Mechanism of ASC-mediated apoptosis: Bid-dependent apoptosis in type II cells

M. Hasegawa, K. Kawase, N. Inohara, R. Imamura, W-C. Yeh, T. Kinoshita, and T. Suda

ASC is an adaptor molecule that mediates apoptotic and inflammatory signals, and implicated in tumor suppression. However, the mechanism of ASC-mediated apoptosis has not been well elucidated. Here, we investigated the molecular mechanisms of ASC-mediated apoptosis in several cell lines using a CARD12-Nod2 chimeric protein that transduces the signal from muramyl dipeptide into ASC-mediated apoptosis. Experiments using dominant-negative mutants, small-interfering RNAs, and peptide inhibitors for caspases indicated that caspase-8 was generally required for ASC-mediated apoptosis, while a requirement for caspase-9 depended on the cell type. In addition, CLARP/FLIP (a natural caspase-8 inhibitor) suppressed ASC-mediated apoptosis, and *Clarp-/-* mouse embryonic fibroblasts were highly sensitive to ASC-mediated apoptosis. Bax-deficient HCT116 cells were resistant to ASC-mediated apoptosis as reported previously, although we failed to observe colocalization of ASC and Bax in cells. Like Fas-ligand-induced apoptosis, the ASC-mediated apoptosis was inhibited by Bcl-2 and/or Bcl-XL in type-II but not type-I cell lines. Bid was cleaved upon ASC activation, and suppression of endogenous Bid expression using small-interfering RNAs in type-II cells reduced the ASC-mediated apoptosis. These results indicate that ASC, like death receptors, mediates two types of apoptosis depending on the cell type, in a manner involving caspase-8.

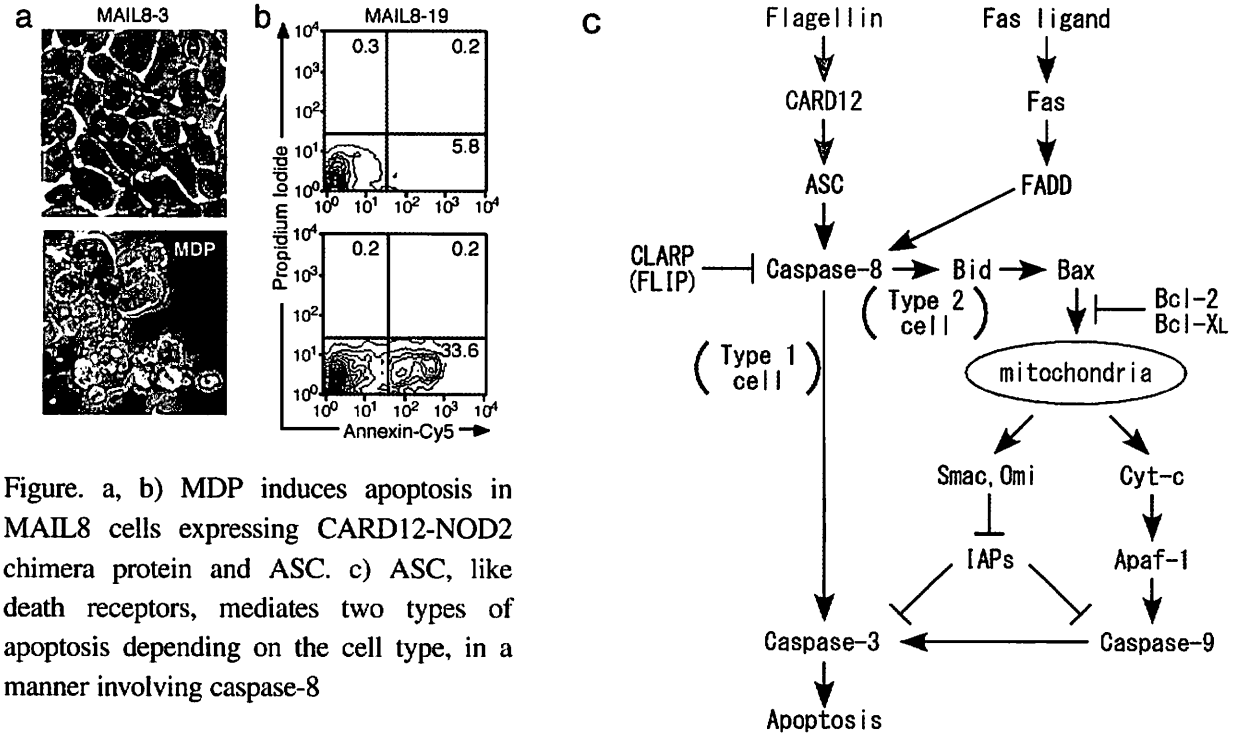

Figure. a, b) MDP induces apoptosis in MAIL8 cells expressing CARD12-NOD2 chimera protein and ASC. c) ASC, like death receptors, mediates two types of apoptosis depending on the cell type, in a manner involving caspase-8