Curcumin enhances ibrutinib induced killing effect against TP53-mutated Chronic Lymphocytic Leukemia cells in vitro

Yunxia Zhang , Xutao Guo, MD , Ru Feng, MD , Xiaolei Wei, MD , Yongqiang Wei, MD , Haohao Lei
Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, China
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Introduction

CLL bearing del(17p) showed inferior OS and time to next treatment compared to non-del(17p) [1]. A significant proportion of patients treated with single-agent ibrutinib experienced CLL progression[2].Curcumin has anti-tumor effect and can induce apoptosis and inhibit the proliferation of a series of tumor cells[3].Curcumin may inhibit TP53-mutated CLL cells, and eliciting a strong synergistic cytotoxic effect in combination with ibrutinib.

Contents and Methods

- The inhibitory effect of ibrutinib combined with curcumin on the proliferation of TP53-mutated Chronic Lymphocytic Leukemia cells was detected by CCK8, and the apoptosis of TP53-mutated CLL cells induced by ibrutinib combined with curcumin was detected by flow cytometry.
- The differences of gene and signal pathway expression among different treatment groups were analyzed by RNA-seq.
- Western blot technology was used to verify the expression of related pathway proteins, and to explore the mechanism of curcumin enhancing the killing effect of ibrutinib against TP53-mutated Chronic Lymphocytic Leukemia cells.

Results

- The CCK8 results showed that the IC50 of ibrutinib at 24 h was 40.61 ± 2.35 μM, while the IC50 of curcumin at 24 h was 26.6± 0.94 μM(Figure 1). The IC50 of ibrutinib at 48 h was 14.6 ±0.8307μM, and the IC50 of curcumin at 48 h was 15.7± 2.53μM. The combination index（CI） of ibrutinib combined with curcumin at 24 h and 48 h was calculated by CompuSyn software. The results showed that the CI values of ibrutinib combined with curcumin were less than 1 at 24 h and 48h, suggesting that ibrutinib combined with curcumin has a synergistic killing effect on CLL cells with TP53 mutation;
- Flow cytometry showed that the IC50 of ibrutinib at 24 hours was 67.20± 2.86 μM, while the IC50 of curcumin at 24 hours was 144±6.6 μM(Figure2). The CI value of ibrutinib combined with curcumin was less than 1 at 24 h, suggesting that ibrutinib combined with curcumin has a synergistic promoting apoptosis on CLL cells with TP53 mutation;
- Compared with the control group, the down-regulated genes of the combined group were mainly enriched in PI3K/AKT and cytokine-cytokine receptor signaling pathway by RNA-seq and KEGG analysis（Figure 3）.
- The results of Western blot showed that the protein expression levels of P65、PI3K and p53 in the single drug group and the combination group were down-regulated in varying degrees, especially in the combination group(Figure 4).

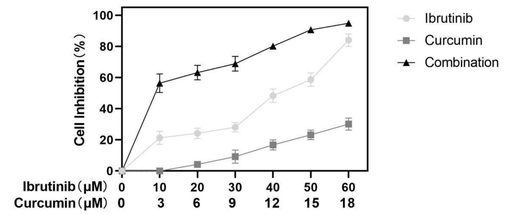

Figure 1 Cell Inhibition of MEC-1 cell line that were exposed to various drug concentration of Curcumin combine with Ibrutinib for 24h.

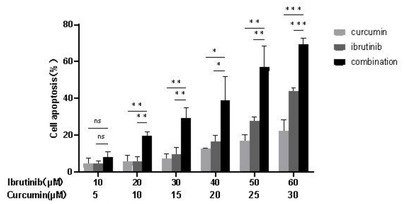

Figure 2 Cell apoptosis of MEC-1 cell line that were exposed to various group for 24h.

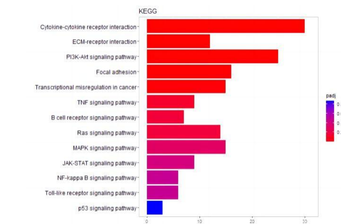

Figure 3 KEGG pathways enrichment analysis of MEC-1 cells, following the treatment with Ibrutinib combine with Curcumin .

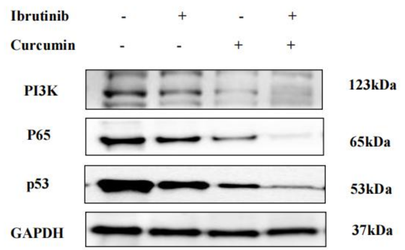

Figure 4 The expression of PI3K、p53 and P65 after the treatment of Ibrutinib and Curcumin for 24h.

Conclusion

- Ibrutinib combined with curcumin has synergistic killing effect on TP53- mutated CLL cells.
- Curcumin enhances the killing effect of ibrutinib on TP53-mutated CLL cells by inhibiting PI3K and NFκB pathway, and degrading p53mt protein.

References

[1]Mato AR, et al.A clinical practice comparison of patients with chronic lymphocytic leukemia with and without deletion 17p receiving first-line treatment with ibrutinib.Haematologica. 2022 Nov 1;107(11):2630-2640.
[2]Ahn IE, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia.Blood. 2017 Mar 16;129(11):1469-1479.
[3]Willenbacher E, Khan SZ, Mujica SCA, et al. Curcumin: New Insights into an Ancient Ingredient against Cancer [J].Int J Mol Sci,2019,20(8): 1808.

Contact information

Coauthor：Xutao Guo: Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, 510515, China.
Email: gxt827@126.com.
First Author: Yunxia Zhang: Department of Hematology, Nanfang Hospital, Southern Medical University, Guangzhou, 510515, China.
Email: zyxseven7@163.com