Lecture V. Cell Birth and Death

Bio 3411
Wednesday
September 12, 2012

Readings

NEUROSCIENCE: 5th ed, pp 477-506
4th ed, pp 596-609

References posted:


†(pdfs on course websites: [http://www.nslc.wustl.edu/courses/Bio3411/bio3411.htm])
Cited A


Cited B

Cited C


What the last Lecture was about

- **Mesoderm** induces **neuroectoderm** in overlying ectoderm that gives rise to **neuronal** or **epidermal** cells.
- The “default” state of **neuroectodermal** cells is **neuronal**.
- **Neuroectoderm** secretes Bone Morphogenic Protein-4 (**BMP-4**), a **signaling molecule** that blocks the neuronal fate in neighboring **neuroectodermal** cells.
- **Mesoderm** secretes proteins - **Chordin**, **Noggin**, **Follistatin** - that block **BMP-4** and **neuroectodermal** cells continue as **neuronal** progenitors.
- This **inductive mechanism** is conserved between vertebrates and invertebrates.
- These, and other similar, signaling mechanisms are used by the developing nervous system to control other events later in development.
- **BMP-4** is a member of the Transforming Growth Factor-beta (**TGF-β**) family of **signaling molecules**.
What this Lecture is about

- Cell Death – Necrosis vs Apoptosis
- Promoting growth and survival – “trophism”
- Inhibition of the “death mechanism”
- Broader implications: neuroembryology; cancer
- Different critters - Same genes
- Molecular models
- Connection of Trophic Factors to cell death

Apoptosis

from Greek
“apo” meaning “separation”
&
“ptosis” for “falling off”

Types of Cell Death

**Necrosis (Provoked)**
- Not Self-Initiated
- Not Stereotypic
- Can Be Slow
- “Messy” (injury can spread)

**Apoptosis (Programmed)**
- Cell-Autonomous
- Stereotypic
- Rapid
- “Clean” (dead cells eaten)


Removing a neuron’s targets, leads to its death

Hamburger, V. (1958, 1977)
Neuronal death is central for normal NS development

- Lateral Motor Column (40% Loss)
- Ciliary Ganglion (54% Loss)
- Trochlear Nucleus (57% Loss)

Neuron survival correlates with target innervation

Motor neurons

Axon Outgrowth

Target Innervation

Target Muscles

Neuronal Loss

Not all neurons innervate targets
Target innervation determines which neurons survive

More targets (more neurons)  Fewer targets (fewer neurons)

Development Progresses

Mouse tumor (sarcoma) transplanted next to developing chick spinal cord causes axon sprouting consistent with a diffusible factor - a nerve growth factor

Levi-Montalcini, R., & Hamburger, V. (1951)
A quantitative functional assay for Nerve Growth Factor (NGF) activity, using explanted cultures of sensory ganglia

NGF is the founding member of a large gene family of Neurotrophins (NTs), distantly related to insulin

NGF binds as a dimer to its receptor
NGF/Neurotrophins Signal through Trk (tyrosine kinase) Receptors

Multiple Signaling Pathways
Via kinases and scaffolding proteins

Apoptosis pathway

Intracellular Ca\textsuperscript{2+} release, modulation of ion channels

Gene Activation/Repression

Trk Receptors
(TrkA, TrkB, TrkC, p75)

NGF/NT

(PLC/PKC kinase)

(Ras/MAP kinase)

(PIK3/AKT kinase)

C. elegans is the model organism for molecular genetic studies

Programmed Cell Death of single identified neurons can be followed in live worms

Sulston, J. E., & Horvitz, H. R. (1977)

2 Classes of *C. elegans* Cell Death Mutants

WT

(pro-survival genes + pro-apoptosis genes) → (normal number of cells)

Mutant class I

(pro-survival genes + pro-apoptosis genes) → (fewer cells)

Mutant class II

(pro-survival genes + pro-apoptosis genes) → (extra cells)
Genetic analysis of cell death genes in *C. elegans* defines a genetic pathway

<table>
<thead>
<tr>
<th>ced-9 (pro-survival) gene</th>
<th>ced-3 (pro-apoptosis) genes</th>
<th>Cell Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>ced-9(lf)</td>
<td>ced-3(lf)</td>
<td>excessive cell death (fewer cells)</td>
</tr>
<tr>
<td>ced-9(lf) ced-3(lf)</td>
<td></td>
<td>reduced cell death (extra cells)</td>
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<tr>
<td>ced-9(lf) ced-4(lf)</td>
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</tr>
</tbody>
</table>


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t(14;18) Chromosomal Translocation Causes Human B-Cell Leukemia by Overexpression of Bcl-2

Stanley Korsmeyer

The “core” Cell Death genes found in *C. elegans* are conserved as *multigene* families in vertebrates

**ced-9 / Bcl-2:**
- *Bcl-2: B-Cell Leukemia.*
- “Pro-survival” protein.
- Inhibits release of cytochrome C from mitochondria (vertebrates).
- Sequesters CED-4 from cytoplasm (worms).

**ced-4 / Apaf:**
- *Apaf: Apoptosis activity factor.*
- “Adaptor” or “scaffold” protein.
- Aggregates inactive procaspase, causing auto-activation by proximity.
- Requires cytochrome C, and ATP for multimerization (vertebrates).

**ced-3 / Caspase:**
- *Caspase: Cysteine active-site, aspartate cleavage-site, Protease.*
- “Terminator” protein.
- Protease activity when activated by proteolysis.

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Molecular Model for Apoptosis

- Bcl-2: Inactive Procaspase recruited
- Apaf: Apaf aggregation
- Caspase: Activated caspase (cascade)
- Cytochrome C: (*Catalysis of the removal of auto-inhibitory caspase domain*)
- Single BH3 domain protein (egl-1): (BH3 domains)
- Mitochondria: (procaspase recruitment)
NGF is only one of multiple pathways to the “core” death mechanism, through many single-BH3 proteins.

Initiation of apoptosis by extracellular ligands (FAS, TNF)

* “Core” apoptotic components


Apaf/Cytochrome C Aggregate into a 7-Spoke Apoptosome Complex (“Wheel of Death”)

Single-BH3 domain molecules integrate multiple signals that trigger apoptosis.

Mitochondria integrate “Pro-survival” and “Pro-death” signals from a family of Bcl-2-like genes.

“Pro-death” Single-BH3 domain proteins complex with Bcl-2 to release cytochrome C from mitochondria through “giant” mitochondrial ionic channels.

Molecular Animation of Cell Death Mediated by the FAS pathway

What this Lecture was about

• Programmed cell death (apoptosis) is a physiological mechanism distinct from necrotic cell death.

• Apoptosis occurs widely during normal development of the nervous system.

• Isolation of specific molecules involved in promoting growth and survival – "trophism," e.g., Nerve Growth Factor (NGF).

• What is the "death mechanism" that NGF (and other neruotrophins) inhibit?

• Broader implications: controlled cell death in neuroembryology vs uncontrolled cell growth of cancer.

• Gene homologies between organisms - humans and worms (nematodes)

• Molecular models for apoptosis

• How do trophic factors connect to this cell death pathway(s)?
END