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Withaferin A Sensitizes Human Renal Cancer Cells to TRAIL-Mediated Apoptosis through DR5 Up-Regulation and c-FLIP Down-Regulation

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Withaferin A (Wit A) reportedly shows cytotoxicity in a variety of tumor cell lines. Here, we show that co-treatment with subtoxic doses of Wit A and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) induces apoptosis in human renal cancer cells, Caki cells, but not in human normal mesangial cells. Moreover, the combined treatment with Wit A and TRAIL dramatically induces apoptosis in various cancer cell types, suggesting that this combined treatment may offer an attractive strategy for safely treating human cancers. Treatment of Caki cells with Wit A up-regulated DR5 in a CHOP-dependent manner. siRNA-mediated CHOP suppression significantly attenuated Wit A-induced DR5 up-regulation and the cell death induced by Wit A plus TRAIL. Interestingly, Wit A-induced increase in ROS levels preceded the up-regulation of CHOP and DR5. The involvement of ROS in CHOP-mediated DR5 up-regulation was confirmed by the result that pretreatment with an antioxidant NAC inhibited Wit A-induced up-regulation of both CHOP and DR5. We also found that Wit A treatment down-regulated c-FLIP via NF-kB-mediated transcriptional control as well as ROS signaling pathways. Taken together, our results show that DR5 up-regulation and c-FLIP down-regulation contribute to the effective sensitizing effect of Wit A on TRAIL-mediated apoptosis in cancer cells.

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Rottlerin Induces Apoptosis via Death Receptor5 (DR5) Up-Regulation through CHOP-Dependent and PKC d-Independent Mechanism in Human Malignant Tumor Cells

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Rottlerin has been shown to induce anti-proliferation and apoptosis of human cancer cell lines. In this study, we demonstrate a novel mechanism of rottlerin-induced apoptosis via death receptor 5 (DR5) upregulation. We found that treatment with rottlerin significantly induces DR5 expression both at its mRNA and protein levels. Down-regulation of DR5 expression with siRNA efficiently attenuated rottlerin-induced apoptosis, showing that the critical role of DR5 in this cell death. Rottlerin-induced DR5 upregulation was accompanied by CCAAT/enhancer-binding protein-homologous protein (CHOP) protein expression and rottlerin-induced increase of DR5 promoter activity was diminished by mutation of a CHOP-binding site of DR5 promoter. Although rottlerin is known to be as an inhibitor of novel isoforms of protein kinase C (PKC), specifically PKC d, not only suppression of PKC d expression by siRNA but also overexpression of WT-PKC d or DN-PKC d did not affect the rottlerin-mediated induction of DR5 in our study. These results suggest that rottlerin induces up-regulation of DR5 via PKC d-independent pathway. Furthermore, subtoxic dose of rottlerin sensitizes human cancer cells, but not normal cells, to TRAIL-mediated apoptosis. Thus, DR5-mediated apoptosis, which is induced by rottlerin alone or by the combined treatment with rottlerin and TRAIL, may offer a new therapeutic strategy against cancer.