



REG γ promotes mantle cell lymphoma cell apoptosis by downregulating NF- κ B signaling

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Background: Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL). REG γ is important for tumor occurrence and development, but understanding of the specific role of REG γ in MCL is lacking. We aimed to identify REG γ effects on the proliferation and apoptosis of MCL cells and clarify the underlying mechanisms.

Methods: JEKO-1 cells stably transfected with a doxycycline-inducible Tet-On system expressed high levels of REG γ . JEKO-1 cells stably expressing shRNA-REG γ to reduce REG γ levels were constructed. Cell proliferation, apoptosis, and p-NF- κ B, NF- κ B, I κ B, REG γ , p-STAT3, STAT3, and PSMB5 levels in transfected cells and in transfected cells treated with Stattic, that is a nonpeptidic small molecule exhibited to selectively inhibit signal transducer and activator of transcription factor 3 through blocking the function of its SH2 domain, were analyzed using western blotting.

Results: The proliferation of JEKO-1 cells was inhibited, and apoptosis was enhanced by increased expression of REG γ ($P < 0.01$). REG γ inhibited MCL cell proliferation in a mouse tumor xenograft model by promoting apoptosis, increased the expression of the three I κ B subunits and inhibited NF- κ B signaling. Overexpressed REG γ inhibited STAT3 and downregulated PSMB5 expression in MCL cells. Stattic downregulated PSMB5 and nuclear factor-kappa B (NF- κ B) expressions and upregulated I κ B ϵ expression in JEKO-1 cells.

Conclusions: We found that REG γ regulated p-STAT3 expression by accelerating its half-life and downregulated the NF- κ B signaling pathway to promote MCL cell apoptosis by negatively regulating STAT3-mediated PSMB5 expression and subsequently upregulating I κ B expression.

Keywords: REG γ ; mantle cell lymphomas; NF- κ B; apoptosis

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Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL), which accounts for 4–9% of NHLs worldwide (1). MCL is characterized by hallmark features such as t(11; 14)(q13; q32)

chromosomal translocation and over-expression of cyclinD1, which lead to a poor prognosis (2). With the rapid development of molecular biology, targeted drugs such as bortezomib, ibrutinib, and lenalidomide have improved outcomes of conventional chemotherapy while

avoiding long-term toxicities (3). However, MCL remains refractory to treatment owing to drug resistance. Therefore, it is necessary to identify new therapeutic targets.

Despite impressive achievements in targeted drugs, MCL has remained refractory to treatment due to tumoral resistance. The proteasome activator REG γ (also known as PSME3, PA28 γ , and Ki antigen) is implicated in the progression of cancer. As a promoter of cellular growth and a key regulator of several tumor suppressors, a recent study has linked REG γ overexpression with tumor formation and suggested the REG γ -proteasome as a potential target of new cancer-drug development (4). REG γ is a member of the 11S proteasome. REG γ is present constitutively within the cell nucleus and is responsible for the degradation of most intracellular proteins in an ATP- and ubiquitin-independent manner by binding to and activating the 20S proteasome (5,6). REG γ is overexpressed in breast (7), thyroid (8) and colorectal cancers (9,10) and is correlated with poor prognosis in patients with MCL (6,11). However, the molecular mechanisms and specific roles of REG γ in MCL are unclear.

Previous studies demonstrated that REG γ promotes cell proliferation and migration and inhibits cell apoptosis

by downregulating the nuclear factor-kappa B (NF- κ B) signaling pathway in bowel inflammation (12), colon cancer (10), and multiple myeloma cells (13). Moreover, REG γ promotes inflammation in testicular Leydig cells via I κ B ϵ signaling (14). However, studies on the functional role and molecular mechanisms of REG γ in MCL remain scarce.

In this study, the JEKO-1 cell line was used to construct stable REG γ -knockdown and REG γ -overexpressing cell lines, and the effects and mechanisms of REG γ in MCLs were investigated. We present the following article in accordance with the ARRIVE reporting checklist (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-2045/rc>).

Methods

Animals and cell culture

All animals were purchased from Yongnuo Biotechnology Co. Ltd. (Guangzhou, China). Human JEKO-1 and REC-1 cells (American Type Culture Collection (ATCC), Rockefeller, MD, USA) were cultured in RPMI 1640 medium with 10% fetal bovine serum (FBS) in an incubator at 37 °C and 5% CO₂. Liposome 2000 reagent (Invitrogen, Carlsbad, CA, USA) was used to transfect cells in log phase growth. Experiments were performed under a project license (No. 2021-KZ-170-01) granted by Guangdong Second Provincial General Hospital Ethics Committee, in compliance with China's national or institutional guidelines for the care and use of animals.

Cell transfection and treatment

In the presence of doxycycline (Dox, Pfizer Inc., New York, NY, USA), JEKO-1 cells stably transfected with the Tet-On system (CloneTech, Palo Alto, CA, USA) expressed high levels of REG γ . pLXSN-REG γ and pEGFP-N1 plasmids were digested with XhoI and XmaI restriction endonucleases, ligated with T4 ligase, and transformed into *E. coli* DH5 α cells. The pEGFP-N1-REG γ plasmid was digested with XhoI and SalI, and pRevTRE was digested with SalI, ligated with T4 ligase, and transformed into *E. coli* DH5 α cells. Plasmid DNA was then circularized with T4 ligase and transformed into DH5 α cells to obtain pRevTRE-REG γ . pRevTet-on, pRevTRE, and pRevTRE-REG γ were transfected individually into the viral packaging cell line PT67 using polyethylenimine reagent. Stable cell

Highlight box

Key findings

- The proteasome activator REG γ inhibits mantle cell lymphoma (MCL) cell proliferation by promoting cell apoptosis. REG γ inhibits MCL cell growth in mice by promoting apoptosis. REG γ -overexpression induces I κ B accumulation and inhibits Nuclear factor-kappa B (NF- κ B) signaling. REG γ controls NF- κ B signal via STAT3-PSMB5 axis.

What is known and what is new?

- With the rapid development of molecular biology, targeted drugs of MCL have improved outcomes of conventional chemotherapy while avoiding long-term toxicities. The proteasome activator REG γ is implicated in the progression of cancer. As a promoter of cellular growth and a key regulator of several tumor suppressors, REG γ -proteasome as a potential target of new cancer-drug development.
- We found that REG γ regulated p-STAT3 expression by accelerating its half-life and downregulated the NF- κ B signaling pathway to promote MCL cell apoptosis by negatively regulating STAT3-mediated PSMB5 expression and subsequently upregulating I κ B expression.

What is the implication, and what should change now?

- Our discovery of the mechanism of REG in MCL provides a potential therapeutic target for its treatment.

lines PT67 pRevTRE and pRevTRE-REG γ were obtained through selection with G418 and hygromycin. The supernatant containing the virus was concentrated, purified, and then used to infect JEKO-1 cells. JEKO-1 cells with high REG γ expression were obtained by selection with G418 for 24 h.

Construction of stably transfected REG γ -knockdown cells using short hairpin RNA (shRNA) against REG γ . The special complementation fragments of REG γ (shN, 5'-UUCUCCGAACGUGUCACGUTT-3'; shREG γ , 5'-CAGAAGACUUGGUGGCAAA-3') were constructed and inserted into the lentiviral vector pHBLV-u6-puro (Huada, Guangzhou, China). Then, the lentiviral viruses were packaged by transfecting 293T cells for 24 h. Then, the obtained stably transfected cells were selected by treatment with 1 μ g/mL of puromycin. The transfection efficiency of shRNA-REG γ on JEKO-1 and REC-1 cells was determined using western blotting.

Cell proliferation assay

The Cell Counting Kit 8 (CCK-8, Beyotime, Beijing, China) assay was used to detect JEKO-1 cell proliferation. First, JEKO-1 cells were seeded in 96-well plates (100 μ L, 2,000 cells per well) at different time points after transfection, and then 10 μ L CCK-8 solution was added to each well. The optical density of each well at 450 nm was measured using a spectrophotometer (Bio-Rad, Hercules, CA, USA) after 4 h of incubation.

Cell apoptosis assay

After transfection, JEKO-1 cell apoptosis was measured using a flow cytometer (BD Biosciences, Franklin Lakes, NJ, USA). Briefly, JEKO-1 cells in each group were suspended in 1 \times binding buffer 48 h after transfection. The cells were then stained with 5 μ L Annexin V (Kegen, Nanjing, China, KGA1022) and 7 μ L 7-AAD reagent (Invitrogen, MA, USA, 2115592). After incubation in the dark for 15 min, apoptosis levels were measured using a flow cytometer.

Western blot assays

The total proteins of JEKO-1 and REC-1 cells were leached using a lysis buffer. After centrifugation (12,000 rpm) at 4 $^{\circ}$ C for 15 min, 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis was used to separate the proteins. Proteins were then transferred to polyvinylidene fluoride membranes

(Millipore, Billerica, MA, USA). Membranes were blocked using 5% skim milk for 1 h at room temperature, and the specific diluted primary antibodies (REG γ , Caspase3, Cleaved Caspase3, PARP, Cyclin D1, BCL-2, Tubulin, p-NF- κ B, NF- κ B, I κ B ϵ , I κ B β , I κ B α , p-STAT3, STAT3, and PSMB5) were incubated with the membranes overnight at 4 $^{\circ}$ C. The membranes were washed three times with tris-buffered saline containing 0.1% Tween 20 solution, and then, horse radish peroxidase-conjugated secondary antibody (1:5,000, Abcam, Waltham, MA, USA) was incubated with the membranes for 1 h at 25 $^{\circ}$ C. Finally, the protein bands were visualized using an ultra-violet (UV) gel imager (FluorChem M, Globalebio, Beijing, China). Cells were treated with or without STAT3 inhibitor Stattic (HY-13818, MCE, Shanghai, China) and then collected, and the protein expression of the NF- κ B signaling pathway was detected by western blot assays. Separately, JEKO-1 cells were treated with cycloheximide (1 mg/mL) for 3–6 h, and the half-life of p-STAT3 was detected by western blot assays.

TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assays were used to evaluate cell apoptosis. First, the cells grown on coverslips were fixed with 4% paraformaldehyde, followed by washing with phosphate-buffered saline. Second, cells were treated with 0.3% H₂O₂ to inhibit the activity of endogenous peroxidase and then with the TUNEL reaction solution (Sigma-Aldrich, Merck KGaA, Germany) for 1 h at 37 $^{\circ}$ C. Finally, they were imaged using a laser confocal microscope (Nikon A1 confocal microscope, Nikon Co., Japan).

Statistical analyses

All experiments were repeated three times. The data are presented as mean \pm standard deviation (SD) and were analyzed using SPSS version 17.0 (SPSS Inc., Chicago, USA). The differences between two groups were compared using a *t*-test and those between more than three groups were compared using one-way analysis of variance (ANOVA). Differences were considered statistically significant at P values less than 0.05.

Results

REG γ inhibits MCL cell proliferation by promoting cell apoptosis

To assess the effect of REG γ on JEKO-1 cells, we

constructed stable REG γ -knockdown (shR) and REG γ -overexpressing (+Dox) cell lines. Reducing levels of REG γ significantly promoted cell proliferation in a time-dependent manner ($P < 0.01$), and higher expression of REG γ suppressed the proliferation of JEKO-1 cells (Figure 1A,1B) ($P < 0.01$). Furthermore, the flow cytometry assay showed that the apoptotic rate was significantly lower in the shR JEKO-1 cells ($P < 0.001$, Figure 1C) and significantly higher in the +Dox JEKO-1 cells than that in their corresponding controls ($P < 0.001$, Figure 1D). These results indicate that silencing REG γ may efficiently promote cell proliferation by inhibiting apoptosis of JEKO-1 cells, and the higher expression of REG γ suppressed the proliferation of JEKO-1 cells by inducing apoptosis *in vitro*. However, flow cytometry assays revealed that REG γ had no effect on the cell cycle of MCL cells (Figure S1).

To further investigate the molecular mechanisms underlying the effects of REG γ on the cell cycle and apoptosis, we investigated the expression of proteins related to the cell cycle and apoptosis in shR and +Dox JEKO-1 cells. The silencing of REG γ in JEKO-1 reduced Caspase3-PARP-mediated apoptosis (Figure 1E). In contrast, higher expression of REG γ promoted Caspase3-PARP-mediated apoptosis (Figure 1F) and REG γ had no effect on cyclin D1 and BCL2 expression (Figure S2).

REG γ inhibits MCL cell growth in mice by promoting apoptosis

Based on our finding that REG γ inhibits MCL cell proliferation by promoting apoptosis *in vitro*, we next investigated the influence of REG γ on apoptosis *in vivo*. ShR and control (shN) JEKO-1 cells were inoculated subcutaneously into the flanks of nude mice, and tumor volume was measured every four days. We observed variability in tumor volumes generated from shR and shN JEKO-1 cells beginning on day 8 after the appearance of the tumors, and tumors generated from shR JEKO-1 cells grew more rapidly than tumors generated from shN JEKO-1 cells ($P < 0.01$, Figure 2A,2B). The TUNEL assay was used to detect apoptotic cells in xenograft tumor tissues, and the results showed that more apoptotic cells were observed in tumor tissues in the shN group than in the shR group (Figure 2C).

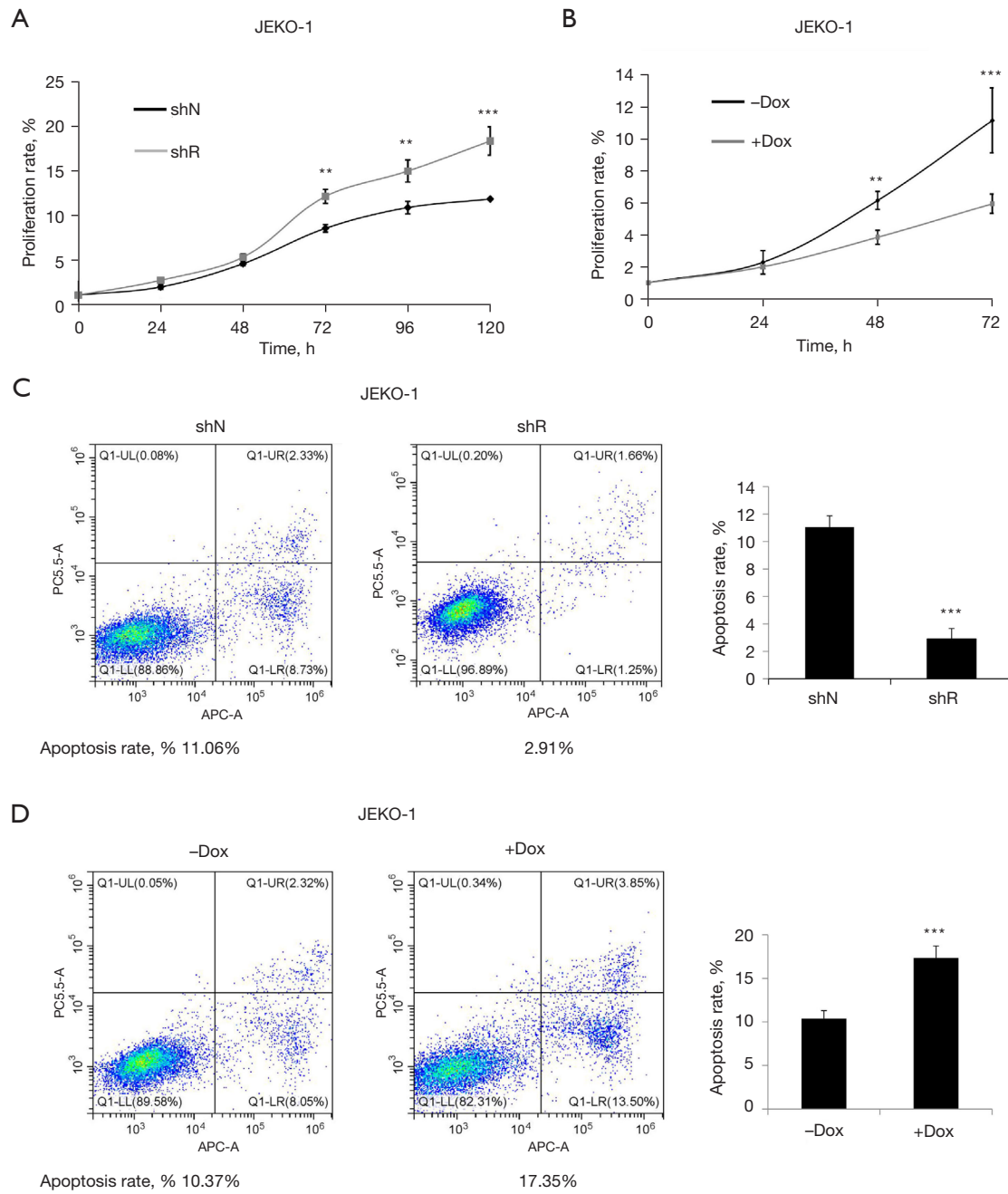
REG γ -overexpression induces I κ B accumulation and inhibits NF- κ B signaling

To explore the underlying mechanisms of REG γ -mediated

MCL cell (JEKO-1 and REC-1) apoptosis, the protein expressions of key molecules involved in the NF- κ B signaling pathway were investigated. Western blot assays revealed decreased expressions of the three types of I κ B subunits and significantly increased levels of NF- κ B in the shR group compared with those in the shN group (Figure 3A,3B), and increased expression of I κ B and significantly decreased levels of NF- κ B in the +Dox group compared with those in the control (-Dox) group (Figure 3C). NF- κ B normally interacts with I κ B, which leads to NF- κ B being sequestered in the cytoplasm to inhibit its signaling (15). These results suggest that REG γ promotes MCL cell apoptosis by upregulating I κ B expression to inhibit NF- κ B activation. However, REG γ reduces I κ B ϵ and promotes NF- κ B signaling in other cell types, including human lymphoma U937 cells, Burkitt lymphoma Namalwa cells, and mouse embryonic fibroblasts (Figure S3). These results indicate that the regulation of NF- κ B and I κ B expression by REG γ is cell-specific.

REG γ controls NF- κ B signal via STAT3-PSMB5 axis

STAT3 is an important molecule in cancer therapy, as it interacts with the NF- κ B signaling pathway and promotes tumor progression (16-18). PSMB5 is the molecular target of bortezomib, used clinically to ameliorate relapsed multiple myeloma and MCL. Activated STAT3 levels in cancers may presumably circumvent the effect of bortezomib regimen through up-regulation of PSMB5 protein. These observations suggest that STAT3 modulates PSMB5 expression in MCL (19). To further elucidate the specific molecular mechanism by which REG γ regulates the NF- κ B pathway, we examined STAT3 and PSMB5 expression in MCL cells with overexpressed and silenced REG γ . The results revealed that REG γ knockdown activated STAT3 and upregulated PSMB5 expression (Figure 4A), whereas REG γ overexpression inhibited STAT3 and downregulated PSMB5 expression in JEKO-1 cells (Figure 4B). We explored the role of REG γ in the STAT3-PSMB5 signaling axis regulating NF- κ B signaling pathways by treating shN and shR JEKO-1 cells with the STAT3 inhibitor Stattic. The results demonstrated that Stattic downregulated PSMB5 expression, upregulated I κ B ϵ expression, and downregulated NF- κ B expression in JEKO-1 cells, in both shN and shR cells (Figure 4C). We found that REG γ is involved in the regulation of p-STAT3 levels by accelerating its half-life (Figure 4D). Taken together, these results show that REG γ regulates NF- κ B



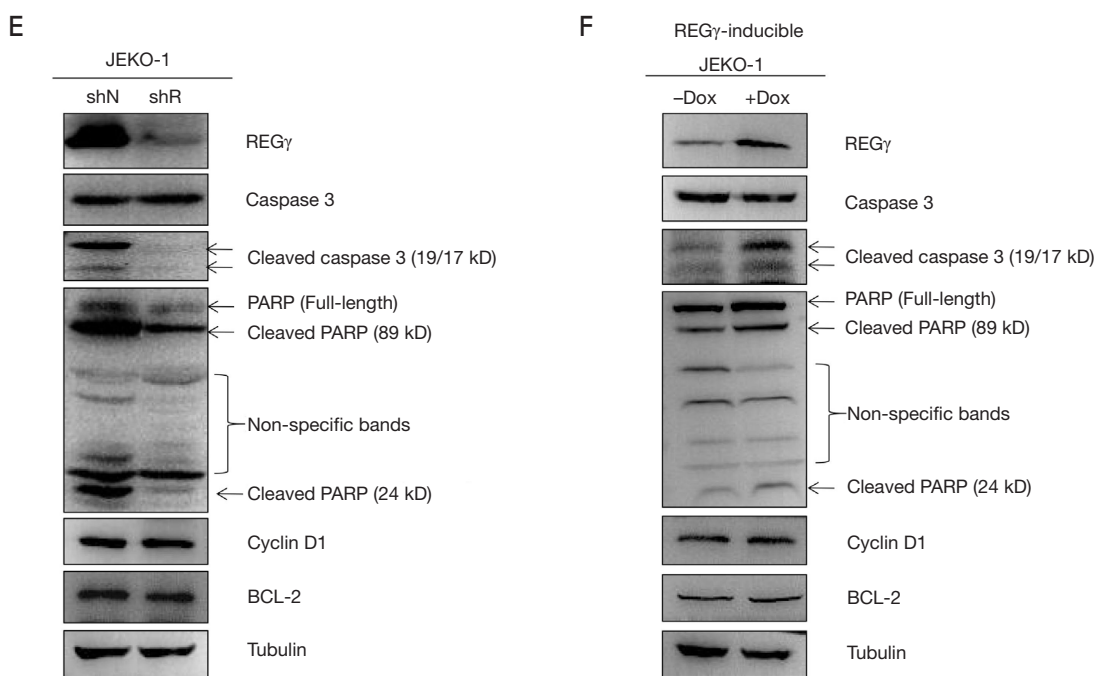


Figure 1 Jeko-1 cells proliferation and apoptosis. Cells were transfected with shRNA against REG γ or harbored doxycycline-inducible REG γ , and then, the proliferation and apoptosis were assessed. (A,B) Proliferation rates of REG γ -knockdown and REG γ -overexpressing cells were detected using CCK-8 assay. (C,D) Percentages of apoptotic REG γ -knockdown and REG γ -overexpressing cells were determined using flow cytometry. (E,F) Levels of the cell cycle-associated proteins and apoptosis-associated proteins in REG γ -knockdown and REG γ -overexpressing cells were detected using western blot assays. **, $P < 0.01$; ***, $P < 0.001$. shRNA, short hairpin RNA; REG γ , the proteasome activator REG γ (also known as PSME3, PA28 γ , and Ki antigen); CCK-8, Cell Counting Kit-8.

signaling by downregulating STAT3-mediated PSMB5 expression, subsequently influencing the expression of I κ B.

Discussion

REG γ is important for tumor occurrence and development, but understanding of the specific role of REG γ in MCL is lacking. In this study, we found that REG γ inhibits MCL cell proliferation by promoting cell apoptosis, and the downregulated NF- κ B signaling is closely related to the regulation of apoptosis. But REG γ reduces I κ B ϵ and promotes NF- κ B signaling in other cell types (human lymphoma U937 cells, Burkitt lymphoma Namalwa cells, and mouse embryonic fibroblasts). REG γ downregulated PSMB5 expression by affecting the level of p-STAT3, accelerating its half-life, upregulating I κ B expression, and subsequently inhibiting NF- κ B signaling to promote MCL cell apoptosis.

REG γ is a member of the 11S proteasome and is upregulated in several types of cancers, such as thyroid,

breast, lung, colorectal, and liver cancers (11,20). In previous reports, the overexpression of REG γ at the cellular level not only promoted cell proliferation and migration of lung cancer cells but also increased the number of cells in the S and G2/M phases (21). Silencing of REG γ suppressed cell proliferation, migration, and invasiveness of endometrial cancer cells (22). Surprisingly, our findings revealed the opposite result in MCL cells. We found that REG γ inhibits MCL cell proliferation by promoting apoptosis, suggesting that upregulation of REG γ may be a potential therapeutic strategy for MCL. In addition, REG γ had no effect on the MCL cell cycle.

The NF- κ B signaling pathway plays a crucial role in the proliferation and apoptosis of MCL cells. Thus, targeting the NF- κ B pathway might be a potential approach in treating MCL (23,24). In this study, REG γ knockdown significantly increased the expression of I κ B and decreased the levels of NF- κ B. The results clearly indicate that silencing REG γ resulted in decreased expression of the three types of I κ B subunits and significantly increased levels

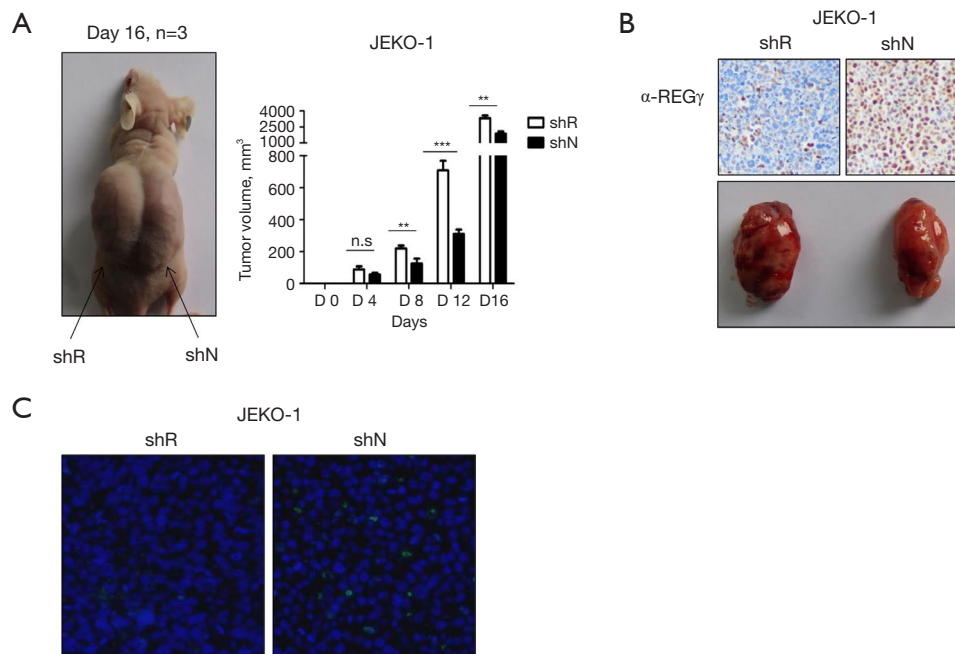


Figure 2 Effect of REG γ on the mantle cell lymphoma cell apoptosis in mice. (A,B) Tumor volumes and growth rate generated from shR and shN JEKO-1 cells were compared after the appearance of the tumors (HE staining, $\times 200$). (C) Apoptosis of shN and shR JEKO-1 cells in the xenograft tumors was assessed using TUNEL staining (DAPI: blue, TUNEL: green, $\times 400$). n.s, no statistical difference; **, $P < 0.01$; ***, $P < 0.001$. REG γ , the proteasome activator REG γ (also known as PSME3, PA28 γ , and Ki antigen); shR, REG γ -knockdown; shN, control; HE, hematoxylin-eosin; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; DAPI, 4', 6-diamidino-2-phenylindole.

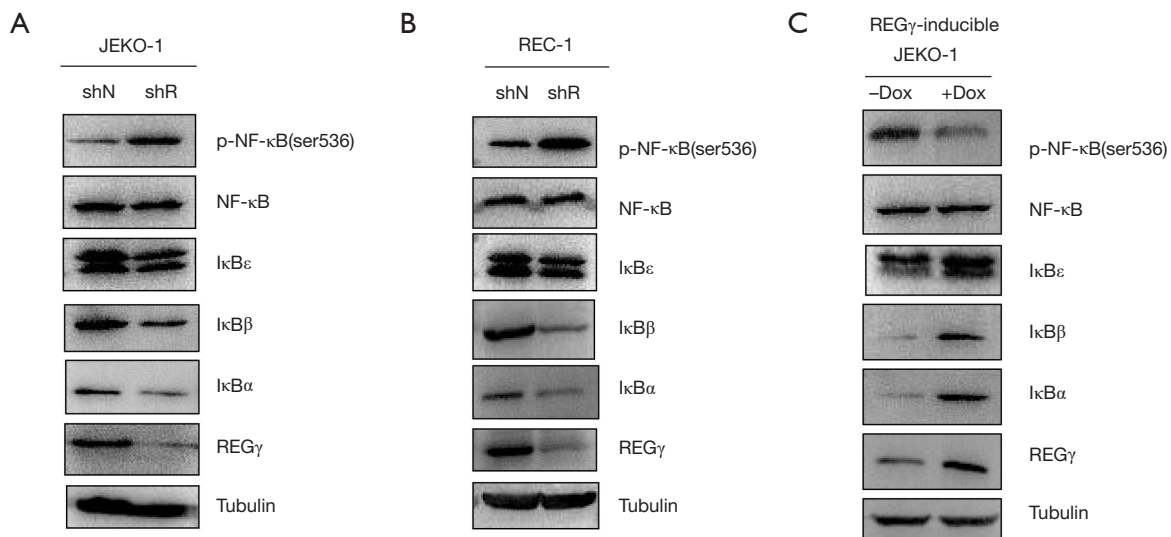


Figure 3 Potential mechanisms of REG γ in JEKO-1 and REC-1 cells. (A,B) Levels of p-NF- κ B(ser536), NF- κ B, I κ B α , I κ B β , I κ B ϵ , and REG γ in shN and shR JEKO-1 and REC-1 cells were determined using western blot assays. (C) The levels of p-NF- κ B(ser536), NF- κ B, I κ B α , I κ B β , I κ B ϵ , and REG γ in JEKO-1 cells harboring doxycycline-inducible REG γ were determined using western blot assays. REG γ , the proteasome activator REG γ (also known as PSME3, PA28 γ , and Ki antigen); p-NF- κ B(ser536), phospho-nuclear factor-kappa B; NF- κ B, nuclear factor-kappa B; I κ B α , inhibitor kappa B alpha; I κ B β , inhibitor kappa B beta; I κ B ϵ , inhibitor kappa B epsilon; shR, REG γ -knockdown; shN, control.

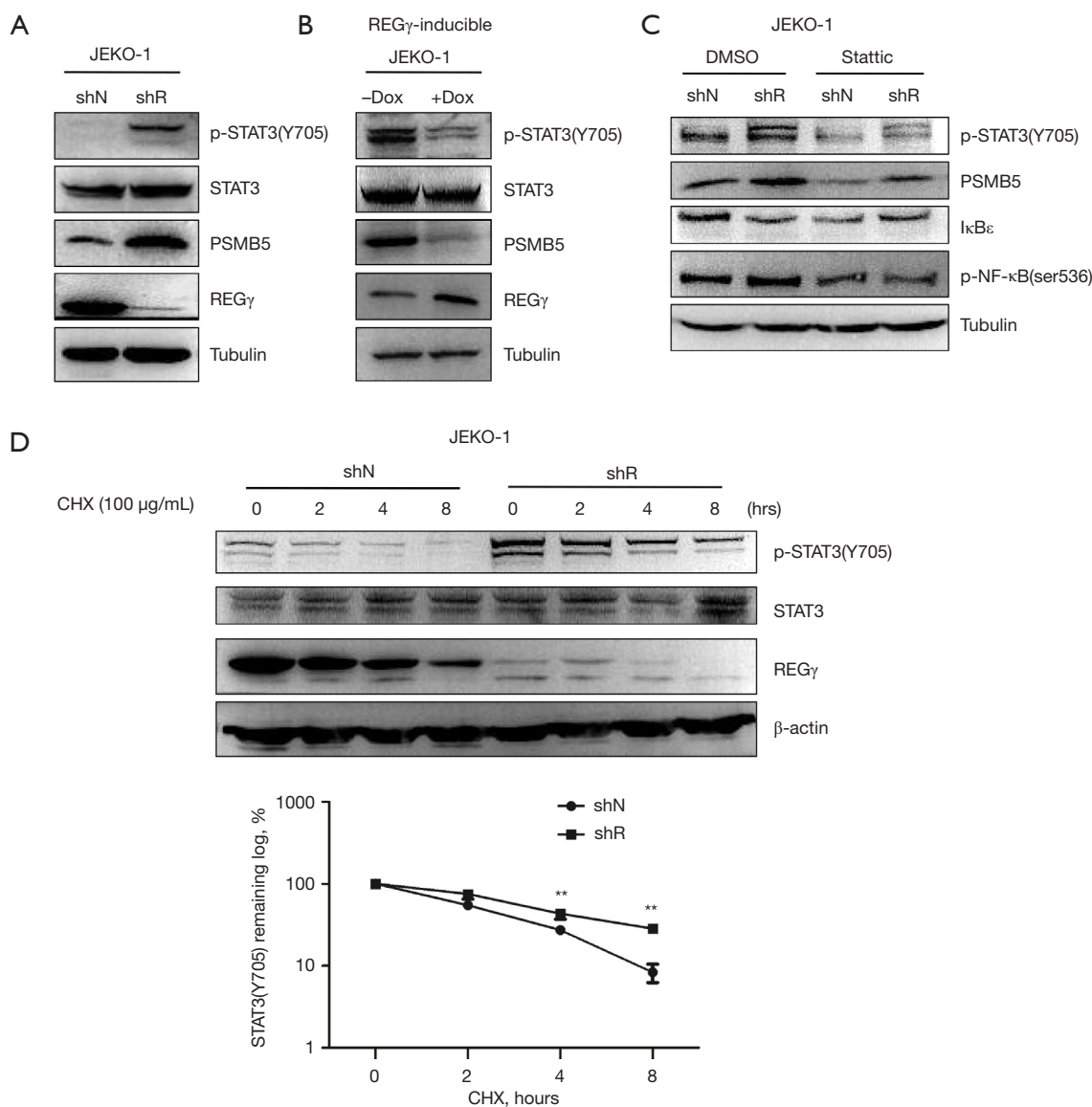


Figure 4 Specific mechanism of REG γ in regulating the NF- κ B pathway. (A) Levels of p-STAT3(Y705), STAT3, PSMB5, and REG γ in shN and shR JEKO-1 cells were detected using western blot assays. (B) Levels of p-STAT3(Y705), STAT3, PSMB5, and REG γ in JEKO-1 cells harboring doxycycline-inducible REG γ were determined using western blot assays. (C) Levels of p-STAT3(Y705), PSMB5, I κ B ϵ and p-NF- κ B(ser536) in shR and shN JEKO-1 cells treated with the STAT3 inhibitor Stattic, was detected using western-blot assays. (D) Degradation of p-STAT3(Y705) and STAT3 in shN and shR JEKO-1 cells. **, P<0.01. REG γ , the proteasome activator REG γ (also known as PSME3, PA28 γ , and Ki antigen); p-STAT3(Y705), phospho signal transducer and activator of transcription 3; STAT3, signal transducer and activator of transcription 3; PSMB5, proteasome subunit beta 5; I κ B ϵ , inhibitor kappa B epsilon; shR, REG γ -knockdown; shN, control; p-NF- κ B(ser536), phospho-nuclear factor-kappa B.

of NF- κ B. Upregulation of I κ B expression causes inhibition of the NF- κ B signaling pathway, leading to reduced proliferation and increased apoptosis of MCL cells (25). These studies agree with our findings, which further show that the inhibitory effect of REG γ on MCL cells is closely related to the downregulation of the NF- κ B signaling. We found that REG γ decreased I κ B ϵ and promoted NF- κ B signaling in other cell types, which indicates that the regulation of NF- κ B and I κ B expression by REG γ is cell-specific.

STAT3 plays a crucial role in the formation and metastasis of cancer, as well as the development of drug resistance (6,11,20). In tumor cells, STAT3 often interacts with NF- κ B to advance tumor progression (16-18). Additionally, it has been suggested that STAT3 could potentially have a role in the biological function of MCL (26). In this study, REG γ knockdown activated STAT3 and upregulated PSMB5 expression, whereas REG γ overexpression inhibited STAT3 and downregulated PSMB5 expression in JEKO-1 cells. Treating the shN and shR JEKO-1 cells with the Stattic downregulated PSMB5 expression, upregulated I κ B ϵ expression, and downregulated NF- κ B expression in JEKO-1 cells, independent of REG γ expression, suggesting that REG γ regulates NF- κ B signaling pathways to promote the apoptosis of JEKO-1 cells by downregulating STAT3-mediated PSMB5 expression and subsequently influencing the expression of I κ B. Meanwhile, p-STAT3 degradation in shR JEKO-1 cells was slower than that in the control group, indicating that REG γ is involved in the regulation of p-STAT3 expression by accelerating its half-life. Previous research has established that STAT3 knockdown significantly reduces expression of the transcription factor PSMB5, and I κ B expression is negatively regulated by PSMB5 (18). Collectively, our results further suggest that REG γ inhibits NF- κ B signaling pathways to promote apoptosis of MCL cell by inhibiting STAT3 activity through shortening half-life of p-STAT3, which downregulates PSMB5 expression and subsequently influences the expression of I κ B. However, several studies have indicated that REG γ promotes tumorigenesis and the development of cancers by promoting degradation of target proteins, which regulate multiple signaling pathways (27-29). This is in contrast to our present findings indicating that REG γ inhibits the growth and proliferation of MCL cells.

Conclusions

REG γ may inhibit cell proliferation while promoting apoptosis of MCL cells by downregulating the NF- κ B signaling pathway. In parallel, this process is potentially highly relevant to the downregulation of STAT3 and PSMB5 expressions and upregulation of I κ B ϵ expression in MCL cells. However, the present study was mainly limited to specific cells and one animal model. The mechanism of action of REG γ in MCL still requires further investigation.

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Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-2045/rc>

Data Sharing Statement: Available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-2045/dss>

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <https://tcr.amegroups.com/article/view/10.21037/tcr-22-2045/coif>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are

appropriately investigated and resolved. Experiments were performed under a project license (No. 2021-KZ-170-01) granted by Guangdong Second Provincial General Hospital Ethics Committee, in compliance with China's national or institutional guidelines for the care and use of animals.

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