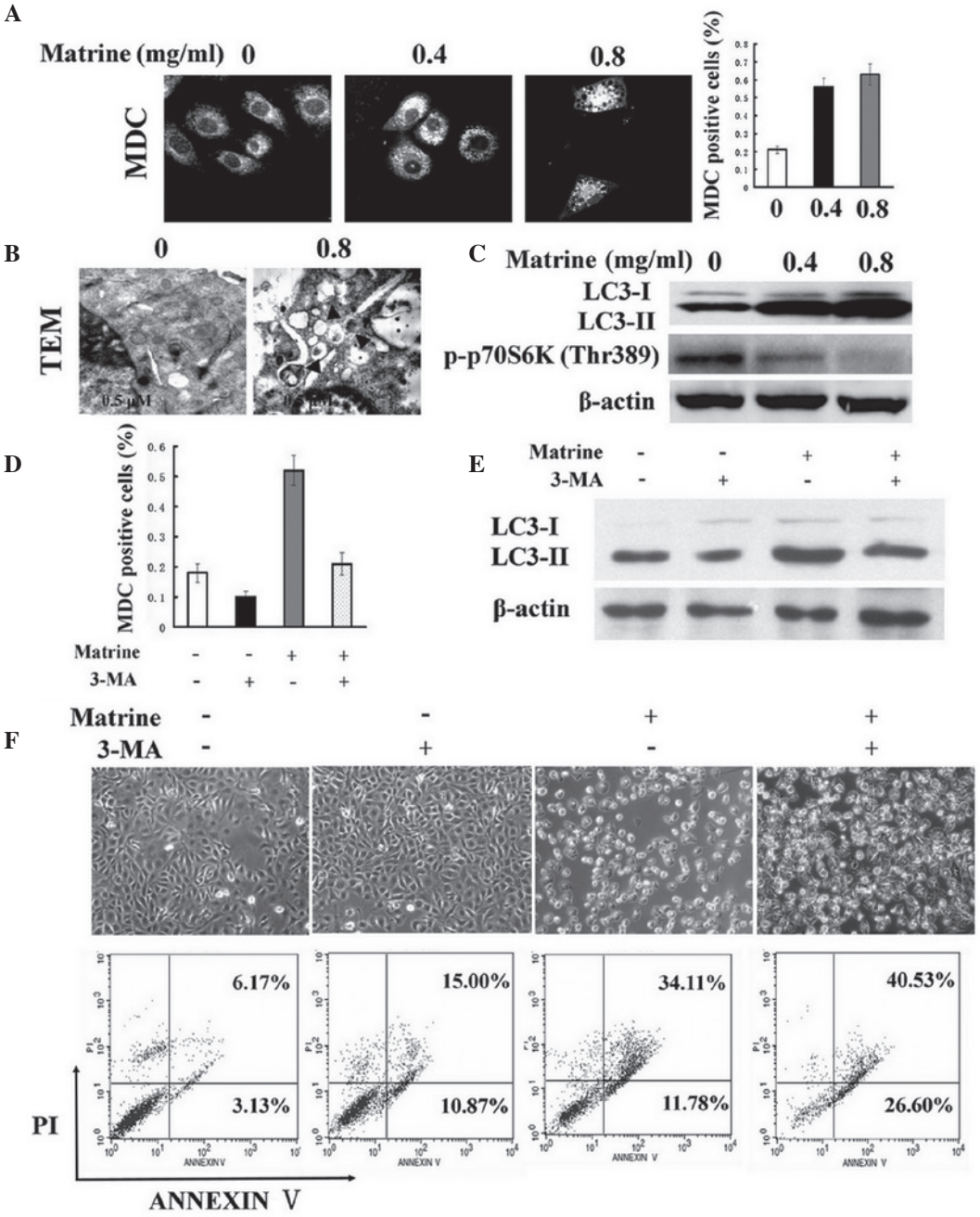

Figure 2. Matrine promotes autophagy in WB-F344 cells, which inhibits apoptosis. (A) Following exposure to matrine at 0.4 (0.4) and 0.8 mg/ml (0.8) for 24 h, MDC staining dots were accumulated in the treated cells, in contrast to the control (0). Representative confocal microscopic images (magnification, x600) were obtained. A significant difference between MDC-positive cells (%) in matrine-treated cells and untreated cells was present. (B) Representative electron microscopic images were obtained from matrine-treated cells at 0.8 mg/ml. The typical autophagosomes or autolysosomes are denoted by arrows (original magnification, x10,000). (C) Western blot analysis of LC3-II and p-p70S6K (Thr389) in the cells subsequent to exposure to matrine at 0.4 (0.4) and 0.8 mg/ml (0.8) for 24 h. β-actin was employed as a protein loading control. (D) The cells were treated with matrine for 24 h in the presence or absence of 5 mM 3-MA. MDC-positive cells (%) are presented as the mean ± standard error of three independent experiments, and a significant difference was found between the combination group (Matrine + 3-MA) and the matrine group (Matrine) at the level of $P<0.05$. (E) Immunoblotting results represent the decrease of LC3-II levels under matrine treatment in combination with 3-MA (Matrine + 3-MA), as compared with matrine alone. (F) Representative light microscopic images (magnification, x100) and Annexin V/PI analysis revealed that apoptosis was increased in the combined group (Matrine + 3-MA) as compared with matrine alone (Matrine). LC3, microtubule-associated protein 1 light chain 3; PI, propidium iodide; TEM, transmission electron microscopy; MDC, monodansylcadaverine; 3-MA, 3-methyladenine.

(S6K1, also known as p70S6K) phosphorylation, which has been widely described to reflect the mechanistic target of rapamycin (mTOR) activity, was downregulated in the treated cells as compared with the control (Fig. 2C). Altogether, these findings confirmed that autophagy was stimulated by matrine in an mTOR-dependent manner in rat oval cells. It is disputed whether autophagy induction is protective or toxic. 3-MA, a general inhibitor of autophagy (13), was used to analyze the role of matrine-induced autophagy in WB-F344 cells. It was presented in Fig. 2D that the increase of MDC-positive dots induced by matrine was overcome by the addition of 3-MA. Similarly, immunoblotting analysis indicated that the increase of the LC3-II/β-actin ratio by matrine was attenuated following the addition with 3-MA (Fig. 2E). Subsequently,

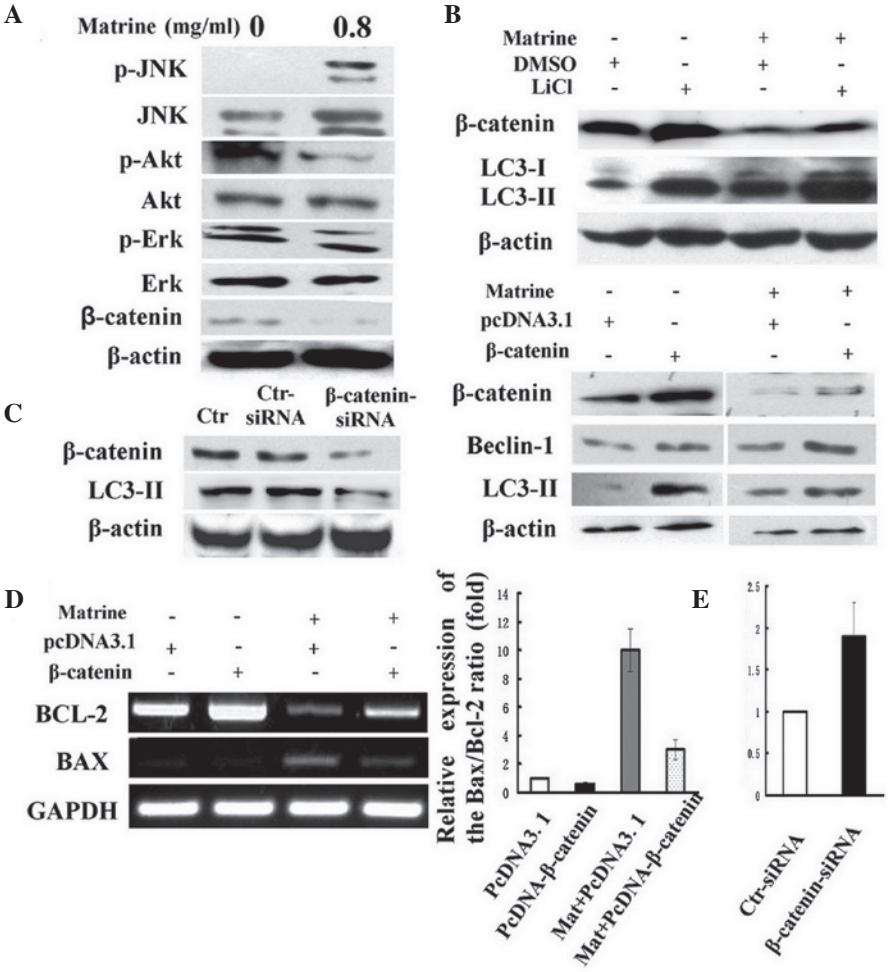

Figure 3. The wnt/β-catenin pathway is involved in matrine-induced autophagy and apoptosis. (A) The cells were treated with matrine at 0.8 mg/ml (0.8) for 24 h. Immunoblotting results represent the levels of p-JNK, p-Akt, p-ERK and β-catenin in WB-F344 cells. (B) In the upper panels, the cells were treated with matrine at 0.8 mg/ml for 24 h in the presence or absence of 20 mM LiCl. Control cells were treated with dimethylsulfoxide. Immunoblotting analysis of the increase in LC3-II levels in matrine-treated cells cotreated with LiCl. In the lower panels, the cells were transiently transfected with constitutively active β-catenin plasmid. The pCDNA3.1 plasmid was used as a control. After 24 h, the transfected cells were incubated with matrine at 0.8 mg/ml for 24 h. Immunoblotting analysis of the increase of LC3-II and beclin-1 levels in matrine-treated cells transfected with β-catenin overexpression plasmid. (C) β-catenin was suppressed by siRNA in the WB-F344 cells and β-catenin loss led to a decline in LC3-II levels as indicated by immunoblotting. The scrambled siRNA was used as a control. (D) RT-PCR and qPCR analysis of the Bax/Bcl-2 ratio in matrine-treated cells transfected with β-catenin overexpression plasmid. The ΔΔCt value of the Bax/Bcl-2 ratio in untreated cells was normalized to 1 as a control. (E) qPCR analysis of the Bax/Bcl-2 ratio in WB-F344 cells transfected with β-catenin siRNA. The scrambled siRNA was used as a control. p-JUN, phosphorylated-c-Jun N-terminal kinase JNK; ERK, mitogen-activated protein kinase 1; LiCl, LC3, microtubule-associated protein 1 light chain 3; siRNA, small interfering RNA; RT-PCR, real-time polymerase chain reaction; qPCR, quantitative PCR; Bcl-2, B-cell lymphoma 2; Bax, Bcl2-associated X protein; Mat, matrine; Ctr, untreated control cells.

3-MA cotreatment induced more significant levels of apoptotic cells compared with matrine alone, as indicated by the Annexin-V-FITC/PI assay (Fig. 2F). These results indicated that suppression of autophagy augmented matrine-induced apoptosis, indicating that the matrine-stimulated autophagic process serves as an oval cell survival mechanism.

β-catenin is involved in matrine-induced autophagy and apoptosis in WB-F344 cells. Accumulating data indicate that Akt, ERK, JNK and Wnt/β-catenin signaling pathways have significant roles in apoptosis or autophagy (14). As shown in Fig. 3A, immunoblotting analysis revealed that matrine treatment suppressed the phosphorylation of Akt and ERK, but significantly increased the phosphorylation of JNK. β-catenin, the major downstream effector of the canonical Wnt signaling pathway, was attenuated by matrine in WB-F344 cells.

The Wnt/β-catenin pathway has a crucial role in stem cell development and renewal. Thus, the role of β-catenin protein in matrine-induced autophagy and apoptosis was analyzed next. LiCl, activating Wnt signaling selectively via the β-catenin/T-cell factor pathway (15), led to an increase in the ratio of LC3-II/β-actin, which was enhanced upon the addition of matrine (Fig. 3B). Similarly, upregulation of beclin-1, the angiotensinogen (Atg)6 mammalian analogue, and the LC3-II/β-actin ratio, was further received in WB-F344 cells transiently transfected with the β-catenin overexpression plasmid, indicating that β-catenin activation stimulated autophagy (Fig. 3B). Additionally, the decline in Bcl-2/Bax ratio under matrine treatment was reversed by β-catenin overexpression in contrast to the control plasmid (Fig. 3D). In addition, it was tested whether inactivation of β-catenin inhibits autophagy activation in WB-F344 cells. For this purpose, RNA interference was used to deplete the β-catenin protein. The

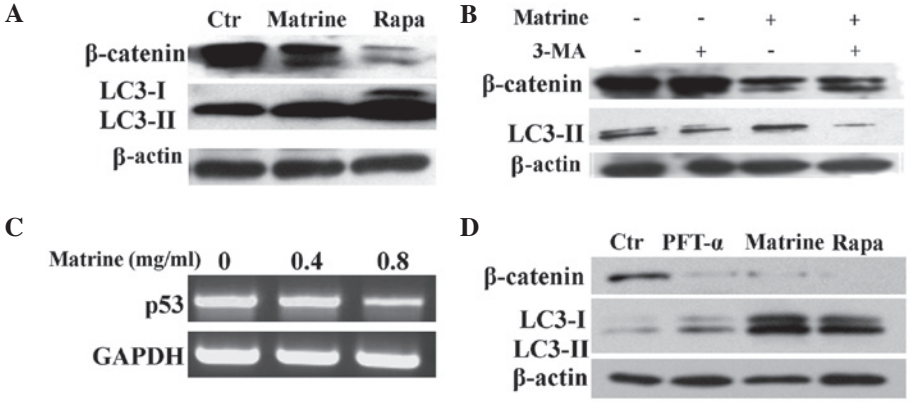

Figure 4. Autophagy is regulated by β-catenin and p53 in WB-F344 cells. (A) Immunoblotting analysis of the expression of β-catenin following incubation with matrine (0.8 mg/ml) and Rapa (10 nM) in WB-F344 cells, as compared with the untreated cells (Ctr). (B) The cells were treated with matrine at 0.8 mg/ml for 24 h in the presence or absence of 5 mM 3-MA. Immunoblotting results indicated the increase of β-catenin levels in the combination group (Matrine + 3-MA) as compared with matrine alone. (C) RT-PCR analysis of p53 expression following matrine incubation at concentrations of 0.4 (0.4) and 0.8 mg/ml (0.8) for 24 h in WB-F344 cells. (D) Immunoblotting analysis of β-catenin and LC3-II levels in the cells treated with 20 μM PFT-α, 10 nM Rapa and 0.8 mg/ml matrine, as compared with the untreated cells (Ctr). RT-PCR, real-time polymerase chain reaction; LC3, microtubule-associated protein 1 light chain 3; Rapa, rapamycin; 3-MA, 3-methyladenine; PFT-α; pifithrin-α; Ctr, untreated control cells.

amount of β-catenin protein and the ratio of LC3-II/β-actin in the cells were reduced by the specific siRNA duplex as compared to the control siRNA (Fig. 3C). Furthermore, the Bax/Bcl-2 ratio was increased in WB-F344 cells transfected with β-catenin siRNA (Fig. 3E). These results indicated that β-catenin is involved in matrine-induced autophagy and apoptosis, possibly through the Bax/Bcl-2 regulation.

β-catenin is modulated by autophagy and p53 in WB-F344 cells. It has been recently reported that β-catenin is downregulated by autophagy (14). As expected, β-catenin was significantly attenuated by matrine and rapamycin (Fig. 4A), a foregone chemical agonist of autophagy (16). However, 3-MA partially reversed the inhibitory effect of β-catenin levels by matrine (Fig. 4B), indicating that β-catenin degradation was negatively modulated by matrine-induced autophagy. Additionally, the Wnt/β-catenin pathways can be modulated by the tumor suppressor p53, which can turn autophagy on or off (17). Next, it was investigated whether p53 was implicated in the β-catenin signal transduction via increased autophagy in liver oval cells. RT-PCR revealed that matrine attenuated the levels of p53 (Fig. 4C). Subsequently, PFT-α, a pharmacological inhibitor of p53 (18), was used to quantify the effect of p53 levels on Wnt signaling and autophagy. PFT-α caused a significant decline of β-catenin levels, but exerted little or no effect on the LC3-II/β-actin ratio. While β-catenin levels decreased, an increase in the LC3-II/β-actin ratio was detected following exposure to rapamycin and matrine (Fig. 4D). These results indicated that p53 interfered with the β-catenin signaling pathway, possibly not via autophagy in WB-F344 cells. β-catenin degradation may be separately modulated by autophagy and p53.

Discussion

Matrine has been shown to exhibit the ability to promote hepatocytic differentiation of rat liver oval cells, one of the origins of HCC, and thereby, to have the potential to be applied in HCC prevention. Besides hepatocytic differentiation, matrine was identified to inhibit rat oval cell proliferation, possibly not via autophagic cell death, but via apoptosis. In rat hepatic oval cells, blockage of the cell cycle at the G0/G1-phase and stimulation of apoptosis was presented following exposure to matrine.

Morphological observations revealed that autophagy was stimulated under matrine treatment. Results on LC3 expression and mTOR inhibition further confirmed these observations. In matrine-treated C6 glioma cells, besides apoptosis, autophagy has been regarded as an additional process of cell death (6). Conversely, other studies have demonstrated that autophagy protects against apoptosis and serves as a cell survival mechanism (19-24). The present study identified that chemical inhibition of autophagy enhanced matrine-induced apoptosis, indicating that autophagy functions as a mechanism of cell survival under matrine treatment. Additionally, it was demonstrated that the activation of β-catenin promotes autophagy in rat hepatic oval cells. The activation of the Wnt/β-catenin signaling pathway has been reported to account for cell survival and proliferation *in vitro* (25). An aberrant activation of β-catenin, which has a role in cell survival or stem cell renewal, was detected in hepatic adenoma and HCC (26). It is conceivable that β-catenin-induced autophagy conduces to oval cell proliferation and adaptation.

A pathway contributing to apoptosis involves the Wnt/catenin signaling cascade (27). Matrine treatment enhanced β-catenin degradation and the Bax/Bcl-2 ratio in rat liver oval cells, indicating that β-catenin may be associated with the induction of apoptosis by matrine. Subsequently, β-catenin inactivation by RNA interference upregulated the Bax/Bcl-2 ratio, whereas β-catenin activation reversed the stimulative effect of the Bax/Bcl-2 ratio by matrine. Possibly, β-catenin loss is essential for matrine-induced stem cell apoptosis. Furthermore, the present study demonstrated that β-catenin inactivation was downregulated by the induction of autophagy by matrine. Under metabolic stress, autophagy induction was previously observed to negatively regulate the Wnt signaling cascade through accelerating segment polarity protein dishevelled homolog-2 turnover (28). It appears that autophagy-mediated β-catenin inactivation induces rat oval cell apoptosis. The canonical Wnt

signaling possibly functions as a crosstalk between autophagy and apoptosis in rat hepatic oval cells.

Autophagy and apoptosis pathways are usually regulated by several common factors, including Beclin-1 and Atg5 (29,30); however, the underlying mechanisms remain to be elucidated. β-catenin activation was indicated to enhance LC3-II levels, but reduce the Bax/Bcl-2 ratio in oval cells, whereas inactivation of the Wnt cascade decreased LC3-II levels, but increased the Bax/Bcl-2 ratio, indicating that β-catenin is linked to matrine-induced autophagy and apoptosis, possibly via the Bax/Bcl-2 proteins. The anti-apoptotic protein Bcl-2 can also inhibit autophagy by a direct interaction with beclin-1 (31). The intersection between autophagy and apoptosis is presumably cross-linked via β-catenin, which conjugates or dissociates Bcl-2 from the complex with beclin-1 through a mechanism that remains to be elucidated.

It has been previously reported that p53 can adversely modulate the Wnt/β-catenin signaling (32). The present study demonstrated that β-catenin inactivation and p53 loss was detected concurrently in matrine-treated cells. Thus, this elimination of β-catenin is likely to be exerted by the autophagy process induction by p53 inactivation. However, chemical inhibition of p53 decreased β-catenin accumulation, but failed to affect the autophagic activities. Therefore, it is likely that p53 did not exert its effect via the autophagic flux. The existence of parallel pathways, a canonical cascade involving components of the Wnt pathway and a second mechanism involving seven in absentia homolog 1, Siah-interacting protein and Ebi (32), may contribute to β-catenin degradation by p53 in oval cells.

In conclusion, matrine-induced apoptosis, which is involved in β-catenin inactivation, contributes to oval cell suppression, while β-catenin activation is associated with the autophagic flux. The canonical Wnt pathway, which can be regulated by autophagy and p53, is likely to be a novel intersection between autophagy and apoptosis in hepatic stem cells.

Acknowledgements

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