Neurodegenerative Disorders: Mechanisms and Emerging Therapies

Lennart Mucke, MD

Director, Gladstone Institute of Neurological Disease
Joseph B. Martin Distinguished Professor of Neuroscience
Professor of Neurology
University of California, San Francisco

48th Annual Recent Advances in Neurology
UCSF

Learning Objectives
Increase Understanding of:
- Scientific, medical and socioeconomic issues raised by neurodegenerative disorders
- Need for better understanding of underlying mechanisms
- Values and limitations of experimental models
- Multifactorial pathogenesis of neurodegenerative disorders
- Likely requirement for a multipronged therapeutic approach
  - Stage dependent?
  - Stratified (disease and patient subtypes)?
- Developing better treatments: the long and short of it

Disclosures

Scientific Advisory Board:
Neuropore Therapies

Consulting:
AbbVie, Catenion

Honoraria for lectures:
AbbVie

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Bristol-Myers Squibb

Patents:
Coinventor on Gladstone/UCSF-owned patents relating to Alzheimer’s disease

Note:
No external financial support received for this lecture.
All drugs mentioned in this lecture are investigational relative to the potential indications discussed and have not yet been approved for these indications by the FDA.

Alzheimer’s Disease
Alzheimer’s Association: Facts and Figures

In US:
- 5.4 Million People with AD
- >15 Million by 2050
World:
- >36 Million Now
- ?? by 2050

Current US Cost: $200 Billion/yr
By 2050: >$1 Trillion/yr?

Change in Number of Deaths
2000–2008

Breast Cancer
Prostate Cancer
Heart Disease
Stroke
HIV
Alzheimer’s Disease

Medicare $125.5B
Medicaid $104.6B
Out-of-Pocket $33.3B
Other $12.8B

-3% -5% -13% -20% -29%
A mutation in APP protects against Alzheimer’s disease and age-related cognitive decline


Chromosomal Location of First Genes Linked to AD

All affect Aβ production or deposition.

Adapted from Lendon et al. JAMA (1997)

Drug Targets Relating to the Amyloid Precursor Protein

Aβ levels in medium of APP-transfected 293T cells
Drug Targets Relating to the Amyloid Precursor Protein

Inhibiting γ-Secretase May Affect the Function of Other Substrates

Table 2

<table>
<thead>
<tr>
<th>Selected γ-secretase Substrates</th>
<th>Other Functions</th>
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<tbody>
<tr>
<td>γ-protocadherin</td>
<td>Voltage-gated Sodium</td>
</tr>
<tr>
<td>APLP1</td>
<td>Channel 1 β2 Subunit</td>
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<td>APLP2</td>
<td>N-Cadherin</td>
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<td>Nectin-1β</td>
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<td>CD43</td>
<td>Notch NRADD</td>
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<td>CD44</td>
<td>P75</td>
</tr>
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<td>DCC</td>
<td>Syndecan-1</td>
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<tr>
<td>DELTA</td>
<td>Tyrosinase</td>
</tr>
<tr>
<td>E-Cadherin</td>
<td>Tyrosinase-related</td>
</tr>
<tr>
<td>ErbB-4</td>
<td>Proteins 1 and 2</td>
</tr>
<tr>
<td>Jagged</td>
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</tbody>
</table>


Immunization Against Aβ Clears Cerebral Amyloid Plaques in hAPP Mice and Humans with AD
Immunization Against Aβ Clears Cerebral Amyloid Plaques in hAPP Mice and Humans with AD

Anti-Aβ antibodies have failed in Phase III trials. Possibly subtle benefits in patients with early AD.

Dementia Is Caused by Different Diseases that Probably Start Decades Before Cognitive Decline Becomes Evident

Impact on clinical trials!

Will Effective Treatment Require Very Early Intervention?

BACE1 inhibitors
Selective γ-secretase inhibitors or modulators
Glutaminyl cyclase inhibitors
Anti-Aβ (oligomer) antibodies
Aggregation blockers
Others

Think of stroke, heart attack, diabetes, cancer and AIDS! Early treatment is key.
Effect of APOE Genotype on the Risk of Caucasians to Develop Alzheimer’s Disease Around the Age of 60

Yadong Huang, MD, PhD
Senior Investigator, Gladstone Institutes
Associate Professor of Neurology, UCSF

Robert Mahley, MD, PhD
Senior Investigator, Gladstone Institutes
Professor of Pathology and Medicine, UCSF

Our ApoE Experts

ApoE4 Probably Contributes to AD through Aβ-dependent and Aβ-independent Mechanisms

Amyloid hypothesis

Aβ Peptide

↑ APP

↑ Aβ generation

↓ Aβ clearance

ApoE4 proteolysis hypothesis

Apolipoprotein E4

Age

↑ ApoE4 fragments

Neurodegeneration

Cognitive decline

Huang & Mucke Cell (2012)

Proteolytic Cleavage of ApoE (4>3/2) Results in Fragments that Impair Mitochondria

* Aging
* Oxidative Stress
* Trauma
* Aβ Deposition

Nucleus

Neuron
ApoE4’s Neurotoxicity and Increased Susceptibility to Cleavage Depend on Intramolecular Domain Interaction and Can Be Reduced by “Structure Correctors”

Chen et al. JBC 287: 5253–5266 (2012)

Fluorescence Resonance Energy Transfer in Neuronal Cells: Small Molecule Disruption of ApoE4 Domain Interaction

Water Maze Test

Mice use spatial cues in the room to locate escape platform hidden under opaque water.

Preliminary Data: PY-101 Corrects ApoE4-Impaired Learning and Memory

Probe (Memory)

Number of Crosses

Latency (s)

Hidden Platform / Days 1–5

***p < 0.005
Multiple, Stage-Dependent Causes of Cognitive Decline in AD

The Clinical Impact of Cause-specific Treatments Will Depend on the Relative Impact of the Cause
Induced Pluripotent Stem Cells and Direct Reprogramming Technologies

Use human skin or blood cells to generate human brain cells. Use resulting brain cells to study (or treat) Alzheimer's disease and related conditions.

Keck Foundation Program in Brain Cell Engineering

Steven Finkbeiner and Sheng Ding

Subtypes of mature human brain cells

Biochemical Phenotype of iPS cell-derived Neurons from Patients with Inherited or Sporadic AD


Examining the Biological Activity of Specific Factors (e.g., Human APP/Aβ) in Transgenic Mouse Models

Lots going on here

7 day movie
Essentially, all models are wrong, but some are useful.

George Box

Even Phase II trials in humans often do not reliably predict the outcome of Phase III trials in humans.

Mark Geyer

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### Analyzing Multifactorial Human Diseases in Transgenic Mouse Models

Example: Alzheimer’s Disease with Causes A–E (or Z?)

---

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
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<tbody>
<tr>
<td>Aβ</td>
<td>Tau</td>
<td>ApoE4</td>
<td>Inflam.</td>
<td>Vascular</td>
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<tr>
<td>a</td>
<td>h</td>
<td>o</td>
<td>v</td>
<td>cc</td>
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<tr>
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<td>i</td>
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<td>hh</td>
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<td>g</td>
<td>n</td>
<td>u</td>
<td>bb</td>
<td>ii</td>
</tr>
</tbody>
</table>
Analyzing Multifactorial Human Diseases in Transgenic Mouse Models
Example: Alzheimer’s Disease with Causes A–E (or Z?)

Human Disease

<table>
<thead>
<tr>
<th>Model Phenotype</th>
<th>Human Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>A' APP/Aβ</td>
<td>Aβ</td>
</tr>
<tr>
<td>a</td>
<td>h</td>
</tr>
<tr>
<td>b</td>
<td>i</td>
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<tr>
<td>c</td>
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<td>f</td>
<td>m</td>
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<tr>
<td>g</td>
<td>n</td>
</tr>
</tbody>
</table>

Synaptic deficits | a | h | o | v | cc |
| Tau pathology? | b | i | p | w | dd |
| Memory problems | c | j | q | x | ee |
| Neuritic plaques | d | k | r | y | ff |
| Neuronal loss? | e | l | s | z | gg |
| Calbindin depletion | f | m | t | aa | hh |
| Nav1.1 depletion | g | n | u | bb | ii |

Analyzing Multifactorial Human Diseases in Transgenic Mouse Models
Example: Alzheimer’s Disease with Causes A–E (or Z?)

Selection of Molecular Abnormalities that Correlate with Cognitive Deficits in hAPP-J20 Transgenic Mice and Also Occur in Humans with AD

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Location</th>
<th>Change*</th>
<th>Validated in AD</th>
</tr>
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<tbody>
<tr>
<td>A2A Receptors</td>
<td>Hipp, Astrocytes</td>
<td>↑</td>
<td>✓</td>
</tr>
<tr>
<td>Calbindin</td>
<td>Dentate gyrus</td>
<td>↓</td>
<td>✓</td>
</tr>
<tr>
<td>Collagen VI</td>
<td>Dentate gyrus</td>
<td>↑</td>
<td>✓</td>
</tr>
<tr>
<td>EphB2</td>
<td>Dentate gyrus</td>
<td>↓</td>
<td>✓</td>
</tr>
<tr>
<td>GIVA-cPLA2 (AA)</td>
<td>Hippocampus</td>
<td>↑</td>
<td>✓</td>
</tr>
<tr>
<td>Klotho</td>
<td>Hippocampus</td>
<td>↓</td>
<td>✓</td>
</tr>
<tr>
<td>Metenkephalin</td>
<td>Hipp and EC</td>
<td>↑</td>
<td>✓</td>
</tr>
<tr>
<td>Nav1.1</td>
<td>Parietal Cortex</td>
<td>↓</td>
<td>✓</td>
</tr>
</tbody>
</table>

* vs. wildtype controls
Aging is the best established risk factor for Alzheimer’s disease (AD). Can factors that extend lifespan and promote healthy aging counteract AD-related cognitive deficits?

Dena Dubal, Ph.D.
Assistant Professor of Neurology, UCSF

Klotho: Suppressor of Aging

- Mutation accelerates aging
- Over-expression extends lifespan
- High expression in choroid plexus and kidney
- Klotho variation in humans modulates longevity and incidence of age-related disease

Klotho mutant: short lifespan, pulmonary emphysema, arteriosclerosis, osteoporosis, hypokinesis

Klotho Is Depleted in the Hippocampus of hAPP-J20 Mice and of Humans with AD

Klotho Elevation Enhances Synaptic Plasticity in Mice With or Without hAPP (LTP in Dentate Gyrus)
NMDA Receptors Involved?

- Klotho regulates ion channel function in kidney
- NMDARs mediate forms of synaptic plasticity thought to underlie learning and memory
- Overexpression of the NR2B subunit of the NMDAR enhances normal cognition
- Dysfunction of NR2B contributes to Aβ and tau-mediated toxicity.
- Does klotho increase NR2B expression and function?

Klotho Elevation Increases NR2B (GluN2B) within Postsynaptic Densities (PSD)

Blocking NR2B (GluN2B) with Ifenprodil Abolishes Klotho-mediated Enhancement of Learning and Memory in a Fear Conditioning Paradigm

In Humans, the KL-VS Variant of KLOTHO Is Associated with Higher Levels of Klotho in Serum
The KL-VS Variant of **KLOTHO** Is Associated with Better Cognition in Three Independent Human Cohorts

- **UCSF Hillblom Aging Cohort**
  - 41 carriers
  - 179 non-carriers

- **Rush Memory & Aging Cohort**
  - 135 carriers
  - 331 non-carriers

- **UCLA Normal Aging Cohort**
  - 12 carriers
  - 20 non-carriers

Klotho Enriches Cognition-Enhancing Glutamate Receptors in Synapses and Improves Cognitive Functions in Mice and Humans

- **Therapeutic Opportunities?**

  - Complementary approaches:
    - Inhibit klotho turnover
    - Simulate klotho activities
    - Activate downstream mediators
    - Block counteracting pathways

Should Neuroinflammation Be Targeted Therapeutically?

HIV-associated Dementia (HAD), an Instructive “Model”?

- Plenty of evidence for neuroinflammation
- No effective anti-inflammatory intervention has emerged. Not even as adjunctive therapy
- Effectively prevented and at least partly reversed by antiretroviral combination therapy
- Are Aβ and tau in AD analogous to HIV-1 proteins in HAD?

Should Neuroinflammation Be Pursued as a Therapeutic Target for Alzheimer’s Disease?

Human Studies
Pathology:
- Innate > adaptive immune activation
Biomarkers:
- Innate > adaptive immune activation
Epidemiology:
- NSAIDs, TBI
Genetics:
- GWAS results

Experimental Models
APP/PS1, Tau, ApoE4:
- Innate immune activation
- Genetic or pharmacological immune modulation:
  - Modulation of AD-related outcome measures

Some Caveats
Association vs. causality
Non-immune functions of “inflammatory mediators” in CNS (e.g., MHC)
Inflammation developed for good reasons

Inflammation in Neurodegenerative Disease—A Double-Edged Sword

“Since many of these responses can exert potent beneficial effects, directing and instructing the inflammatory machinery may be a better therapeutic objective than suppressing it.”
Immunotherapy Can Reduce Aβ, Tau and α-Synuclein Accumulation in the Brain
Schenk et al. Nature 1999 and many other studies thereafter

Alzheimer’s Disease Anti-inflammatory Prevention Trial (ADAPT)
- 2,528 elderly persons randomized to naproxen or celecoxib versus placebo
- NSAIDs may have an adverse effect in later stages of AD
- Asymptomatic individuals treated with NSAIDs may have reduced AD incidence, but only after 2 to 3 years.
- Treatment effects may differ at different stages of disease.


Possible reasons for discrepancy between epidemiological studies and prospective clinical trials:
- Too little, too late, not long enough
- Drugs not optimized for most relevant target
- Heterogeneity of patient populations
- Confounding factors (comorbidities, socio-economic, etc.)
Targeting Glial Activities and Pathways
Neuroinflammation or Neuromodulation?

For Example: Astrocytosis

- Overproduction of GABA by reactive astrocytes in APP/PS1 mice

- Reactive astrocytes overexpress A2A receptors in AD.
- Conditional ablation in APP mice improves memory.
- Activation of astrocytic signaling pathway impairs memory.
- A2A receptor antagonists in phase III trials for PD.

The Astroglial Network

Astrocytes are abundant, interconnected and dynamic

Credit: Jeff Lichtman

Astrocytes Regulate Diverse Aspects of Brain Function

Metabolism and homeostasis
Neurovascular dynamics
Development and synaptogenesis
Injury and inflammation
Synaptic transmission and plasticity
Behavior and cognition?

Credit: Jeff Lichtman
**A2A Receptors Regulate Glial Functions and Modulate Neuropathological Processes**

Adenosine

Glial cells

- Regulation of microglia
- Regulation of astrocytes
- Neuropathological alterations

**Do A2A receptors have a role in AD pathology?**

**Increased Expression of Adenosine A2A Receptors on Astrocytes Correlates with Disease Progression in AD**

**Aging, But Not Young, hAPP Mice Also Have Increased A2A Receptor Levels in Astrocytes**

17 months old

3 months old

14 months old

Thioflavin-S

NTG
hAPP
NTG
hAPP
Thioflavin-S
**Two-Pronged Approach**

1. Does astrocytic $G_s$-coupled signaling affect learning and memory?
2. Does the astrocytic A2A receptor affect learning and memory?

**Transgenic Mice with Rs1 Expression in Astrocytes**

Rs1 expression induced in adulthood to avoid effects on development

**Transgenic Mice with Rs1 Expression in Astrocytes**

Rs1 expression was restricted to astrocytes

**Learning and Memory in the Morris Water Maze**

**Rs1 Activation by Ligand Had Minimal Effect on Learning**
Rs1 Activation by Ligand Impaired Long-Term Memory

**Two-Pronged Approach**

1. Does astrocytic Gα耦合 signaling affect learning and memory?
   - Yes, it promotes memory loss.

2. Does the astrocytic A2A receptor affect learning and memory?
   - Genetic ablation approach using Cre/loxP

Training:
- Day 1
- Day 2
- Day 4

Probe:
- Day 1
- Day 2
- Day 4

- Con - Saline
- GFAP-Rs1 - Saline
- GFAP-Rs1 - Rs1 ligand

Additional results:
- Effect was reversible
- A single injection 4 hrs prior to probe had no effect.
Preservation of Neuronal A2A Receptors in Conditional Knockout Mice

- The loxP-A2A line was kindly provided by Jiang-Fan Chen (Boston U); described by Bastia et al. (2005) and others.
- The GFAP-Cre line was generated by Bajenaru et al. (2002) and has been used by Karasinska et al. (2013); Stenzel et al. (2011); Yamakawa et al. (2008); Drogemuller et al. (2006); Matos et al. (2013) and others.

Ablation of Astrocytic A2A Receptors

- Enhanced Memory
  - Transgenic mice expressing Cre recombinase and loxP-A2A genes

Ablation of Astrocytic A2A Receptors Reduced Memory Loss in Aging hAPP Mice

- Morris water maze, 15–17-month-old mice

Ablation of Astrocytic A2A Receptors Reduced Memory Loss in Aging hAPP Mice

- Open-field habituation, 15–17-month-old mice
Two-Pronged Approach

1. Does astrocytic G\textsubscript{s}-coupled signaling affect learning and memory?
   - Yes, it promotes memory loss.

2. Does the astrocytic A\textsubscript{2A} receptor affect learning and memory?
   - Yes, it promotes memory loss.

Potential Roles of Astrocytes in Memory Regulation

1. Indirect function
   Memory = neuronal network

2. Integrated function
   Memory = integrated networks

3. Parallel function
   Memory = two separate networks

A\textsubscript{2A} Antagonists in Clinical Trials for PD

Istradefylline
Preladenant
Tozadenant

Orr et al., Nat. Neurosci.(2015)
Inactivation of astrocytic A\textsubscript{2A} receptors improved memory in mice with or without hAPP/A\beta.

Could A\textsubscript{2A} receptor antagonists be beneficial in AD?
Preclinical trial in hAPP mice in progress
If positive, repurposing Phase IIa trial in humans
Aβ Causes Tau-dependent Alterations in Neuronal Activities

Substrate of Cognitive Decline and Entry Point for New Therapies

Enrollment
- MCI
- AD
- DLB
- FTD
- Normal controls

Measures
- 36-hour video-EEG
- MEG/EEG
- Fluctuation scores, imaging, genetics, etc.

Abnormal Activity in the Entorhinal Cortex and the Hippocampus in Humans with aMCI during a Cognitive Task

Adapted from Bakker A et al, Neuron, 2012
Levetiracetam Normalizes Abnormal Activity in the Entorhinal Cortex and the Hippocampus in Humans with aMCI

Gladstone/UCSF Phase IIa Levetiracetam Trial for MCI/AD with Associated Epileptic Activity

- **Enrollment**
  - Early-onset AD (age-at-onset <70)
  - Mild impairment (MMSE 18-26)
  - Epileptiform activity on initial screen

- **Measures**
  - Target engagement: M/EEG
  - Cognitive outcomes: memory, executive function

Design of Phase IIa Levetiracetam Trial Currently Ongoing at UCSF

Developing Better Treatments for AD
The Long and Short of It

Identification of potential drug targets
Validation in experimental models
Relevance to disease

Anti-apoE4 drugs
Klotho boosters
A(2A) antagonists
Anti-epileptics

Generation of novel compounds
Screening of drug libraries
Repurposing of drugs in use

Confirm drug works in brain,
Toxicity/safety trials

Proof-of-concept trials
Efficacy trials
Multifactorial Diseases Often Require the Combination of Different Treatments

**Hypertension**
- Salt restriction
- Weight loss
- Diuretics
- Enzyme inhibitors
- Receptor/channel blockers

**Alzheimer’s disease**
- Exercise?
- Anti-Aβ drugs?
- Anti-Tau drugs?
- Anti-ApoE4 drugs?
- Neuromodulators?
- Immune modulators?
- Others?

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