

Wogonin Inhibits Growth of Mantle Cell Lymphoma Cells through Nuclear Factor- κ B Signaling Pathway

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To the Editor: Mantle cell lymphoma (MCL) is a non-Hodgkin's lymphoma (NHL) subtype and considered one of the most aggressive lymphomas with worse prognosis than other subtypes of NHL.^[1] Despite the great progress over the last several decades, treatment of MCL still has troubles in survival, relapse, and drug resistance. Hence, extensive efforts are still needed in developing new agents for MCL. Recently, wogonin, as a major active ingredient of *Scutellaria*, has attracted considerable attention because of its antineoplastic properties and low toxicity.^[2] Previous studies have demonstrated that wogonin has cytotoxic effects on several cancer cell lines. However, the antitumor effect of wogonin on MCL cells has seldom been reported so far. In this study, we report that MCL cells, including Mino, JeKo-1, and REC-1 cells, are sensitive to wogonin and the possible involved mechanisms are also explored.

Various cell lines, including Jurkat, Raji, Mino, JeKo-1, and REC-1 cells, were treated with different concentrations of wogonin for 24 h. The cytotoxic effects of wogonin on different cells were detected using CCK-8 assay. The cell viability of all cells decreased with increasing concentration of wogonin, and the cell viability of MCL cells (Mino, JeKo-1, and REC-1 cells) decreased more notably compared with Jurkat (acute T lymphoblastic leukemia cell line) and Raji cells (Burkitt's lymphoma cell line) [Figure 1a]. Wogonin inhibited the cell growth in a concentration-dependent manner, and the median inhibition concentrations (IC50s) for Jurkat, Raji, Mino, JeKo-1, and REC-1 cells were 89.04, 105.00, 25.98, 45.30, and 48.90 μ mol/L, respectively. Obviously, MCL cells are more sensitive to wogonin than Jurkat and Raji cells.

The effects of wogonin on the apoptosis and cell cycles of MCL cells were examined by flow cytometry (FCM) analysis. As illustrated in Figure 1b, after treatment with wogonin at the dose of IC50s for 24 h, Mino, JeKo-1, and REC-1 cells had increased apoptosis rates compared with those treated with dimethyl sulfoxide (DMSO) ($P < 0.05$). As shown in Figure 1c, wogonin-treated cells in the G0/G1 phase of the cell cycle were significantly accumulated, whereas those in the S phase were greatly reduced in comparison to DMSO-treated cells ($P < 0.05$). These findings indicate that wogonin could induce MCL cells arrested at the G0/G1 phase and retard the transition from G1 to S phase.

Given that some evidences show that the nuclear factor- κ B (NF- κ B) signaling pathway is involved in the anticancer activity of wogonin, and NF- κ B regulates cell proliferation and apoptosis, the expression of NF- κ B and its downstream cell cycle-related (cyclin D1) and apoptosis-related genes (Bcl-2, Bax, and caspase-3) was analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and Western blotting to explore the possible mechanism of the anticancer effects of wogonin on MCL cells. As Figure 1d shows, the mRNA expression of NF- κ B p65, cyclin D1, and Bcl-2 was significantly downregulated in wogonin-treated cells compared with DMSO-treated ones ($P < 0.05$). By contrast, wogonin-treated cells had higher mRNA levels of Bax and caspase-3 than cells treated with DMSO ($P < 0.05$), which resulted in an elevated ratio of Bax/Bcl-2 in the wogonin group. The results of Western blotting exhibited the same variation tendency as RT-PCR [Figure 1e]. The protein expression of NF- κ B p65, cyclin D1, and Bcl-2 was decreased in cells treated with wogonin, whereas the protein levels of Bax and cleaved caspase-3 were increased significantly ($P < 0.05$). Particularly, the phospho-NF- κ B p65 proteins were remarkably reduced, which inhibited the activation of NF- κ B and NF- κ B signaling pathways.

NF- κ B has been found to play a vital role in tumor development in recent years, which can regulate cell proliferation and apoptosis which are essential cellular alterations for tumorigenesis.^[3] The most commonly detected NF- κ B dimer is a heterodimer of p50 and p65, and the latter is responsible for the strong transcription activating potential of NF- κ B. Therefore, p65 and its active form (phospho-NF- κ B p65) were determined in this study. As shown in Figure 1d and 1e, wogonin remarkably blocked the expression and activation of NF- κ B in MCL cells. This finding indicates that NF- κ B signaling pathway played an important role in the anticancer effect of wogonin on MCL cells. However, the detailed molecular mechanism remains to be further investigated.

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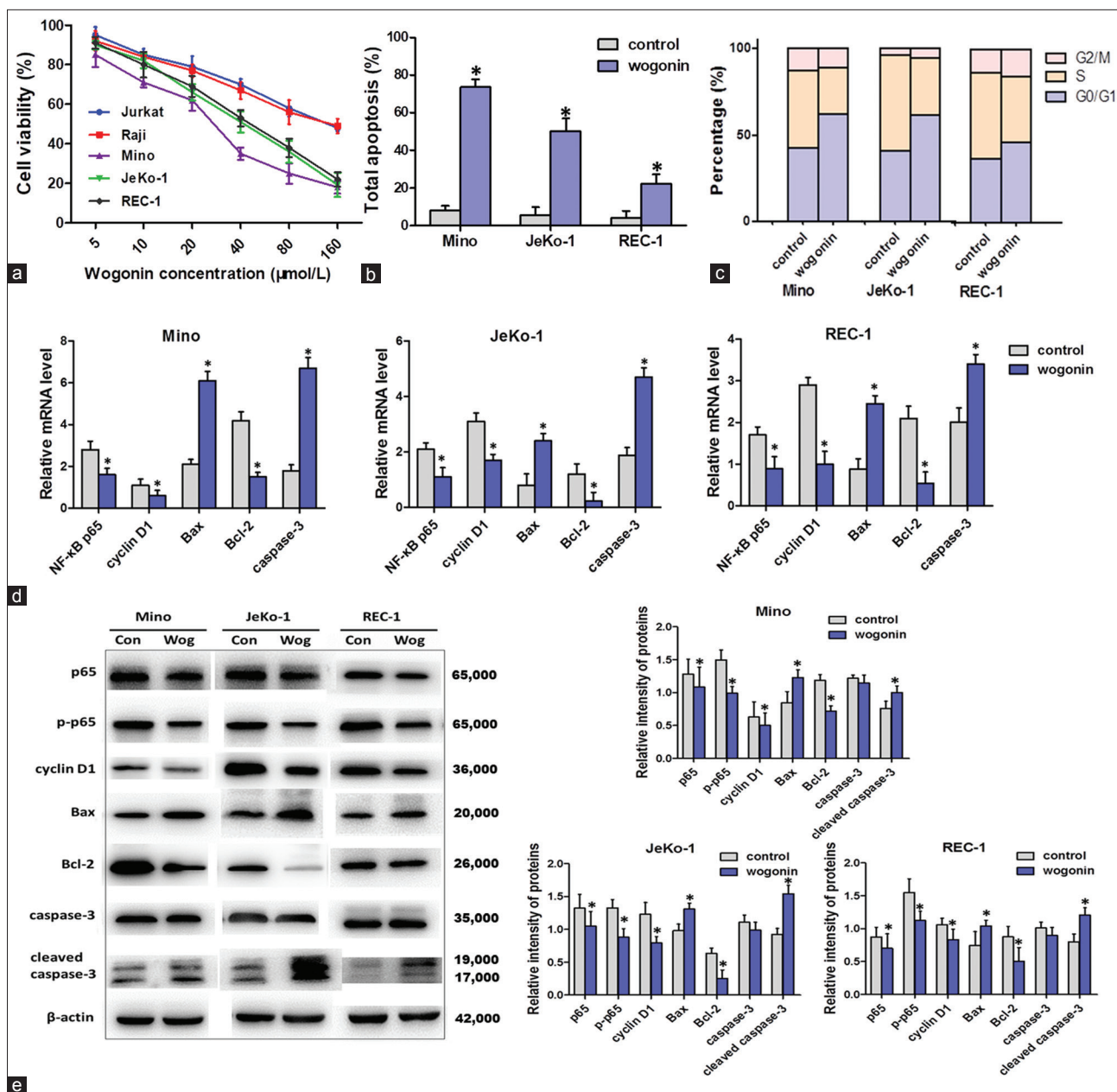


Figure 1: The anticancer effects of wogonin on mantle cell lymphoma cells. (a) Growth inhibition effect of wogonin on different cells; (b) wogonin induced the apoptosis of mantle cell lymphoma cells ($*P < 0.05$ when compared with controls); (c) wogonin caused mantle cell lymphoma cell arrest in the G0/G1 phase; (d) wogonin regulated the mRNA expression levels of cell cycle-related and apoptosis-related genes through nuclear factor- κ B signaling pathway. mRNA levels were evaluated by reverse transcription-polymerase chain reaction ($*P < 0.05$ when compared with controls); (e) wogonin regulated the expression of cell cycle-related and apoptosis-related proteins via nuclear factor- κ B signaling pathway. Protein expressions were determined using Western blotting. Bar graphs show the relative expression of proteins ($*P < 0.05$ when compared with controls).

In the study, three MCL cells treated with wogonin were mainly arrested in the G0/G1 phase [Figure 1c]. Downregulation of cyclin D1 has been reported to be associated with G1 phase arrest. Cyclin D1 causes the G1/S cell cycle transition to facilitate progression.^[4] Our results of RT-PCR and Western blotting also showed that the expression of cyclin D1 significantly declined in MCL cells treated with wogonin [Figure 1d and 1e], which further reveals that wogonin could suppress the expression of cyclin D1. In addition, the transcription of cyclin D1 gene can be directly initiated by NF- κ B since the cyclin D1 promoter contains a κ B site. Therefore, these results suggest that wogonin could inhibit MCL cell proliferation through suppressing NF- κ B/

cyclin D1 pathway, thereby causing cell cycle arrest in the G0/G1 phase.

As shown in Figure 1d and 1e, the ratio of Bax/Bcl-2 was elevated in the wogonin-treated cells, and the caspase-3 proteins were significantly cleaved. The Bcl-2 family modulates cell apoptosis by either pro- or anti-apoptotic members. Bax is a pro-apoptotic regulator that improves the permeabilization of the mitochondrial outer membrane and antagonizes the anti-apoptotic effect of Bcl-2.^[5] Cleaved caspase-3 proteins are regarded as the final stage of apoptosis. These results were in accordance with the increased apoptosis in wogonin-treated cells analyzed by FCM [Figure 1b], which

demonstrates that wogonin could induce apoptosis of MCL cells by modulating the expression of apoptosis-related proteins. In addition, the transcription of apoptosis-related genes, including the Bcl-2 family, can be regulated by NF- κ B, thus inhibiting cell apoptosis. This finding further provides evidence for that wogonin could induce MCL cell apoptosis by inhibiting NF- κ B expression and activation, then activating Bax/Bcl-2/caspase-3-related apoptosis pathway.

Overall, the result of the study showed that wogonin has anticancer activity on MCL cells. Wogonin can inhibit MCL cell growth by inducing the G0/G1 phase arrest and apoptosis, in which one of the underlying mechanisms is the inhibition of NF- κ B signaling pathway. Wogonin might cause cell cycle arrest via NF- κ B/cyclin D1-mediated pathway and induce apoptosis by NF- κ B/Bcl-2/caspase-mediated pathway. Therefore, this study suggests that wogonin is a potential candidate for MCL treatment.

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Conflicts of interest

There are no conflicts of interest.

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