

2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid 처리에 의한
TRAIL-mediated signaling을 통한 **apoptosis** 유도

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2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid induces
apoptosis via TRAIL-mediated signaling

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Objectives

2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid is a pentacyclic triterpene compound. It leads to TRAIL-mediated apoptosis signaling pathway. We investigated TRAIL-receptor family and apoptosis-related genes in human colon carcinoma (HCT-116) cells.

Materials and Methods

- Materials - 2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid, TRIZOL reagent, RC DC protein assay kit, Anti-DR4/TRAIL-R1, Anti-DR5/Apo2/TRAIL-R2
- Methods - cell culture, microarray analysis, RT-PCR, western blot analysis

Results

2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid appeared to cooperate with mitochondrial pathway as well as TRAIL-mediated apoptosis signaling pathway in HCT-116 cells. RT-PCR data showed that increased the levels of DR4, DR5 and DcR2 mRNAs. 2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid increased the levels of IAPs, BAD, BAX and caspase-9 mRNAs and decreased the levels of BID and caspase-3 mRNAs. The level of DR5 protein also increased after 6 hrs of incubation with 2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid, compared with untreated control cells. However, treatment with ursolic acid did not change the expression of DR5 protein. Our findings indicates that DR5 induction is a critical event in 2 β , 3 α , 23-trihydroxyurs-12-en-28-oic acid-mediated apoptosis in HCT-116 cells.

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GENE	Primer	Fold	Control	Treated
Bcl-xL	Forward: 5'-ATACAGCTGGAGTCAGTTAGTGAT-3'	0.84		
	Reverse: 5'-CTCTAGGTGGTCATTTCAGGTAAGT-3'			
BID	Forward: 5'-GATGAGTGCATCACAAACCTACT-3'	0.698		
	Reverse: 5'-CGTAGGTTCTGGTTAATAAAATTCA-3'			
BAD	Forward: 5'-ATGTTCCAGATCCCAGAGTTT-3'	1.027		
	Reverse: 5'-ATCTTCTCCAGATGGTGAGTG-3'			
BAX	Forward: 5'-TCTGAGCAGATCATGAAGACA-3'	1.153		
	Reverse: 5'-ATCTTCTCCAGATGGTGAGTG-3'			
Caspase-3	Forward: 5'-CTCGGTCTGGTACAGATGTCGATG-3'	0.822		
	Reverse: 5'-GGTTAACCCGGTAAGAATGTGCA-3'			
Caspase-9	Forward: 5'-CATTATCAACAATGTGAACTTCTGC-3'	1.715		
	Reverse: 5'-AGTAGGACACAAAGATGTCACCTGG-3'			
Smac/DIABLO	Forward: 5'-ATCAGAGCCTCATTCCCTTAGTAGT-3'	1.415		
	Reverse: 5'-GTTTCTGACGGAGCTTCTATCT-3'			
IKK	Forward: 5'-GACTCCATGAAAGACGAGGAGTA-3'	1.274		
	Reverse: 5'-TAGCCATGGATAGAGGCTAAGTGTA-3'			

Figure 1. Effect of 2β , 3α , 23-trihydroxyurs-12-en-28-oic acid on apoptosis-related genes mRNA expression in HCT-116 cells. HCT-116 cells were treated with $50 \mu\text{M}$ of 2β , 3α , 23-trihydroxyurs-12-en-28-oic acid for 6 hrs. After RNA preparation, total RNA was utilized for synthesis of cDNA.

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