ZOO332H1S – Part 1 (Mar. 17, 2003) - AJE

Introduction to Programmed Cell Death* in the Nervous System

1. Developing Brain
2. Mature Neurons

* PCD, *a.k.a.* apoptosis

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Background

Survival of developing neurons depends upon trophic support - now classic description by Viktor Hamburger and Rita Levi-Montalcini

Neurotrophin hypothesis – immature neurons compete for target-derived trophic factors

Cultured neurons 'require' NGF for survival (why use sympathetic neurons)

Block RNA and protein synthesis

Cells not just die of "starvation"

C. elegans – discovery of programmed cell death genes

Cellular suicide program in vertebrate neurons

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Key Molecular Components of PCD

Cysteine proteases – caspases (more than 14)

Mammalian PCD regulated by

- Bcl-2 family of proteins
- adaptor protein APAF-1 ("apoptotic protease-activating factor 1)
- caspase 3, caspase 8, caspase 9

Neurons share same basic apoptosis program with all other cell types

Specificity of regulation: by balance of Bcl-2 and caspase family members

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cont… Key Molecular Components of PCD

Bcl-2 Family:

both anti-apoptotic and pro-apoptotic members

common feature: contain one or more Bcl-2 homology (BH) domain

Anti-apoptotic members: Bcl-2 and Bcl-x_L; located in outer mitochondrial membrane (and endoplasmic reticulum and perinuclear membrane)

Overexpression studies with Bcl-2 – *in vitro* and in transgenic mice (higher expressors; knock out)

$Bclx_L$ embryonic lethal (E13) with massive cell death in developing NS

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cont… Key Molecular Components of PCD

Pro-apoptotic – Bax, Bad (a Bcl-2 family member)

Apaf-1 (CED-4) – transmits apoptotic signals from mitochondrial damage to activate caspases

interacts with cytochrome C released from mitochondria and caspase 9 (complex formation)

>> activation of pro-caspase 9 ("pro" form is inactive)

Activated caspase 9 cleaves pro-caspase 3

"Indisputable" role in neuronal cell death:

1. Caspase inhibitors block NGF-deprivation induced PCD
2. Caspase inhibitors block PCD by other cytotoxic insults
3. K.O.'s of Apaf-1, caspase 9 and caspase 3

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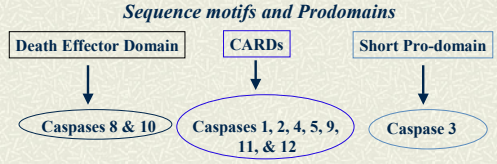
Models and Activation

Simplistic model: neuron-specific caspases not likely

Expressed as catalytically inactive proenzymes,
3 parts: NH_2 terminal pro-domain, larger subunit, small subunit

Sequence motifs and Prodomains



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Caspase 3, 9 and Apaf-1

Caspase 3 and 9 are major caspases involved in neuronal cell death

Severe and similar defects appear during embryogenesis in KO mice

Apaf-1 KO mice – similar phenotype (more severe?)

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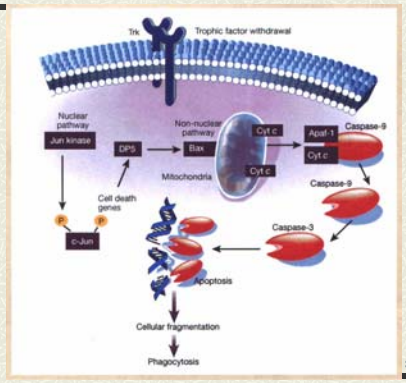
Activation of apoptosis in sympathetic neurons by trophic factor withdrawal



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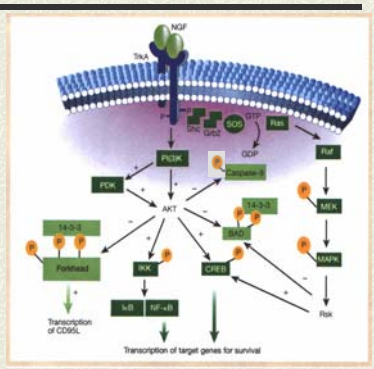
Neuronal Survival Pathways



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PI(3)K-Akt Pathway – Evidence for Involvement in Survival

Inhibitors of PI(3)K block survival effect of NGF

PI(3)K enzymes normally present in cytoplasm and can be activated directly by recruitment to an activated TrkR or indirectly via Ras

Parallel pathway (via activation of GTP-binding protein kinase Ras) leads to reinforcing effects of Akt (via phosphorylation of CREB and Bad

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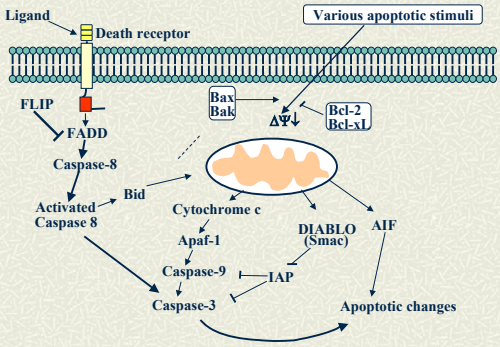
Intrinsic *vs.* Extrinsic Death Signalling Pathways



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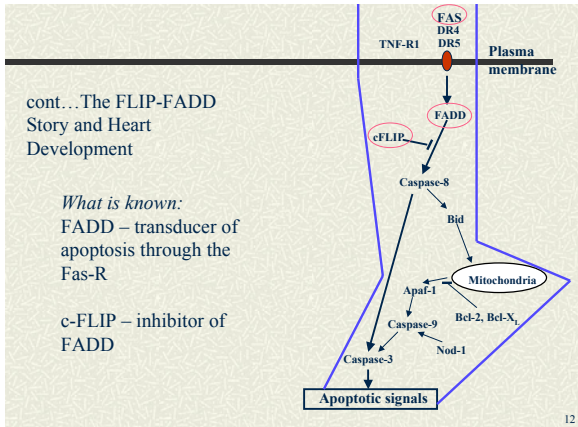

cont…The FLIP-FADD Story and Heart Development

What is known:
FADD – transducer of apoptosis through the Fas-R

c-FLIP – inhibitor of FADD

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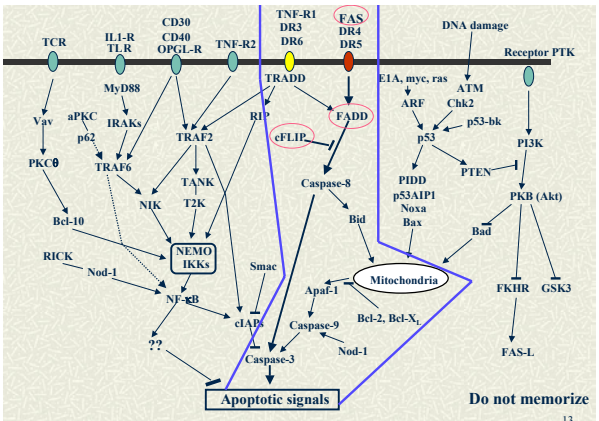

(Some) Signalling Pathways Involved in Cell Life/Death

Putting it all back together…

Because an organism is so much more than just a bunch of genes, molecules, and signalling pathways.

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