

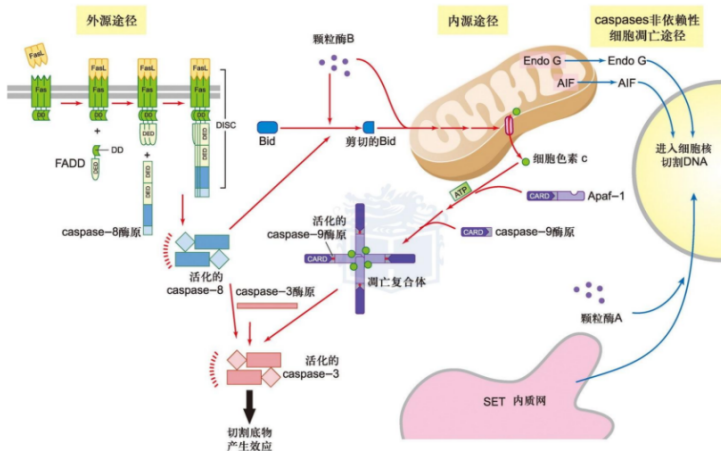
Indetification, Purification, Molecular Cloning and Characterization of Smac

(A second mitochondria-derived activator of caspase)

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A major caspase activation



Major regulators of mitochondria-initiated caspase

- Proapoptotic member
 - Bad
 - Bax
 - Bid
- Anti-apoptotic member
 - Bcl-2
 - Bcl-X

IAPs (Inhibitors of apoptosis proteins) bind to procaspase-9 and inhibit caspases with baculovirus IAP repeat (BIR) domain. The major types of IAPs are:

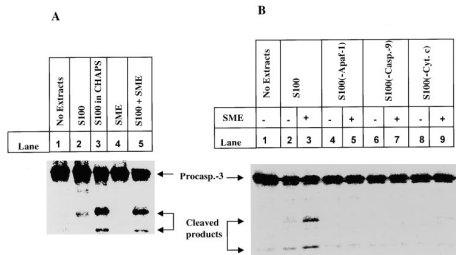
- IAP
- XIAP
- c-IAP-1
- C-IAP-2

- Different response of cells to microinjected Cyt C
- Healthy neurons don't respond to microinjected Cyt C in NGF negative environment

Solutions: An additional caspase-activating protein factor regulated by Bcl-2 family proteins

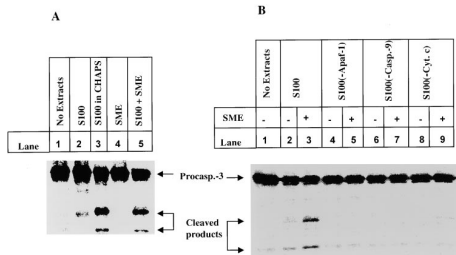
Caspase 3 detection under different conditions:

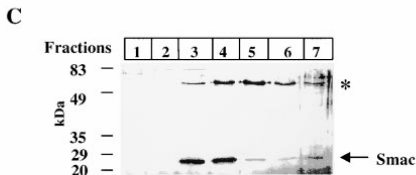
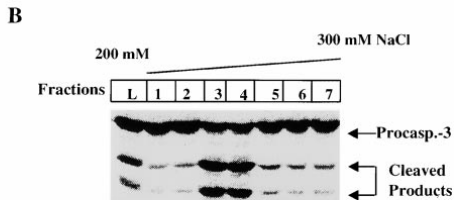
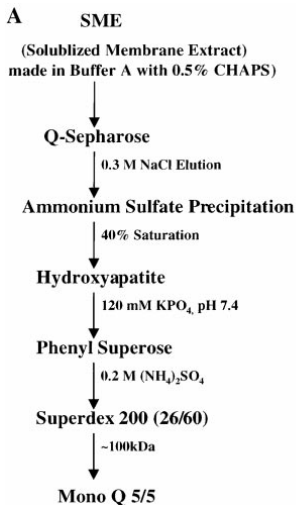
- Detergent(+/-)
- Solublized "membrane" extract(SME)
- S100(+/-1)



Caspase 3 detection under different substrate conditions:

- S100
- Apaf-1
- Caspase-9
- Cyt C

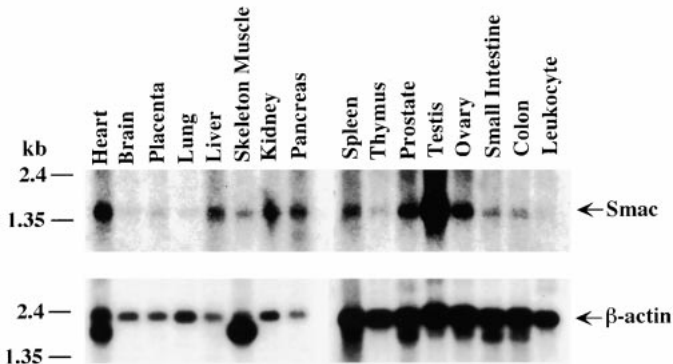




- Tryptic digestion
- Edman degradation
- cDNA clone
- Amino Acid sequence deduction

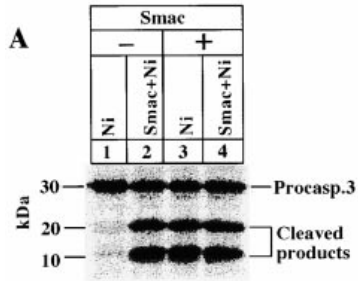
MAALKSWLSRSVTSFFFRYRQCLCVPVVANFKKRCFSELIR 40
PWHKTVTIGFGVTLCAVPIAQKSEPHSLSEALMRRVSL 80
VTDSTSTFLSQTTYALIEAITEYTKAVYTLTSLYRQYTSL 120
LGKMNSEEEDEVQVIIIGARAEMTSKHQEYLLKLETTWMTA 160
VGLSEMAAEAAAYQTGADQASITARNHIQLVKLQVEEVHQL 200
SRKAETKLAEAAQIEELRQKTQEEGEERAESEQEAYLRED 239

Northern blot analyzation for Smac concentration in different tissues



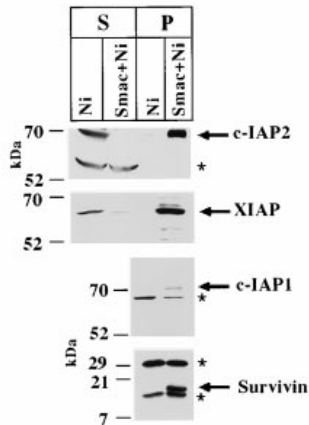
Promote caspase activation or eliminate S100 inhibitor

- Smac-coupled nickel beads (-/+)
- Free Smac (3/4)
- Western blot analysis

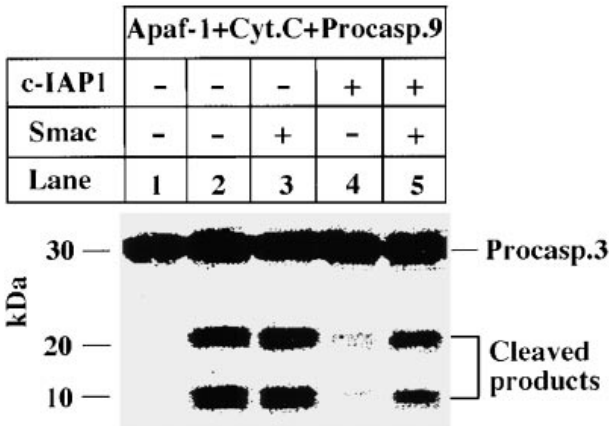


Abundance of IAP family proteins in eluted materials and supernatant are measured with western blot

C



Disprove the situation that Smac activate apoptosis by activate S100 component



Hela cells with or without Smac encoding cDNA are tested under UV irradiation for apoptosis information

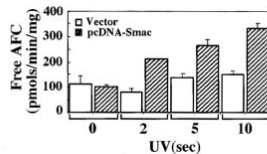
A



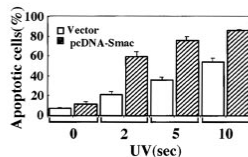
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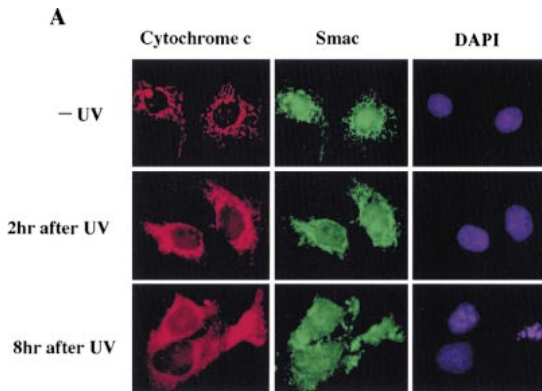
C



D

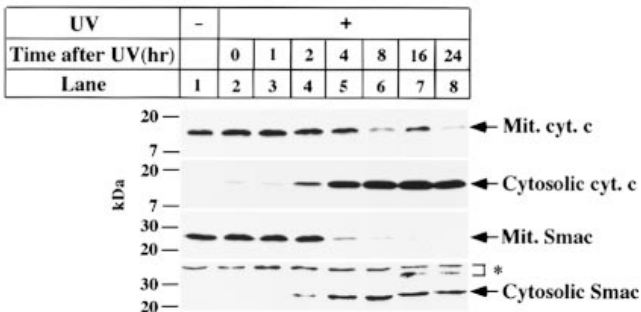


An immunostain method is used to locate Cyt C, Smac and DAPI during apoptosis

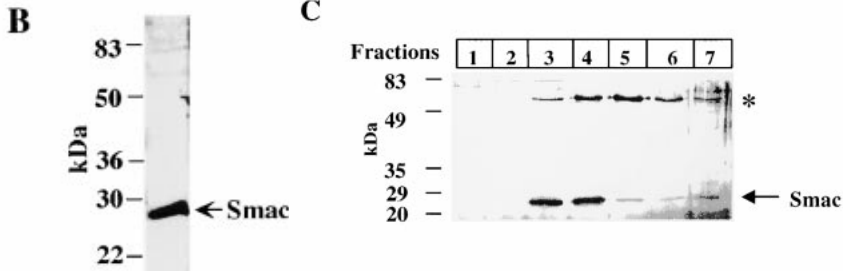


Mitochondria and cytosolic fractions are isolated to test Cyt C and Smac concentration during UV irradiation process

B



Comparasion of molecular weight of Smac before and after maturation



Edman sequencing of mature Smac

MAALKSWLSRSVTSFFRYRQCLCVPVVANFKKRCFSELIR 40

PWHKTVTIGFGVTLCAVPIAQKSEPHSLSSEALMRRAVSL 80

VTDSTSTFLSQTTYALIEAITEYTKAVYTLTSLYRQYTSL 120

LGKMNSEEEDEVQVIGARAEMTSKHQEYLKLETTWMTA 160

VGLSEMAAEAAAYQTGADQASITARNHIQLVKLQVEEVHQL 200

SRKAETKLAEAQIEELRQKTQEEGEERAESEQEAYLRED 239

- Mechanism of apoptosis activation by Smac
- Smac vs Drosophila Reaper, Grim, Hid
- Regulation of Smac
- Explanation for detergent
- *Cyt C releasing route vs Smac releasing route