F113 Induction of p21 and apoptosis by C11 in human hepatocarcinoma cells

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C11. a chloride-containing VK3 analog, acts as a mediator of programmed cell death in SK-Hep-1 cell lines, but its molecular mechanisms linked to cell death are not understood. In this study, we investigated the expression of p21 gene and its relationship to apoptosis induced by C11. In SK-hep-1 cells. the addition of C11 resulted in time-dependent growth suppression and DNA fragmentation characteristics of apoptosis. p21 protein was induced during this process, while the protein level of p53 was not changed at the same condition. This apoptotic cell death with p21 induction was also observed in the Hep3B cells lacking functional p53 after treatment of C11. These results suggest that C11-induced apoptosis is associated with up-regulation of p21 protein in p53-independent pathway. Next. in order to confirm whether the p53-independent p21 induction is required for C11-induced apoptosis. we introduced the p21 gene into Hep3B. Overexpression of p21 did not affect the expression of the bcl-2 gene. but DNA fragmentation and PARP cleavage were significantly increased. These data indicate that p21 is involved in C11-induced apoptosis. Although Bcl-2 has been implicated to interfere with an essential signaling molecule involved in the apoptosis pathway, its molecular mechanism and target molecule are poorly understood. To determine the effects of bcl-2 overexpression on apoptosis and to investigate whether Bcl-2 interferes with the p53-independent p21 pathway. we transfected the bcl-2 expression vector into SK-Hep-1 cells. Overexpression of Bcl-2 prevented C11-induced apoptosis. Taken together, C11-induced apoptosis is regulated by p53-independent p21 pathway and bcl-2 may inhibit functional activity of p21, thereby may inhibit the C11-induced apoptosis.

F201 Two cDNA clones encoding chitinase from the root nodule of *Elaeagnus umbellata*; their structures, expression patterns and putative functions.

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Two cDNA clones encoding chitinase were isolated from a root nodule cDNA library of *Elaeagnus umbellata* by differential hybridization. The two clones, pEuNOD-CHT1 and pEuNOD-CHT2, encoded for 335 and 317 amino acid residues with molecular weight of mature protein of 33.3 kDa and 31.1 kDa, respectively. The two chitinase were similarly consisted of four domains; hydrophobic signal peptide domain, cysteine-rich chitin-binding domain, hinge domain, and catalytic domain. EuNOD-CHT2 had a C-terminal extension involved in targeting into vacuole, which was not present on mature protein. Accordingly, EuNOD-CHT1 and EuNOD-CHT2 were classified into class Ib and Ia, respectively, based on their protein structures and pI values (4.28 for EuNOD-CHT1, 8.26 for EuNOD-CHT2). To better understand the biological roles of these chitinases in root nodule development, Northern hybridization, RT-PCR and *in situ* hybridization techniques were employed. Putative functions of two cloned chitinases will be discussed from these results.