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Acquired TRAIL resistance in human breast cancer cells are caused by the sustained cFLIP and XIAP protein levels and ERK activation

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We established TRAIL-resistant MDA-231/TR cells from MDA-231 parent cells to understand the mechanism of TRAIL resistance in breast cancer cells. Basal expression levels of death receptors (DR4 and DR5), IAP family, and Bcl-2 family were not changed in MDA-231/TR cells. Cleavage of procaspase-8, procaspase-3, and PLC- 1 was occurred in dose-dependent manner in TRAIL treated MDA-231 cells. Whereas, their cleavage was partly inhibited in MDA-231/TR cells after TRAIL treatment and the level of cFLIP protein was sustained in TRAIL resistant cells. In addition, TRAIL-resistant MDA-231/TR cells are cross-resistant to TNF- but sensitive to cytotoxic drugs including 60 M oxaliplatin and 20 g/ml etoposide. Interestingly, XIAP protein level was reduced in a dose-dependent manner in TRAIL-treated MDA-231 cells, which was inhibited by preincubation with z-VAD. However, we cannot detect any changes of mRNA expression in TRAIL-treated cells. These results indicate that reduction of XIAP protein was related to activation of caspase-dependent pathway. Moreover, we found that basal level of phospho-ERK was higher and level of phospho-ERK more sustained after TRAIL treatment in MDA-231/TR than MDA-231 cells. Pretreatment with PD98059 or transfection of MKK1 dominant negative expression vector attenuated TRAIL resistance in MDA-231/TR cells. These results suggest that ERK activation is important for TRAIL resistance in MDA-231 breast cancer cells. Our findings provide the evidence that the sustained expression level of cFLIP and XIAP protein and sustained ERK activation may lead to acquired TRAIL resistance in breast cancer cells.

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ESE-3 transcription factor is involved in the regulation of DR-5 expression through putative Ets sites

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The death receptor 5 (DR-5) is one of receptors for tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) and is able to apoptosis in various tumor cells through an intracellular death domain. The ESE-3 is a member of Ets transcription factors, which regulate expression of a variety of cellular genes by binding to purine-rich GGAA/T core sequence in cooperation with other transcription factors and co-factors. In this study, we demonstrated for the first time that ESE-3 may regulate DR-5 expression through Ets binding sequence within DR-5 promoter region. Using a combination of the electrophoretic mobility shift assay and the luciferase reporter assay, we found that putative Ets sites are responsible for ESE-3 transcriptional activity, and that co-factors, CBP and p300 may be involved to the up-regulation of DR-5 by ESE-3.