Cell Metabolism and Cell Death in Neurodegenerative Disease

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Cell Biology and Death of Neurons

- Subcellular organelles in neurons
- Protein Synthesis and Degradation
- Mitochondria and Oxidative Metabolism
- Cell Death: Necrosis and Apoptosis
- Pathways leading to cell death
- Parkinson’s: Mitochondrial dysfunction+
- Alzheimer’s: Protein aggregation+
• Alzheimer’s Disease: abnormal protein cleavage producing toxic protein aggregation + dysfunction of mitochondria
• Parkinson’s Disease: gene mutations leading to mitochondrial dysfunction + protein aggregation
• ALS: mutation in superoxide dismutase (SOD1) gene leading to mitochondrial dysfunction, oxidative stress, and protein aggregation
• Huntington’s Disease: huntingtintin gene mutation > polyglutamine repeats > mitochondrial dysfunction, oxidative stress, protein aggregation
• Stroke, Epilepsy: glutamate excitotoxicity yielding mitochondrial dysfunction + oxidative stress, apoptosis.
1. **Rough Endoplasmic Reticulum (rER):** stacks of membrane dotted with ribosomes. Function: synthesis of integral membrane proteins. mRNA transcripts bind to ribosomes which *translate* the mRNA blueprint in order to assemble proteins from amino acid sequences.

2. **Mitochondria:** membrane bound “kidney” shaped organelles. Function: cellular respiration where converted proteins, fats, sugars (glucose) and oxygen enter the Krebs cycle to produce reactions that yield adenosine triphosphate (ATP). ATP is released into the cytosol as the energy source of the cell.

3. **Smooth Endoplasmic Reticulum (sER):** membrane without ribosomes. Function: varied, including regulation of intracellular concentrations of Ca++. 

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**Subcellular Organelles of the Neuron**
**rER: Protein Synthesis**  
(integral membrane proteins)

**Protein Degradation**  
(ubiquitin-proteosome sys)

Disruption = Protein Aggregation

Regulatory protein that targets proteins to the proteosome

Proteolysis occurs to break peptide bonds via proteases

Amino acids require energy

Protein complex with a core

Activation

Conjugation

Recognition (ligase)

Chaperone

'Ashtralling proteins (including CDC48/197 in some cases)

Proteasome

Protein Substrate

ADP ATP

Cytosolic peptidases

Peptides
Mitochondrial Oxidative Metabolism

Glucose

- Glycolysis
- Pyruvate

Mitochondrial Functions
respiration-energy

- Fatty-acyl-CoA transport
- Acetyl-CoA
- TCA cycle
- β-oxidation

Oxidative phosphorylation
(electron transport chain)

NADH reducing agent > antioxidants.
Free radicals incr if blocked.

Cyt c = heme protein > apoptosis if released

complex I

Cyt c

complex III

lipid peroxidation

I II III IV V

electron chain protein complexes
Cell death is a normal process during brain development. It is mostly abnormal in the adult brain. Neurons die during acute trauma and chronic disease as well as in aging. There are two basic types of cell death: necrotic cell death and programmed cell death.

Necrotic cell death occurs most commonly when the brain is damaged by impact injury, subarachnoid hemorrhage, ischemic stroke, seizures, and other sudden insults.

Programmed cell death (apoptosis) occurs more commonly in chronic progressive diseases such as Alzheimer’s and Parkinson’s. However, both necrosis and apoptosis can occur in both acute and chronic brain injury. Understanding the mechanisms underlying necrosis and apoptosis has contributed to treatment protocols for these diseases.
Cells Undergoing Necrosis

1. Necrosis; from Greek for “death”.
2. It is a non-programmed cell death occurring due to injury or disease = abnormal, inappropriate.
3. Cell bodies initially swell up leading to rupture of the cell membrane.
4. Nucleolus breaks up and nucleus becomes multi-nucleolated.
5. Significant cytoplasmic vacuolization occurs with breakdown of organelles.
7. This debris produces an inflammatory auto-immune response in brain.
8. Necrosis is triggered in part by endogenous release of intracellular Ca++ produced by ion failure.

(From Squire et al, 2008)
Cells Undergoing Apoptosis

1. Apoptosis; Gr. for “leaves dropping”.
2. It is one form of programmed cell death (PCD) termed type 1. = normal, appropriate in development.
4. Nuclear chromatin condenses, nuclei become pyknotic (shrink).
5. DNA fragmentation occurs, mediated by proteases.
6. Eventually the cytoplasm and nucleus break up, blebs form on membrane and bud off to form apoptotic bodies.
7. Residues are phagocytized by glial cells, macrophages.
8. Triggered by activation of cell-suicide cysteine proteases called caspases.

(From Squire et al, 2008)
Cell Death Summary

Necrosis

- Somatic swelling
- Vacuolization
- Membrane rupture

Toxic release of debris

‘Normal’ genetic program occurring in single cells

Apoptosis

- Blebs
- Dense nucleus condensed chromatin
- Apoptotic bodies
- Macrophage, lysosome degradation

Phagocytosis

Processes in multiple cells lead to toxic content release which causes brain inflammation.

(From Squire et al, 2008)
Cell injury (trauma, ischemia, toxins, hypoglycemia) leads to glutamate excitotoxicity and significant Ca++ influx into a damaged cell (stroke, status epilepticus).

Oxidative stress- ROS generation also lead to release of Ca++ from intracellular stores in the endoplasmic reticulum.

DNA damage activates poly ADP-ribose polymerase which decreases pH and leads to acidosis.

Increased Ca++ and acidosis produce calpain proteases which together with free radicals rupture the membrane of lysosomes which contain ‘digestive’ enzymes.

Release of proteolytic enzymes cathepsin B and D from the lysosome degrade cell structure and metabolism, leading to necrotic cell death.
Pathways to Neuronal Necrosis

Glutamate Excitotoxicity
(excessive depolarization)

- NMDA receptor
- AMPA receptor
- Ca++ channel
- [Ca^{2+}]_i
- DNA strand breaks
- Poly ADP-ribose polymerase
- Cathepsin release
- Acidosis
- ATP
- ADP
- pH 4-5
- "suicide bag"
- Lysosome

Intracellular calcium stores

NMDA receptor

AMPA receptor

Ca++ channel

Necrosis initiating insults

Death

degrade cell organelles

Endoplasmic reticulum

IP3

Ca^{2+} stores

ROS overproduction

zinc

(cysteine protease)

Death

Cell organelles

Cathepsin release

Poly ADP-ribose polymerase

DNA strand breaks

Acidosis

ATP

ADP

pH 4-5

"suicide bag"

Lysosome
Bcl proteins regulate apoptosis by controlling mitochondrial outer membrane permeabilization, in particular by regulating release of cytochrome c.

Anti-apoptotic Bcl-2 proteins in the mitochondrial membrane normally inhibit cytochrome c release.

Pro-apoptotic Bcl proteins (Bax, Bid, Bad) translocate to the mitochondrial outer membrane and promote cytochrome c release during injury and disease.

Upon release from mitochondria cytochrome c binds to APAF-1 and forms a complex with caspase 9 which is called an ‘apoptosome’.

Caspase 9 activates caspase 3 leading to membrane blebbing, chromatin condensation, DNA fragmentation and apoptotic cell death.
Intrinsic-Extrinsic Apoptotic Pathways

Intrinsic Pathway
- Bcl-2 family
- Intracellular death signal
- Cytochrome c release
- BAX
- Cytochrome c
- Cleavage, activation of effector caspases (caspase 3)
- Apoptosome
- Initiator caspase (caspase-9, initially monomeric)
- Mitochondrial Membrane Permeabilization
- Heptamerization of Apaf-1

Extrinsic Pathway
- Tissue Necrosis Factor Receptor (TNFR)
- Death receptor (for example, FAS)
- Ligand (FasL)
- Initiator caspase (caspase-8)
- Active initiator caspase (uncleaved homodimer)
- Active effector caspase (heterotetramer)

BID = BH3 interacting domain death agonist
BAX = Bcl-2–associated pro-apoptotic X- protein

(From Bredesen et al, 2006)
Protein Aggregation Pathways

- Normal protein degradation involves the ubiquitin-proteosome system for recycling, redistribution of protein. Ubiquitin guides the protein, the proteosome degrades it.

- The first pathological step blocking protein degradation is the formation of a misfolded protein.

- Once misfolded, the hydrophobic fragments that are normally buried inside the protein get exposed to the aqueous environment.

- This intermediate has a high tendency to aggregate and become stabilized because the proteosome is unable to degrade the protein.

- Incorporation of additional monomers to the protein gives rise to soluble oligomers, protofibrils, and then ‘mature’ protein aggregates which are insoluble.
Normal Protein Degradation

- Post-translational modification via covalent bond
- Proteolysis occurs to break peptide bonds via proteases

Abnormal Formation of Aggregates (cleavage or phosphorylation)

- Disease protein
- Covalent modification
- Abnormal conformation
- Globular intermediates
- Protofibrils
- Aggregates

- \( \alpha \beta \) 1-40 B sheets
- Oligomers
- Fibrils
Progressive neurodegenerative disorder affecting 0.5 million Americans, about 1% of the pop. over 60.

Symptoms include tremor-at-rest, cogwheel rigidity, postural instability, festinating gate, slow movement (bradykinesia), flat affect, depression.

Pathology includes loss of >70% of dopamine neurons in SN compacta and build-up of intracellular inclusions (Lewy bodies) composed of fibrillar α-synuclein.

Mitochondrial dysfunction (complex I inhibition leading to oxidative stress) may be the initial step in killing DA cells. However, protein aggregation is also involved.

Drug treatments are l-dopa, dopamine agonists, MAO inhibitors. Surgical approach is DBS, usually in the subthalamus.
Parkinson’s Disease Pathology

MRI of pathology in putamen-globus pallidus

- abnormal posture
- festinating gate
- rigidity
- akinesia
- pill-rolling tremor at rest

Loss of dopamine in Parkinson’s brain

Lewy bodies

SN
Mitochondrial Induced Cell Death Cascade in Parkinson’s

- Inhibition of complex I of the electron transport chain impairs mitochondrial respiration, including reduction in NADPH and other antioxidants.
- This leads to increased production of intra- and extra-mitochondrial Reactive Oxygen Species (free radicals such as superoxide anion, hydrogen peroxide, NO).
- ROS increases the soluble pool of cytochrome c in IMS as well as ROS in the extra-mitochondrial cytosol.
- Cytosolic ROS produces DNA fragmentation yielding transcriptional upregulation of pro-apoptotic Bax which translocates to the mitochondrial outer membrane.
- Bax then induces release of cytochrome c which triggers caspase activation and apoptotic cell death.
Mitochondrial Dysfunction in Parkinson’s Cell Death

MPTP → oxidative phosphorylation → increased permeabilization

Complex I (NADH) → ROS → DNA fragmentation

MPP⁺ → oxidative phosphorylation → increased permeabilization

Bax, Bim = Pro-apoptotic proteins

JNK/c-jun → transcription → DNA strand breaks → DNA fragmentation

PTP → translocation → Caspase activation

Bax, Cyt c → Cell death

Lipid peroxidation, Protein nitration → DNA fragmentation

Perier C et al. PNAS 2007;104:8161-8166

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Protein Aggregation in Parkinson’s Disease

Gene Mutations
Protein Misfolding
Abnormal Protein Aggregation
Reactive Oxygen Species Production
Oxidative Stress
Cell Apoptosis

Gene mutations
E3 ubiquitin ligase
disrupts protein guidance

Protein structure

ROS

cytoskeleton
cytochrome c
caspases
apoptosis

ATP
disrupts protein guidance

Oxidative stress

Protein Misfolding
Misfolding
Proteasomal Dysfunction
Protein accumulation

Neurodegeneration

Aggregation (Lewy body)
Alzheimer’s Disease

• Progressive neurodegenerative disease producing severe cognitive decline, memory loss, behavioral disorders.

• Familial early age Alzheimer’s is an autosomal-dominant genetic abnormality in the coding of one or more proteins. Accounts for ~5% of cases. All others are sporadic.

• Pathology characterized by senile plaques of amyloid-β peptide and neurofibrillary tangles consisting of hyperphosphorylated tau protein.

• Genes thought to be involved include amyloid precursor protein (APP) gene and those encoding presenilins 1-2. Presence of the apoE4 gene allele is also a risk factor.

• Acetylcholine and glutamate transmitter systems are also effected. FDA approved drugs target ACHe (Aricept, an inhibitor), or the NMDA receptor (Namenda, a antagonist)

• Ultimate neuronal loss occurs first in hippocampus, basal forebrain, temporal and prefrontal cortex, then broadly.
Protein Aggregation and Oxidative Stress in Alzheimer’s Disease

• In familial early-onset AD three gene mutations have been identified: amyloid precursor protein (APP) and genes (PS1-PS2) encoding presenilins 1 and 2.

• Deposits of amyloid β in extracellular space are produced by cleavage of transmembrane APP by β and γ secretases. Aβ-42 is likely the fragment causing amyloid plaques.

• The abnormal cleavage of APP is produced by a mutation in the presenilin gene. The resulting plaques disrupt cell function.

• Plaques also increase ROS which produces cell membrane damage and mitochondrial oxidative stress leading to apoptosis.

• Neurofibrillary tangles (NFT) also accumulate inside damaged cells. NFT are paired helical filaments consisting of the hyper-phosphorylated microtubule-associated protein tau. These also damage cell function.
Assembly of Plaques and Tangles

- N-APP
- Multi-protein complex with active docking, ATP binding sites
- APP Intracellular cytoplasmic domain
- β-secretase
- BACE = β-site of APP cleaving enzyme
- Amyloid plaques
- Neurofibrillary Tangles
- Hyperphosphorylated Tau
Cell Signaling Pathways in Alzheimer’s Disease

- Aβ Protein Aggregation (senile plaques)
- Hyperphosphorylated Tau (neurofibrillary tangles)

T1 MRI of AD brain

- ROS Formation
- Oxidative Stress
- Bax, Caspases
- Apoptosis

Oxidative degradation by free radicals
Ischemic and Hemorrhagic Stroke

- Stroke = A “Brain Attack” defined as an “abrupt onset of a focal neurologic deficit”. 3rd leading cause of death in the US, no. 1 cause of morbidity.
- Stroke is a vascular insult produced by either obstruction in a blood vessel (embolus, thrombus) or hemorrhage (aneurysm, AVM).
- Stroke causes ischemia (blood loss), hypoxia (↓ oxygen) and infarct (cell death) leading to paralysis, impaired speech, loss of vision, etc.
- Tissue damage is due to glutamate excitotoxicity > ionic failure, excess intracellular Ca+, oxidative stress, etc.
- Within the infarct, the core is largely necrotic while the surrounding penumbra can be reperfused. Without reperfusion, apoptosis occurs in the penumbra.
Core and Penumbra in Stroke

Morphology
- Infarction
- Inflammation and apoptosis

Biochemistry
- Ionic failure
- Anoxic depolarization
- Glucose use
- Glutamate release
- Glucose use
- Protein synthesis
- Acidosis
- Oxygen extraction
- Selective gene expression
- < 20% blood flow
- Depleted ATP
- No energy metabolism
- Necrosis

PET

- Ischemic penumbra (perinfarct zone)
- Excitotoxicity
- Peri-infarct depolarizations
- Inflammation
- Apoptosis

Impact

Minutes

Hours

Days
Detrimental Events in Stroke

• **Glutamate excitotoxicity**: ion channel and reuptake failure produces glu overload > prolonged NMDA and AMPA stimulation. This is one source of Ca+ influx.

• **Calcium Dysregulation**: Ca+ is also released from mitochondria and ER stores and by activation of Na+/Ca+ exchanger.

• **Oxidative Stress**: overload of ROS oxidants from mitochondria (superoxide, hydrogen peroxide, NADPH oxidase, NO) > release of proteases and caspases leading to apoptosis.

• **Spreading Depolarization**: triggered by high levels of glu and K+ disrupts ionic gradients yielding massive depolarization of cells.

• **Inflammation**: release of cytokines (TNF, IL1β), prosto-glandins, produce severe inflammation in the affected area.
Imaging the Ischemic Penumbra In Stroke

Moskowitz et al 2010