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Telmisartan Exerts Cytotoxicity in Scirrhous Gastric Cancer Cells by Inducing G_0/G_1 Cell Cycle Arrest

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Abstract. *Background/Aim: This study aimed to assess the effects of telmisartan (TEL), a potential antitumor agent, and its mechanism of action in the regulation of apoptosis, autophagy, and cell cycle in scirrhous gastric cancer (SGC). Materials and Methods: The effect of TEL on the viability and chromatin condensation of OCUM-2M and OCUM-12 cells was assessed. Protein expression and the cell cycle were analysed using western blotting and flow cytometry, respectively. Results: TEL inhibited cell proliferation in a dose-dependent manner and increased chromatin condensation and autophagy marker LC3-II levels in OCUM-12 cells. TEL also increased the proportion of cells in the G_0/G_1 phase transition. Conclusion: Apoptosis and autophagy are partially involved in the inhibitory effect of TEL on cell proliferation. Additionally, TEL caused G_0/G_1 cell cycle arrest. Therefore, TEL could be a promising treatment for SGC.*

Gastric cancer is the fifth most common cancer (5.6% of the total cases), and the fourth leading cause of cancer-related deaths (7.7% of the total cancer deaths) worldwide (1). Among the different cancer types, scirrhous gastric cancer (SGC) grows diffusely in the submucosa; therefore, its early detection is difficult, and the prognosis remains poor (2). Gastric cancer is mainly treated by surgery, but in advanced cases, radiation therapy and chemotherapy agents such as fluorouracil, cisplatin, oxaliplatin, and paclitaxel are used. Recently, molecular-targeted agents, such as human epidermal growth factor receptor 2 inhibitors and immune checkpoint inhibitors, have been used for its treatment, improving patient outcome (3). However, these strategies are not adequately effective, and novel therapeutic approaches are required to improve the outcome of SGC patients further.

Angiotensin II receptor blockers (ARBs) exert antihypertensive effects by selectively inhibiting angiotensin II type 1 receptors. Moreover, ARBs promote cardioprotection and renal protection. ARBs have also been reported to exert protective effects against various cancers (4, 5), and telmisartan (TEL), an ARB, has been reported to exert antitumor effects on several cancers, including lung (6), prostate (7), and oesophageal (8) cancers. However, the antitumor effect of TEL on SGC is unclear.

Peroxisome proliferator-activated receptor (PPAR) is a member of the nuclear hormone receptor superfamily and has three subtypes: α, γ, and δ. PPARs form a group of transcription factors that are involved in controlling the intracellular metabolism of hydrocarbons, lipids, and proteins, as well as in cell differentiation. Furthermore, PPARγ and PPARδ regulate cell proliferation and differentiation, and have been reported to exhibit antitumor effects on many cancers (9). Importantly, TEL has been shown to activate PPARγ and PPARδ (10, 11).

The present study aimed to examine the antitumor effects of TEL on SGC and elucidate the mechanism of action of this ARB. To this end, we investigated the effect of TEL on SGC cells and examined its involvement in the regulation of apoptosis, autophagy, PPARγ and PPARδ, mitogen-activated protein kinase (MAPK) pathway, Akt pathway, and cell cycle.

Materials and Methods

Chemicals and antibodies. TEL and irbesartan (IRB) were purchased from Wako Pure Chemical Industries (Osaka, Japan) and LKT Laboratories (St. Paul, MN, USA), respectively; they were dissolved in dimethyl sulfoxide (Nacalai Tesque, Kyoto, Japan). GW9662 (a PPARγ inhibitor) and SP600125 (a JNK inhibitor) were obtained

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from Sigma-Aldrich (St. Louis, MO, USA). GSK3787 (a PPARδ inhibitor), U0126 [an extracellular signal-regulated kinase (ERK) 1/2 inhibitor] and Akt inhibitor IV were provided by Merck (Darmstadt, Germany). Rabbit monoclonal anti-human antibodies against LC3A/B (D3U4C, product no. 12741), phospho-SAPK/JNK (Thr183/Tyr185, product no. 4668), phospho-p38 MAPK (Thr180/Tyr182, D3F9, product no. 4511), phospho-ERK1/2 (Thr202/Tyr204; D13.14.4E, product no. 4370), phospho-Akt (Ser473, D9E, product no. 4060), cyclin D1 (E3P5S, product no. 55506), CDK2 (78B2 product no. 2546), CDK4 (D9G3E product no. 12790), CDK6 (D4S8S product no. 13331), β-actin (13E5, product no. 4970), mouse monoclonal anti-human antibodies against cyclin E1 (HE12, product no. 4129), and rabbit polyclonal anti-human antibodies against Bax (product no. 2772), Bcl-2 (product no. 2876), and cyclin E2 (product no. 4132) were procured from Cell Signaling Technology (Danvers, MA, USA).

Maintenance of cell lines and cell cultures. OCUM-2M (12) and OCUM-12 (13) human SGC cell lines were provided by the Department of Surgical Oncology, Osaka City University Graduate School of Medicine (Osaka, Japan). OCUM-2M and OCUM-12 cells were maintained in a continuous culture in Dulbecco's modified Eagle's medium (DMEM, Wako Pure Chemical Industries) supplemented with 10% heat-inactivated foetal bovine serum (FBS, Invitrogen, Thermo Fisher Scientific, Carlsbad, CA, USA) and penicillin (50 U/ml)-streptomycin (50 μg/ml) (Nacalai Tesque) at 37˚C in a humidified atmosphere containing 95% air and 5% CO_2.

Cell viability assay. A total of 5.0×10^5 OCUM-2M cells or 8.0×10^5 OCUM-12 cells per well were seeded onto 96-well plates (Asahi Glass, Tokyo, Japan) with TEL, in the presence or absence of inhibitors for 48 h. Cell viability was determined as previously described (14). Briefly, a Cell Quanti-Blue™ (BioAssay Systems, Hayward, CA, USA) Cell Viability Assay Kit was used for this assay, and fluorescence was measured using a CytoFluor® Series 4000 Fluorescence Multi-Well Plate Reader (PerSeptive Biosystems, Framingham, MA, USA) at excitation/emission wavelengths of 530/580 nm. The 50% growth inhibitory concentrations (IC_{50}) were calculated according to the sigmoid inhibitory effect model. TEL was used at approximately the IC_{50} value in each cell line in the co-treatment experiments.

Detection of chromatin condensation using fluorescence microscopy. Cells were seeded at a density of 3.3×10^5 (OCUM-2M) or 5.2×10^5 (OCUM-12) onto a 60-mm dish and incubated with TEL for 48 h. Chromatin condensation was observed as previously described (14). Briefly, the cells were stained with 80 μg/mL of Hoechst 33342 (Dojindo Molecular Technologies, Kumamoto, Japan) and observed under UV excitation using a fluorescence microscope (VANOX; Olympus, Tokyo, Japan). The cells were imaged at 20× magnification.

Western blot analysis. Cells were seeded at a density of 7.0×10^5 (OCUM-2M) or 1.39×10^6 (OCUM-12) in a 100-mm dish and incubated with TEL for 48 h. Western blot analysis was performed according to a previously described method (14). Briefly, 10 μg of protein (per lane) were loaded onto 6.5 or 12.5% SDS-PAGE gels and electrophoresed. Proteins were then transferred to a polyvinylidene difluoride membrane (GE Healthcare, Chicago, IL, USA) and blocked with Tris-buffered saline, 0.1% Tween® 20 containing 2% ECL Advance™ Blocking Agent (GE Healthcare), for 1 h. The blocked membranes were incubated with primary antibodies (1:10,000 dilution) at 4˚C overnight. The membranes were then incubated with the secondary antibody (1:25,000 dilution) for 1 h at 20˚C, and chemiluminescence was detected.

Cell cycle analysis. Cells were seeded at a density of 7.0×10^5 (OCUM-2M) or 1.39×10^6 (OCUM-12) onto a 100-mm dish and incubated with TEL for 48 h. The cell cycle was analysed as previously described (15). Briefly, cells were stained with propidium iodide (Nacalai Tesque) in the dark at 4˚C for 30 min and analysed using FACSCalibur HG™ (BD Biosciences, Franklin Lakes, NJ, USA).

Statistical analysis. Statistical significance of the difference between groups was established using the Student's or Welch's *t*-test for comparisons of two groups and ANOVA followed by Dunnett's post-hoc test for multiple comparisons. Statistical significance was set at $p<0.05$. Data are expressed as the mean±standard deviation.

Results

Effects of TEL and IRB on cell viability. TEL inhibited cell proliferation in a dose-dependent manner with an IC50 value of 36.9±2.7 μM in OCUM-2M and 78.7±4.4 μM in OCUM-12 cells. In contrast, IRB, another ARB, did not inhibit cell proliferation; the IC_{50} values of IRB were higher than 100 μM (Figure 1).

Involvement of apoptosis and autophagy in the effect of TEL on cell viability. Next, we investigated the role of apoptosis and autophagy in TEL-induced inhibition of cell proliferation. We stained cell nuclei with Hoechst 33342, and observed chromatin condensation, an indicator of apoptosis. TEL significantly increased the proportion of OCUM-12 cells showing chromatin condensation from 4% to 11% ($p<0.05$), while no significant increase was observed in OCUM-2M cells (Figure 2A). We also detected Bax, the pro-apoptotic protein, and Bcl-2, the anti-apoptotic protein in OCUM-12 cells. TEL did not increase the Bax/Bcl-2 expression ratio, indicating that the mitochondrial apoptotic pathway was not involved in TEL-induced inhibition of cell proliferation in OCUM-12 cells (Figure 2B). These results suggested that TEL caused apoptosis that did not involve the mitochondrial pathway.

We next detected the presence of LC3 protein, which has been widely used as a credible autophagy marker (16, 17). When autophagy is induced, LC3-I is converted to LC3-II; however, TEL treatment did not markedly change the levels of LC3-II in OCUM-2M or OCUM-12 cells (Figure 2C). These results suggest that autophagy has limited involvement in TEL-induced cell death.

Involvement of PPARγ and PPARδ in the effect of TEL on cell viability. The effects of TEL, in combination with GW9662 (a PPARγ antagonist) or GSK3787 (a PPARδ antagonist), were examined to evaluate whether TEL activated PPARγ- or PPARδ-mediated cytotoxicity. TEL-induced cytotoxicity was not blocked by GW9662 or GSK3787 in both OCUM-2M and OCUM-12 cells (Figure

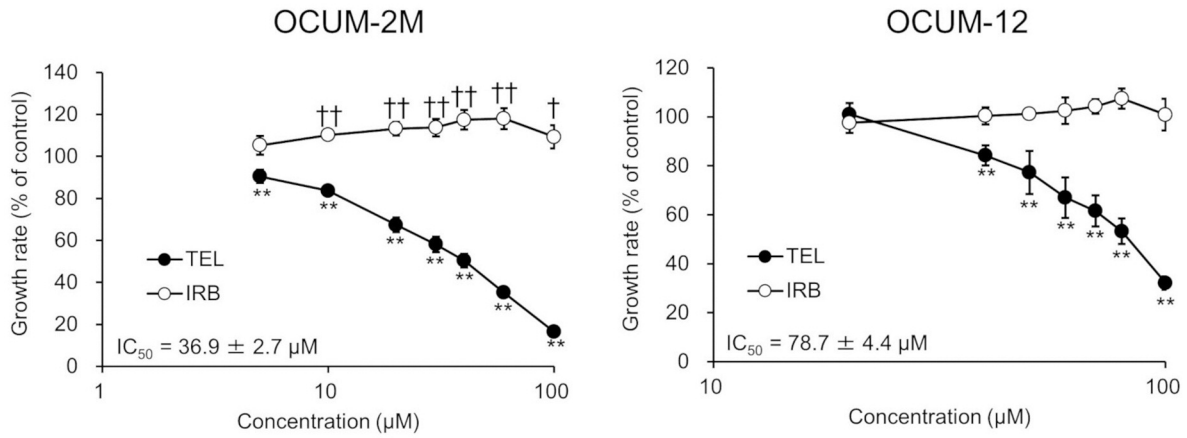

Figure 1. *Cytotoxic effects of TEL and IRB on the SGC cell lines OCUM-2M and OCUM-12. Cell viability was assessed using a fluorescence-based assay; the data are presented as the mean±SD (n=4). Statistical significance was assessed using Dunnett's test (control vs. each concentration of TEL or IRB). **p<0.01 vs. control (for TEL). †p<0.05 and ††p<0.01 vs. control (for IRB). TEL: Telmisartan; IRB: irbesartan; SGC: scirrhous gastric cancer.*

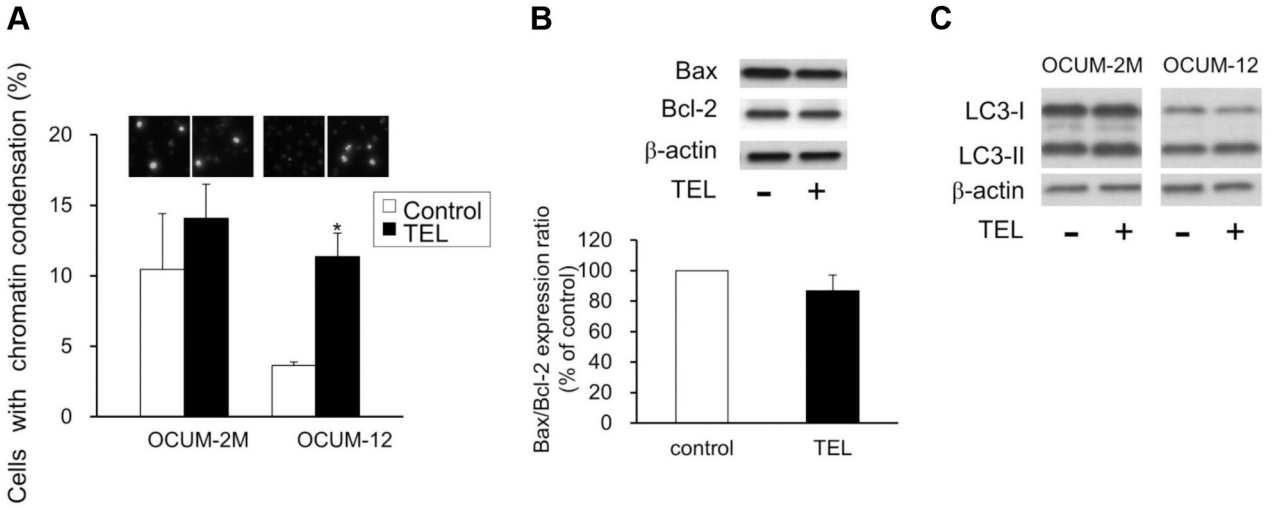

Figure 2. *Involvement of apoptosis and autophagy in TEL-induced cell death. (A) Chromatin condensation induced by TEL. Representative fluorescence microscopy images of cells stained with Hoechst 33342 and the proportion of cells showing chromatin condensation. The data are presented as the mean±SD from three independent experiments. The statistical significance was assessed using t-test. *p<0.05 vs. control. (B) Bax and Bcl-2 protein expression. Bax and Bcl-2 expression levels were normalised to those of β-actin and analysed from three independent experiments. *p<0.05 and **p<0.01 vs. control (t-test). (C) LC3 protein expression. LC3 expression was normalised to that of β-actin and analysed from three independent preparations. TEL: Telmisartan.*

3), suggesting that TEL induced cytotoxicity in a PPARγ- and PPARδ-independent manner.

Involvement of the MAPK signalling pathway in the effect of TEL on cell viability. To clarify whether the MAPK signalling pathway was involved in TEL cytotoxicity, we examined the expression and phosphorylation levels of the representative MAPK subfamily proteins (JNK, p38, and ERK1/2). Phosphorylation of p38 was not affected by TEL treatment in both OCUM-12 and OCUM-2M cells (Figure 4A), suggesting that the contribution of p38 to TEL cytotoxicity was minimal. TEL upregulated JNK and ERK1/2 phosphorylation in OCUM-12 and OCUM-2M cells, respectively (Figure 4A).

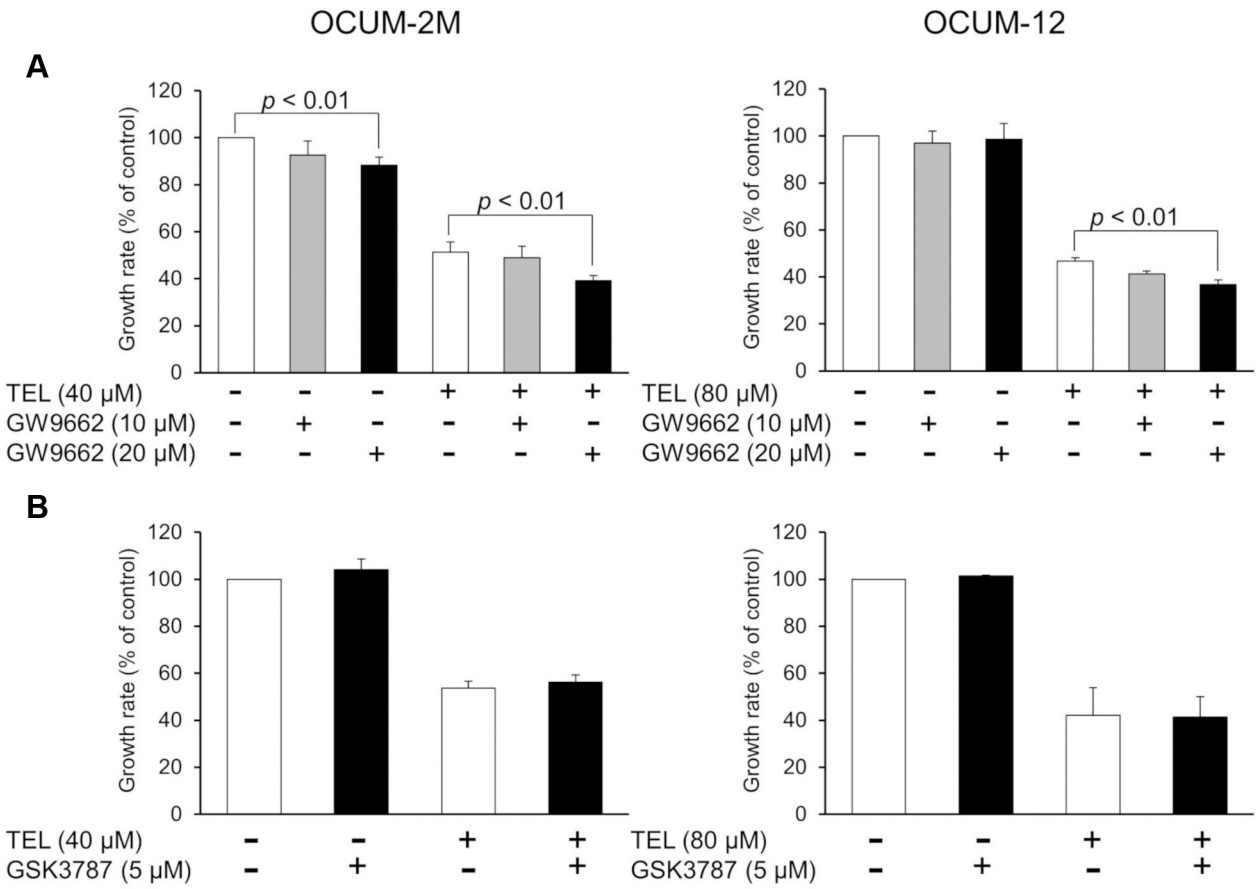

Figure 3. *Effect of PPARγ inhibitor (GW9662) and PPARδ inhibitor (GSK3787) on TEL-induced cell death. Cell viability was assessed using a fluorescence-based assay (n=4-6). The statistical significance was assessed using Dunnett's test (control vs. inhibitors, TEL vs. TEL + inhibitors). TEL: Telmisartan.*

We then treated OCUM-12 cells with the JNK inhibitor SP600125, and OCUM-2M cells with the ERK1/2 inhibitor U0126. SP600125 and U0126 did not suppress TEL-induced cell death (Figure 4B and C). These results suggest that although TEL activates JNK or ERK1/2, they are not involved in TEL-induced cytotoxicity.

Involvement of the Akt pathway in the effect of TEL on cell viability. To examine the involvement of the Akt pathway, we analysed the expression and phosphorylation of Akt protein. Akt phosphorylation was elevated in TEL-exposed OCUM-12 cells (Figure 5A). We then investigated the effect of Akt inhibitor IV on TEL-induced cell death. Akt inhibitor IV significantly decreased the viability of TEL-exposed cells ($p<0.01$, TEL *vs*. TEL + Akt inhibitor IV) (Figure 5B). However, this was not thought of as a meaningful difference. These results indicated that Akt is not involved in TEL-induced inhibition of cell proliferation, although Akt may be activated by TEL.

Involvement of cell cycle regulation in the effect of TEL on cell viability. We examined whether TEL affected cell cycle progression and found that the proportion of cells in the G_0/G_1 phase was significantly increased after TEL exposure ($p<0.01$, control *vs*. TEL) (Figure 6A). TEL increased cyclin D1 levels and decreased those of CDK2 and CDK6 in OCUM-2M cells. In OCUM-12 cells, cyclin D1, CDK2, CDK4, and CDK6 levels were decreased (Figure 6B). These results suggested that TEL induced G_0/G_1 cell cycle arrest.

Discussion

In this study, we showed that TEL treatment inhibited proliferation of SGC cells in a dose-dependent manner, with IC_{50} values ranging between 37-79 μM. IRB did not inhibit proliferation in the examined dose range (Figure 1). In support, TEL has been previously reported to inhibit proliferation of non-SGC cells (18). Furthermore, we showed that TEL was cytotoxic to SGC cells, which have some unique

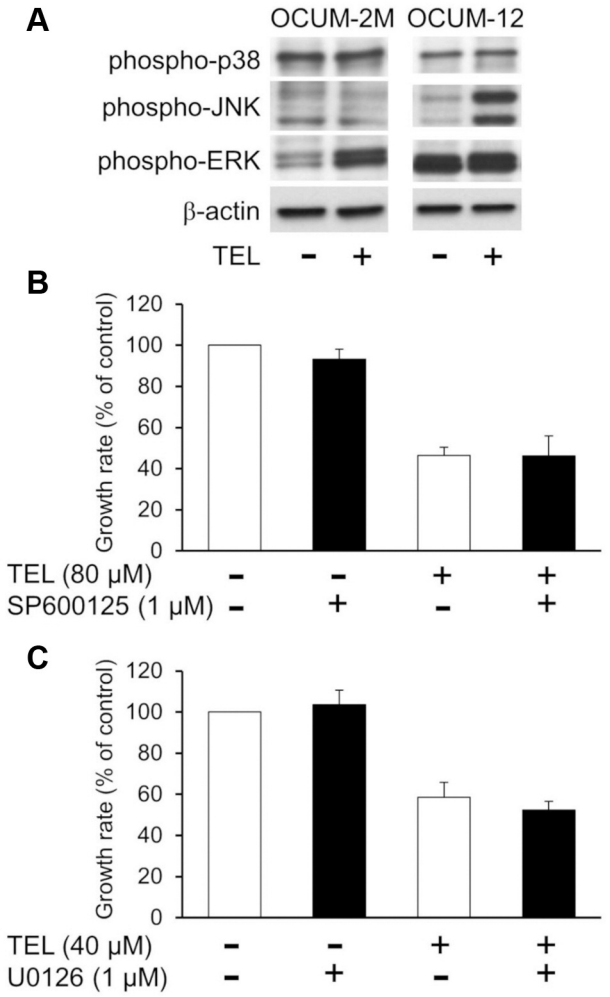

Figure 4. *Involvement of the MAPK signalling pathway in TEL-induced cell death. (A) JNK, p38, and ERK1/2 expression in cells treated with TEL. Characteristic bands showing the phosphorylation of JNK, p38, and ERK1/2 are observed on the western blot. β-actin was used as a loading control. (B) Effect of a JNK inhibitor (SP600125) on TEL-induced death in OCUM-12 cells. (C) Effect of an ERK1/2 inhibitor (U0126) on TEL-induced death in OCUM-2M cells. Cell viability was assessed using a fluorescence-based assay (n=4). The statistical significance was assessed using Dunnett's test (TEL vs. TEL + inhibitors). MAPK: Mitogen-activated protein kinase; TEL: telmisartan; JNK: c-Jun N-terminal kinase; ERK1/2: extracellular signal-regulated kinase 1/2.*

characteristics compared to other gastric cancer types. It has been previously shown that the relative binding affinity of IRB to the angiotensin II type 1 receptor is stronger than that of TEL (19). Furthermore, ARBs have been reported to both increase and decrease the risk of cancer (4). The antitumor effect of TEL seems to be independent of its capacity to block angiotensin II type 1 receptor, which is the typical pharmacological effect of ARBs.

In the present study, to reveal the detailed mechanism underlying TEL-induced cytotoxicity, we first investigated the morphological changes that occurred during cell death. An increase in the proportion of OCUM-12 cells showing chromatin condensation was observed after TEL treatment (Figure 2B), indicating that apoptosis was involved in TEL-induced death in this cell line. However, no change in the proportion of cells with condensed chromatin was observed in OCUM-2M cells (Figure 2B). TEL has been reported to induce apoptosis in various cancer cells, such as lung (6), prostate (7) and renal cancers (20), but not in oesophageal cancer (8, 21), cholangiocarcinoma (22) and non-SGC (18). The involvement of apoptosis in TEL-induced cytotoxicity appears to depend on the cell type. We then investigated the possible role of autophagy in TEL-induced cytotoxicity. TEL had no or minimal effects on the expression of the autophagy marker LC3-II (Figure 2C), suggesting that TEL inhibited cell proliferation without inducing autophagy. We conclude that autophagy does not strongly contribute to TEL-induced cytotoxicity in SGC.

PPARγ and PPARδ are activated by TEL (11, 23), and they have been reported to be involved in the antitumor effects of TEL on other cancer cell lines (7, 24). However, GW9662 and GSK3787, did not block TEL-induced death in either OCUM-2M or OCUM-12 cells (Figure 3), indicating that TEL caused cell death in a PPARγ- and PPARδ-independent manner. Thus, the dependence of TEL-induced cytotoxicity on PPARγ or PPARδ function may vary depending on the type of cancer.

MAPK signalling pathway regulates cell proliferation, growth, and survival, and may be a target for cancer therapy (25, 26). MAPK has been reported to be involved in the antitumor effects of TEL against some cancers (27-29). Three MAPK proteins were detected, and although p38 was not activated by TEL in either OCUM-12 or OCUM-2M cells, JNK was activated in OCUM-12 cells (Figure 4A). Interestingly, TEL also activated ERK1/2 in OCUM-2M cells (Figure 4A), which has been shown to promote cell proliferation (30). To clarify the involvement of JNK and ERK1/2 in TEL-induced cytotoxicity, we examined the effect of the JNK inhibitor SP600125 and the ERK1/2 inhibitor U0126. SP600125 and U0126 had no impact on TEL-induced cytotoxicity in OCUM-12 and OCUM-2M cells, respectively (Figure 4B and C), suggesting that JNK or ERK1/2 were not involved in TEL-induced cell death, although both proteins were activated by TEL. Stresses, such as oxidative and endoplasmic reticulum stresses, have been reported to activate ERK1/2 (31), and TEL might cause such stresses. ERK1/2 activation may have been triggered to protect against TEL-induced stress.

Some studies have reported that TEL affects Akt in certain cancer types (6, 21, 32); therefore, we investigated the involvement of Akt on TEL-induced cytotoxicity in SGC.

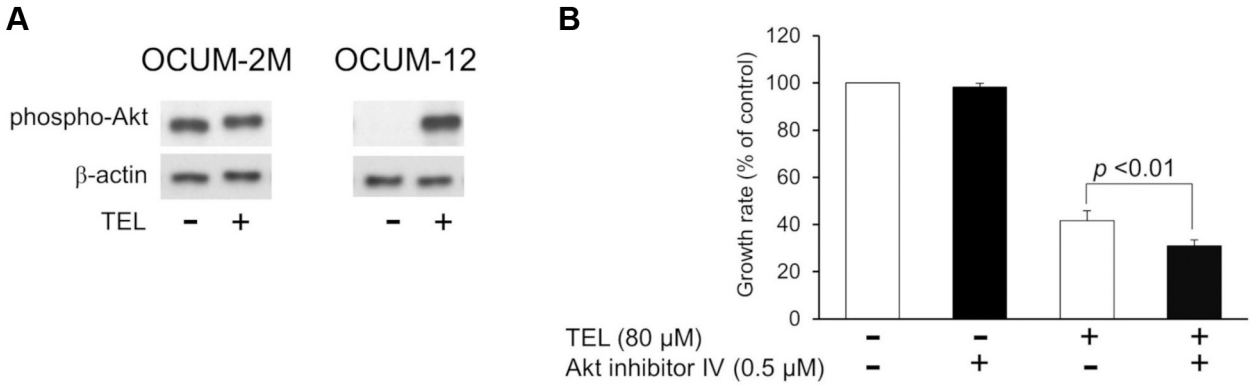

Figure 5. *Involvement of the Akt pathway in TEL-induced cell death. (A) Characteristic bands showing phosphorylation of Akt are observed on the western blot. β-actin was used as a loading control. (B) Effect of Akt inhibitor IV on TEL-induced death in OCUM-12 cells. Cell viability was assessed using a fluorescence-based assay (n=4). The statistical significance was assessed using Dunnett's test (TEL vs. TEL + inhibitors). MAPK: Mitogen-activated protein kinase; TEL: telmisartan.*

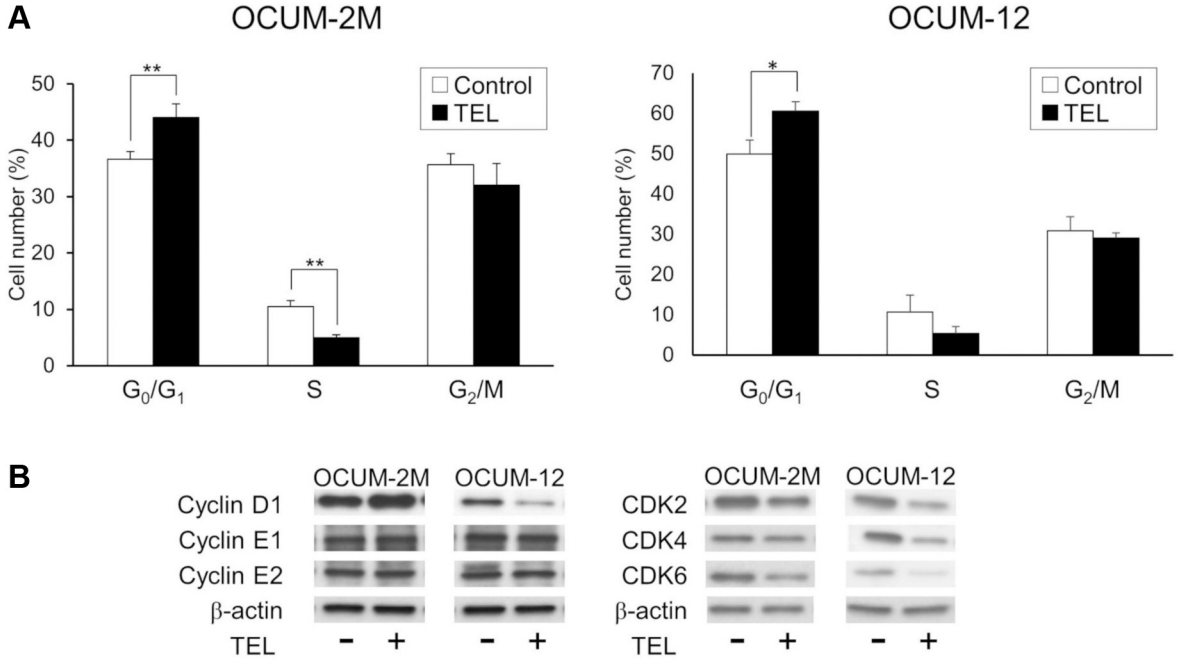

Figure 6. *Effect of TEL on cell cycle progression. (A) The cell number was assessed using flow cytometry. Statistical significance was assessed using t-test. *p<0.05 and **p<0.01 vs. control. (B) G_0/G_1 phase-related protein expression in cells treated with TEL. Typical bands showing cyclin D1, cyclin E1, cyclin E2, CDK2, CDK4, and CDK6 are observed on the western blot. β-actin was used as a loading control. TEL: Telmisartan; CDK: cyclin-dependant kinase.*

Although Akt is known to regulate cell proliferation and be anti-apoptotic, in this case TEL upregulated phospho-Akt protein in OCUM-12 cells (Figure 5A). Next, we examined the effect of Akt inhibitor IV on TEL-induced cytotoxicity. Conversely, Akt inhibitor IV enhanced the effect of TEL (Figure 5B). We concluded that although Akt was not involved in TEL-induced cell death, TEL might have caused stress, which in turn activated Akt in OCUM-12 cells. Along this line, Akt has been reported to be activated by reactive oxygen species *via* phosphatidylinositol-3 kinase (33). Thus, Akt inhibitor IV may have reduced cell viability by suppressing the cell proliferative effect of Akt.

TEL has been reported to regulate cell cycle in various cancer cells (8, 18, 21, 22, 34); in SGC cells, TEL treatment resulted

in an increase in the proportion of cells in the G_0/G_1 phase (Figure 6A). Furthermore, TEL reduced cyclin D1, CDK2, CDK4, and CDK6 levels in OCUM-12 cells, and CDK2 and CDK6 levels in OCUM-2M cells (Figure 6B). Cyclin D and CDK4/CDK6, and cyclin E and CDK2 form a complex and are involved in the transition from G_1 to S phase (35). Therefore, our results suggest that TEL caused a decrease in cyclin-CDK complexes and blocked the transition from G_1 to S phase. TEL-induced G_0/G_1 cell cycle arrest also has been reported in oesophageal adenocarcinoma (21), cholangiocarcinoma (22) and hepatocellular carcinoma (34) cells. Furthermore, Fujita *et al*. reported that TEL arrested non-SGC cells in G_0/G_1 phase transition (18). However, the detailed mechanism underlying TEL-induced cell cycle arrest still needs to be investigated.

In this study, the effects of TEL have only been investigated *in vitro*, and the *in vivo* effects are unknown. In the future, we plan to uncover the detailed mechanism underlying the antitumor activity of TEL, including *in vivo* experiments.

To the best of our knowledge, this is the first study to demonstrate that TEL had antitumor effects on SGC. TEL inhibited cell proliferation in a dose-dependent manner in two SGC cell lines. This study clarified that TEL exhibited PPARγ- and PPARδ-independent cytotoxicity that was partly mediated by apoptosis. TEL cytotoxicity was also independent of the MAPK signalling and Akt pathways. Furthermore, TEL induced cell death by causing G_0/G_1 phase cell cycle arrest. Therefore, the present study shows that TEL might be a promising drug target candidate for the treatment of SGC, which presents an unmet need for therapy.

Conflicts of Interest

The Authors declare no conflicts of interest in relation to this study.

Authors' Contributions

Conception and design: Tsujiya Y, Okamura N; Development of methodology: Tsujiya Y, Okamura N; Acquisition of data: Tsujiya Y, Yamamori M; Analysis and interpretation of data: Tsujiya Y, Okamura N; Writing, review, and/or revision of manuscript: Tsujiya Y, Yamamori M, Yashiro M, Okamura N; Administrative, technical, or material support: Hasegawa A, Yamamori M, Yamamoto Y, Yashiro M; Study supervision: Okamura N.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *71(3)*: 209-249, 2021. PMID: 33538338. DOI: 10.3322/caac.21660

2 Endo K, Sakurai M, Kusumoto E, Uehara H, Yamaguchi S, Tsutsumi N and Ikejiri K: Biological significance of localized Type IV scirrhous gastric cancer. Oncol Lett *3(1)*: 94-99, 2012. PMID: 22740862. DOI: 10.3892/ol.2011.454

3 Song Z, Wu Y, Yang J, Yang D and Fang X: Progress in the treatment of advanced gastric cancer. Tumour Biol *39(7)*: 1010428317714626, 2017. PMID: 28671042. DOI: 10.1177/1010428317714626

4 Afsar B, Afsar RE, Ertuglu LA, Kuwabara M, Ortiz A, Covic A and Kanbay M: Renin-angiotensin system and cancer: epidemiology, cell signaling, genetics and epigenetics. Clin Transl Oncol *23(4)*: 682-696, 2021. PMID: 32930920. DOI: 10.1007/s12094-020-02488-3

5 Mc Menamin ÚC, Murray LJ, Cantwell MM and Hughes CM: Angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in cancer progression and survival: a systematic review. Cancer Causes Control *23(2)*: 221-230, 2012. PMID: 22116540. DOI: 10.1007/s10552-011-9881-x

6 Zhang S and Wang Y: Telmisartan inhibits NSCLC A549 cell proliferation and migration by regulating the PI3K/AKT signaling pathway. Oncol Lett *15(4)*: 5859-5864, 2018. PMID: 29552215. DOI: 10.3892/ol.2018.8002

7 Wu TT, Niu HS, Chen LJ, Cheng JT and Tong YC: Increase of human prostate cancer cell (DU145) apoptosis by telmisartan through PPAR-delta pathway. Eur J Pharmacol *775*: 35-42, 2016. PMID: 26852954. DOI: 10.1016/j.ejphar.2016.02.017

8 Matsui T, Chiyo T, Kobara H, Fujihara S, Fujita K, Namima D, Nakahara M, Kobayashi N, Nishiyama N, Yachida T, Morishita A, Iwama H and Masaki T: Telmisartan inhibits cell proliferation and tumor growth of esophageal squamous cell carcinoma by inducing S-phase arrest *in vitro* and *in vivo*. Int J Mol Sci *20(13)*: 3197, 2019. PMID: 31261874. DOI: 10.3390/ijms20133197

9 Peters JM, Shah YM and Gonzalez FJ: The role of peroxisome proliferator-activated receptors in carcinogenesis and chemoprevention. Nat Rev Cancer *12(3)*: 181-195, 2012. PMID: 22318237. DOI: 10.1038/nrc3214

10 Benson SC, Pershadsingh HA, Ho CI, Chittiboyina A, Desai P, Pravenec M, Qi N, Wang J, Avery MA and Kurtz TW: Identification of telmisartan as a unique angiotensin II receptor antagonist with selective PPARgamma-modulating activity. Hypertension *43(5)*: 993-1002, 2004. PMID: 15007034. DOI: 10.1161/01.HYP.0000123072.34629.57

11 Mikami D, Kimura H, Kamiyama K, Torii K, Kasuno K, Takahashi N, Yoshida H and Iwano M: Telmisartan activates endogenous peroxisome proliferator-activated receptor-δ and may have anti-fibrotic effects in human mesangial cells. Hypertens Res *37(5)*: 422-431, 2014. PMID: 24352213. DOI: 10.1038/hr.2013.157

12 Yashiro M, Chung YS, Nishimura S, Inoue T and Sowa M: Establishment of two new scirrhous gastric cancer cell lines: analysis of factors associated with disseminated metastasis. Br J Cancer *72(5)*: 1200-1210, 1995. PMID: 7577468. DOI: 10.1038/bjc.1995.486

13 Kato Y, Yashiro M, Noda S, Tendo M, Kashiwagi S, Doi Y, Nishii T, Matsuoka J, Fuyuhiro Y, Shinto O, Sawada T, Ohira M and Hirakawa K: Establishment and characterization of a new hypoxia-resistant cancer cell line, OCUM-12/Hypo, derived from a scirrhous gastric carcinoma. Br J Cancer *102(5)*: 898-907, 2010. PMID: 20145613. DOI: 10.1038/sj.bjc.6605543

14 Fujita M, Hasegawa A, Yamamori M and Okamura N: In vitro and *in vivo* cytotoxicity of troglitazone in pancreatic cancer. J Exp Clin Cancer Res *36(1)*: 91, 2017. PMID: 28673319. DOI: 10.1186/s13046-017-0557-6

15 Fujita M, Yagami T, Fujio M, Tohji C, Takase K, Yamamoto Y, Sawada K, Yamamori M and Okamura N: Cytotoxicity of troglitazone through PPARγ-independent pathway and p38 MAPK pathway in renal cell carcinoma. Cancer Lett *312(2)*: 219-227, 2011. PMID: 21903322. DOI: 10.1016/j.canlet.2011.08.010

16 Kabeya Y, Mizushima N, Ueno T, Yamamoto A, Kirisako T, Noda T, Kominami E, Ohsumi Y and Yoshimori T: LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. EMBO J *19(21)*: 5720-5728, 2000. PMID: 11060023. DOI: 10.1093/emboj/19.21.5720

17 Mizushima N, Yoshimori T and Levine B: Methods in mammalian autophagy research. Cell *140(3)*: 313-326, 2010. PMID: 20144757. DOI: 10.1016/j.cell.2010.01.028

18 Fujita N, Fujita K, Iwama H, Kobara H, Fujihara S, Chiyo T, Namima D, Yamana H, Kono T, Takuma K, Hirata M, Kobayashi K, Kato K, Kamada H, Morishita A, Tsutsui K, Himoto T, Okano K, Suzuki Y and Masaki T: Antihypertensive drug telmisartan suppresses the proliferation of gastric cancer cells *in vitro* and *in vivo*. Oncol Rep *44(1)*: 339-348, 2020. PMID: 32627043. DOI: 10.3892/or.2020.7607

19 Unger T: Pharmacology of AT1-receptor blockers. Blood Press Suppl *(3)*: 5-10, 2001. PMID: 11683476. DOI: 10.1080/08037050152518302

20 de Araújo Júnior RF, Leitão Oliveira AL, de Melo Silveira RF, de Oliveira Rocha HA, de França Cavalcanti P and de Araújo AA: Telmisartan induces apoptosis and regulates Bcl-2 in human renal cancer cells. Exp Biol Med (Maywood) *240(1)*: 34-44, 2015. PMID: 25125501. DOI: 10.1177/1535370214546267

21 Fujihara S, Morishita A, Ogawa K, Tadokoro T, Chiyo T, Kato K, Kobara H, Mori H, Iwama H and Masaki T: The angiotensin II type 1 receptor antagonist telmisartan inhibits cell proliferation and tumor growth of esophageal adenocarcinoma *via* the AMPKα/mTOR pathway *in vitro* and *in vivo*. Oncotarget *8(5)*: 8536-8549, 2017. PMID: 28052030. DOI: 10.18632/oncotarget.14345

22 Samukawa E, Fujihara S, Oura K, Iwama H, Yamana Y, Tadokoro T, Chiyo T, Kobayashi K, Morishita A, Nakahara M, Kobara H, Mori H, Okano K, Suzuki Y, Himoto T and Masaki T: Angiotensin receptor blocker telmisartan inhibits cell proliferation and tumor growth of cholangiocarcinoma through cell cycle arrest. Int J Oncol *51(6)*: 1674-1684, 2017. PMID: 29075786. DOI: 10.3892/ijo.2017.4177

23 Erbe DV, Gartrell K, Zhang YL, Suri V, Kirincich SJ, Will S, Perreault M, Wang S and Tobin JF: Molecular activation of PPARgamma by angiotensin II type 1-receptor antagonists. Vascul Pharmacol *45(3)*: 154-162, 2006. PMID: 16765099. DOI: 10.1016/j.vph.2006.05.002

24 Li J, Chen L, Yu P, Liu B, Zhu J and Yang Y: Telmisartan exerts anti-tumor effects by activating peroxisome proliferator-activated receptor-γ in human lung adenocarcinoma A549 cells. Molecules *19(3)*: 2862-2876, 2014. PMID: 24603556. DOI: 10.3390/molecules19032862

25 Braicu C, Buse M, Busuioc C, Drula R, Gulei D, Raduly L, Rusu A, Irimie A, Atanasov AG, Slaby O, Ionescu C and Berindan-Neagoe I: A comprehensive review on MAPK: A promising therapeutic target in cancer. Cancers (Basel) *11(10)*: 1618, 2019. PMID: 31652660. DOI: 10.3390/cancers11101618

26 Liu F, Yang X, Geng M and Huang M: Targeting ERK, an Achilles' Heel of the MAPK pathway, in cancer therapy. Acta Pharm Sin B *8(4)*: 552-562, 2018. PMID: 30109180. DOI: 10.1016/j.apsb.2018.01.008

27 Ishiguro H, Ishiguro Y, Kubota Y and Uemura H: Regulation of prostate cancer cell growth and PSA expression by angiotensin II receptor blocker with peroxisome proliferator-activated receptor gamma ligand like action. Prostate *67(9)*: 924-932, 2007. PMID: 17440964. DOI: 10.1002/pros.20571

28 Saber S, Khodir AE, Soliman WE, Salama MM, Abdo WS, Elsaeed B, Nader K, Abdelnasser A, Megahed N, Basuony M, Shawky A, Mahmoud M, Medhat R and Eldin AS: Telmisartan attenuates N-nitrosodiethylamine-induced hepatocellular carcinoma in mice by modulating the NF-ϰB-TAK1-ERK1/2 axis in the context of PPARγ agonistic activity. Naunyn Schmiedebergs Arch Pharmacol *392(12)*: 1591-1604, 2019. PMID: 31367864. DOI: 10.1007/s00210-019-01706-2

29 Takahashi S, Uemura H, Seeni A, Tang M, Komiya M, Long N, Ishiguro H, Kubota Y and Shirai T: Therapeutic targeting of angiotensin II receptor type 1 to regulate androgen receptor in prostate cancer. Prostate *72(14)*: 1559-1572, 2012. PMID: 22430461. DOI: 10.1002/pros.22505

30 Wee P and Wang Z: Epidermal growth factor receptor cell proliferation signaling pathways. Cancers (Basel) *9(5)*: 52, 2017. PMID: 28513565. DOI: 10.3390/cancers9050052

31 Salaroglio IC, Mungo E, Gazzano E, Kopecka J and Riganti C: ERK is a pivotal player of chemo-immune-resistance in cancer. Int J Mol Sci *20(10)*: 2505, 2019. PMID: 31117237. DOI: 10.3390/ijms20102505

32 Wang C and Wang WB: Telmisartan induces osteosarcoma cells growth inhibition and apoptosis via suppressing mTOR pathway. Open Life Sci *13*: 242-249, 2018. PMID: 33817089. DOI: 10.1515/biol-2018-0029

33 Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y and Dong W: ROS and ROS-mediated cellular signaling. Oxid Med Cell Longev *2016*: 4350965, 2016. PMID: 26998193. DOI: 10.1155/2016/4350965

34 Oura K, Tadokoro T, Fujihara S, Morishita A, Chiyo T, Samukawa E, Yamana Y, Fujita K, Sakamoto T, Nomura T, Yoneyama H, Kobara H, Mori H, Iwama H, Okano K, Suzuki Y and Masaki T: Telmisartan inhibits hepatocellular carcinoma cell proliferation *in vitro* by inducing cell cycle arrest. Oncol Rep *38(5)*: 2825-2835, 2017. PMID: 29048654. DOI: 10.3892/or.2017.5977

35 Asghar U, Witkiewicz AK, Turner NC and Knudsen ES: The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov *14(2)*: 130-146, 2015. PMID: 25633797. DOI: 10.1038/nrd4504

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