

Cinchonine induces apoptosis of HeLa and A549 cells through targeting TRAF6

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Abstract

© 2017 The Author(s). Background: Cancer cells are known to over-express TRAF6 that is critical for both AKT and TAK1 activations. The Really Interesting New Gene (RING) domain of TRAF6 is believed to be responsible for the E3 ligase activity, ZINC fingers of TRAF6 provide critical support for the activity of the RING domain which is critical for both AKT and TAK1 activations. Methods: We employed computational docking program to identify small molecules that could effectively and competitively bind with the RING domain of TRAF6, which is believed to be responsible for its E3 ligase activity. MTT assay and flow cytometry were employed to analyze apoptosis of cancer cells. Signaling pathways were detected using immunoprecipitation and western blotting, and immunofluorescence was pursued to assess the nature of binding of cinchonine to TRAF6. We also performed animal experiments to test effect of cinchonine in vivo. Results: Cinchonine, a naturally occurring Cinchona alkaloid identified from the docking study, could bind to TRAF6 in HeLa and A549 cells and induce apoptosis of these cancer cells. We found that AKT ubiquitination and phosphorylation as well as phosphorylation of TAK1 were decreased. These activities would lead to subsequent suppression anti-apoptotic protein Bcl-2, while elevating pro-apoptotic protein Bax. Immunofluorescence staining unambiguously demonstrated the binding of cinchonine specifically at the RING domain of TRAF6 in cells, thereby validating the computational modeling. Animal experiments showed that cinchonine could suppress tumor growth in mice without showing significant acute toxicity. Conclusion: These investigations suggest that through competitive binding with the RING domain of TRAF6, cinchonine could induce apoptosis via inhibiting AKT and TAK1 signaling pathways.

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Keywords

AKT and TAK1 activations and phosphorylations, Cinchonine, Immunofluorescence staining, RING domain of TRAF6, Ubiquitination

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