Dissecting the causes of neurodegeneration

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Outline

• Overview of common neurodegenerative disorders
• How can mice be genetically modified to create models of human disease?
• Mouse models of neurodegeneration – examples, pros and cons.
• What are some shared features of neurodegeneration?

Neurodegeneration

• Neurodegenerative diseases are common, incurable disorders associated with progressive degeneration or death of neurons, affecting function.
• The greatest risk factor is advanced age.
• Can have an inherited component, but the etiology of most is complex and includes multiple genetic and/or environmental factors.
• Genetic forms provide insight into cellular pathways.

Alzheimer’s disease (AD)

• Most common neurodegenerative disease:
  – 60-70% of dementia cases.
  – In 2015, ~44 million people worldwide had AD; only 25% of affected individuals are diagnosed.
• Pathological hallmarks include plaques (Aβ) and tangles (tau).
• Familial forms most commonly caused by mutations in amyloid precursor protein (APP) or TAU.

Parkinson’s disease (PD)

• Second most common neurodegenerative disorder:
  – In 2013, ~10 million people had PD.
• Early (<50 years of age) and late-onset forms.
• Pathological hallmark is presence of Lewy bodies in neurons (insoluble protein aggregates that include α-synuclein).
• Dopaminergic neurons earliest and most severely affected.
• Mutations in at least 6 different genes can cause or increase susceptibility to PD: α-synuclein, parkin, LRRK2, PINK1, DJ-1, ATP13A2, GBA.

Huntington’s disease

• A rare disease (5-10 cases/100,000 persons) that affects muscle coordination and leads to mental decline and behavioral symptoms.
• Is an autosomal dominant neurodegenerative disease caused by a mutation in Huntingtin (HTT).
  – The mutation is an expansion of a trinucleotide repeat sequence (CAG, which encodes the amino acid glutamine) in exon 1.
  – Normally, people have 10-35 repeats. People with 36-39 repeats may or may not be affected. Individuals with>39 CAG repeats will develop HD, and the larger the repeat, the earlier the onset of HD.
• No treatment or cure, but presymptomatic genetic testing can be performed.
Making and using mouse models to study neurodegeneration

- Animals models can be used to test:
  - Whether a mutation (or variant) contributes to disease (and be used to investigate mechanism).
  - The combinatorial effects of different genetic (and environmental) factors.
  - Therapeutic strategies and agents.
- Mouse is most commonly used model because:
  - It is a mammal with high genetic similarity to humans.
  - It has a relatively short lifespan (aging studies).
  - It has been possible to genetically modify mice for decades and many sophisticated tools exist.
  - Many well-characterized mouse mutants are already available.

When aren’t mice good models?

- Some aspects of human mental function are difficult to assess in mice, especially cognition/ dementia.
  - i.e., what is parallel (for mice) to the Mini-Cog?
  1) 3 word registration
  2) Clock drawing
  3) 3 word recall
- Mouse lifespan is much shorter than human.
- Genetic models don’t always recapitulate human disease.

What is a gene?

- Exons: DNA that encodes protein sequence
- Introns: DNA in between exons; “non-coding” (gets cut out of mRNA)
- Promoter: “drives” gene expression (factors bind that initiate transcription of DNA into RNA)

How do genes make proteins?

- When promoter “turned on”, RNA copy of DNA is made
- 5’UTR (untranslated region): part of mRNA before the start codon
- 3’UTR: sequence following stop codon
- Polyadenylation (pA) signal: add A’s to mRNA
- Start codon: where the protein-coding sequence begins
- Stop codon
- RNA
How do genes make proteins?

- Introns are spliced ("cut") out, leaving mRNA = complimentary to exon (coding) DNA.
- mRNA gets translated into protein (amino acids).

Important points to remember

- cDNA is a DNA copy of mRNA
- All cells have DNA (gene), but only make protein if the gene is turned on (promoter is "active") and there are no mutations that affect gene expression
- Promoters can be on/off in different cell types or at different times during development

Types of genetic modifications in mice

- Transgene insertion
- Knock-out
- Conditional knock-out
- Knock-in
- Gene-trap insertion
- Mutagenesis
  - Directed (knock-out, knock-in)
  - Random (radiation, chemicals)

Transgene Insertion

- Random insertion of “mini-gene” (promoter, protein-coding sequence, pA sequence) into the mouse genome
- Provides over-expression gene of interest using specific promoter (i.e., in a specific tissue or cell type)
  - Sequence can be wildtype, mutant, different species
- Expression of some transgenes is controlled by the presence of a second transgene and/or drug treatment

Adapted from: Gunn & Canine, Chapter 5, Molecular & Genetic Basis of Neurological & Psychiatric Disease, 5th Ed.
Example: Alzheimer’s 5XFAD model
Over-express human amyloid precursor protein (APP) and presenilin 1 (PS1) containing mutations associated with familial Alzheimer’s disease

5XFAD mice develop intraneuronal amyloid deposits and gliosis by 2 months of age
Figure (copyrighted material): panels from Figure in Oakley et al. 2006 showing Aβ and GFAP expression in Tg brains

5XFAD mice show neuron loss and progressive memory impairment
Figures (copyrighted material) from figures in Oakley et al. 2006

PD models: α-synuclein Tg mice
• Several lines of transgenic mice have been generated that over-express wildtype or PD-mutant α-synuclein.
  – Promoters include PDGF-β, Thy-1, TH, Pip and prion
  – Variable results; some developed intraneuronal inclusions/protein aggregates, gliosis, motor coordination defects. None displayed dopaminergic neuronal death in substantia nigra.
  – Many of the pathological changes were observed in spinal cord, with motor neurons most severely affected.
  – α-synuclein expression levels correlated with severity of behavioral and neuropathological phenotypes.

Getting more relevant expression
• Standard transgenes use a specific, well-characterized promoter to drive gene expression.
• Promoter regions can be large and involve many regulatory elements.
• Bacterial and yeast artificial chromosomes (BACs and YACs) are very large segments of DNA that can contain entire genes –including their regulatory elements –and can be replicated and manipulated (in bacteria or yeast).
  – The coding region can be replaced with another gene to drive expression in a more specific pattern, replace mouse sequence with human (wildtype or mutant), etc.
  – Typically provide lower (closer to endogenous) expression levels.

BACHD model
• Transgenic mice that express neuropathic, full-length human mutant Htt containing 97 CAA-CAG repeats. [mixed repeat is more stable]
• Develop aggregates of mutant HTT fragments in the nucleus and cytoplasm in neurons, in a pattern similar to that observed in HD patients.
• Exhibit progressive motor deficits, neuronal synaptic dysfunction and late-onset neuropathology.

Figure (copyrighted material) showing cortical atrophy, from Gray et al. 2008
**Knock-out mice**

- Generate a mutation that ablates gene expression (no protein is made).
  - May involve deletion of entire coding region or just a critical part of the gene.
- Primarily used to assess gene function *in vivo*.
- Traditionally generated by manipulating DNA in ES cells (“gene targeting”). Modified ES cells are injected into blastocysts and can incorporate into the developing embryo.
  - If ES cells contribute to germ line (sperm), the mutation can be passed on to progeny.

**Gene Targeting**

Figure (copyrighted material): Fig. 5.3 in Gunn & Canine

Adapted from Gunn & Canine, Chapter 5, Rosenberg’s Molecular & Genetic Basis of Neurological & Psychiatric Disease, 5th Ed.

**Conditional Knock-out**

- Makes use of bacterial recombination system
  - Cre recombinase recognizes specific DNA sequence (loxP site).
  - When 2 loxP sites are present, Cre promotes recombination between them

Figure (copyrighted material): Gunn & Canine Fig. 5.4B

From: Gunn & Canine, Chapter 5, Rosenberg’s Molecular & Genetic Basis of Neurological & Psychiatric Disease, 5th Ed.

**Conditional Knock-out**

- Gene targeting is used to insert loxP sites, usually flanking critical exon.
- Mice carrying “floxed” allele are mated to transgenic mice expressing Cre recombinase.
  - Knock-out allele is generated only in cells/tissues expressing the Cre transgene

Figure (copyrighted material): Gunn & Canine Fig. 5.4A

From: Gunn & Canine, Chapter 5, Rosenberg’s Molecular & Genetic Basis of Neurological & Psychiatric Disease, 5th Ed.

**Knock-out example: parkin**

- Mutations in parkin (PARK2) are the second most common known cause of Parkinson’s disease (PD).
- Loss-of-function mutations in parkin cause autosomal recessive, juvenile-onset PD.
- Mutations in parkin or inactivation of parkin (caused by oxidative, dopaminergic or nitrosative stress) are implicated in sporadic PD.
- Parkin knock-out mice do not develop symptoms of PD or show loss of dopaminergic neurons. They do show subtle mitochondrial dysfunction, and parkin was subsequently shown to play a role in ridding cells of damaged mitochondria.

**Conditional knock-out example: parkin**

- To test whether developmental compensatory mechanisms mitigate the effects of loss of parkin, parkin conditional knock-out mice were used to ablate parkin from adult ventral midbrain of mice.
- Cre recombinase was delivered to the brains of 6-8 week-old parkin<sup>fl/fl</sup> mice by stereotactic injection of GFP-fused Cre recombinase lentivirus (Lenti-GFPCre).
- Control injections were performed with lentivirus expressing GFP (Lenti-GFP).

Figure (copyrighted material) From Stevens et al. 2015

Conditional knock-out example: parkin

- Within 4 weeks, parkin was “absent in the ventral midbrain of parkin\(^{fl/fl}\) mice injected with Lenti-GFPCre.
- Adult deletion of parkin led to age-dependent loss of dopamine neurons and defective mitochondrial biogenesis.

Knock-in example: zQ175 (HD)

- In zQ175 mice, mouse Htt exon 1 is replaced with human HTT exon 1 sequence containing a ~190 CAG repeat tract.
  - Expression of mutant HTT is under control of endogenous regulatory elements
  - Heterozygous mice develop disease symptoms consistent with HD in humans

Motor coordination defects, striatal atrophy...

- ...and other phenotypes consistent with HD phenotypes in humans

Knock-in mice

- Uses the same approach as knock-out but instead of deleting DNA sequences, it replaces them.
  - Can be used to generate “humanized” allele
  - Can be used to model the effects of specific human mutations
  - A key advantage is that the gene is expressed using the endogenous promoter and regulatory elements, providing “normal expression pattern and levels

zQ175 heterozygotes show nuclear accumulation of mHTT

Figure (copyrighted material) from Carty et al. 2015

Generating mutations using nucleases

Figure (copyrighted material): Gunn & Canine Fig. 5.5
Insights into neurodegeneration from mouse models

- A common feature of neurodegenerative disorders is the accumulation of proteins in abnormal conformations
  - Aβ and Tau (AD)
  - α-synuclein (PD)
  - Prion protein (Transmissible Spongiform Encephalopathies)
- There is mounting evidence that many of these protein aggregates can spread from cell-to-cell

Prion diseases

- Transmissible Spongiform Encephalopathies – Scrapie, “Mad cow disease,” Kuru
  - Misfolded prion protein (PrPSc) converts normally folded prion protein (PrPC); this process is called “seeding”
  - PrPSc aggregates can be transferred between cells

Are all neurodegenerative disorders “prion” diseases?

- Neuronal accumulation of protein aggregates appears to spread throughout the brain as disease progresses.
- Several studies have demonstrated that Tau, Aβ, α-synuclein and mutant HTT can form fibrils in vitro.
- In cell culture studies, misfolded proteins could be transferred from cell-to-cell.

Are all neurodegenerative disorders “prion” diseases?

- Human studies —“suggestive” information
  - Individuals given growth hormone —some that developed iCJD (prion disease) also showed Aβ pathology (Jaunmuktane et al. (2015) Nature 525(7568):247-50).

Mouse studies confirm cell-to-cell spread of α-synuclein

- Mouse cortical neuronal stem cells were labeled with GFP and injected into the brains of transgenic mice expressing human α-synuclein in neurons or nontransgenic controls.
- 1 week post-injection, ~2.5% of transplanted cells in Tg+ mice showed human α-synuclein immunoreactivity. At 4 weeks, 15% were positive for human α-synuclein.

α-synuclein pathology is transmissible

- Intracerebral injection of preformed α-synuclein fibrils or brain homogenates from older α-synuclein transgenic mice (exhibiting α-synuclein pathology) into young, presymptomatic transgenic mice accelerated Lewy body formation and onset of neurological symptoms.

Figures (copyrighted material) from Luk et al., 2012


Luk et al. (2012),JEM 209(5):975-86.
Multiple System Atrophy & \( \alpha \)-synuclein

- Multiple System Atrophy (MSA) is a progressive disorder characterized by loss of autonomic nervous system function and other signs of Parkinsonism.
  - Neuropathological hallmark is glial cytoplasmic inclusions of \( \alpha \)-synuclein filaments.
- 14 human brain homogenates from MSA patients were injected into the brains of transgenic mice expressing human mutant (A53T) \( \alpha \)-synuclein.
  - Mice homozygous for transgene developed neurological dysfunction within ~4 months, associated with deposition of \( \alpha \)-synuclein within neuronal cell bodies and axons.
- Supports hypothesis that MSA is a synucleinopathy but that the aberrant conformer of \( \alpha \)-synuclein is different from the one associated with PD.

AD, HD

- Similar studies have shown that mutant human tau and A\( \beta \) aggregates can spread between cells and seed aggregate formation that can incorporate endogenous protein.
- Some data suggests mutant HTT aggregates may also be actively transferred between cells.

In vivo imaging: another advantage of mice

- Can be diagnostic, used to follow progression or effect of treatment.
  - Can be used to detect plaques & tangles (AD)
- Common approaches:
  - Magnetic Resonance Imaging (MRI)
  - Computer Tomography (CT)
  - Nuclear imaging (Positron Emission Tomography (PET), Single-Photon Emission Computed Tomography (SPECT))

In vivo imaging - Bioluminescence

\[
\begin{align*}
D - \text{luciferin} + \text{ATP} - \text{Mg}^{2+} + \text{O}_2 &\rightarrow \text{luciferase} \\
\text{Oxyluciferin} + \text{CO}_2 + \text{AMP} + \text{PP}_1 - \text{Mg}^{2+} + \text{photon} &\rightarrow \text{luciferase} \\
\end{align*}
\]

- Oxidation of luciferin by luciferase generates light.
- When this reaction occurs in living tissues, the photons can be detected and quantified by a highly sensitive charge-coupled device (CCD) camera, allowing for non-invasive analysis of biomolecular reactions.
  - System can also detect and quantify fluorescent signals.

In vivo imaging - Bioluminescence


Figures showing IVIS setup and example (copyrighted) from Hachgrafe & Mandelkow, 2013
Advantages & Disadvantages of BLI

• The good
  – Easy to perform the imaging.
  – Non-invasive (does require anesthesia).
  – Relatively cost effective.
  – No need for radioactive tracers.
  – Allows for linear (timecourse) studies of specific individuals.

• The bad
  – Lack of spatial resolution (due to tissue absorption and light scattering).
  – Signal intensity depends on depth of source.

Reporter mice

• For bioluminescent imaging, transgenic mice expressing luciferase are used.
  – Expression driven by promoter that will be “activated” in specific situation.
  – Luciferase fused to gene-of-interest.

• Example: Most forms of neurodegeneration are associated with gliosis. Luciferase expressed from GFAP promoter will increase as GFAP expression increases.

Inducible Tau-luciferase mouse model

• Transgenic expression of aggregation-prone mutant human Tau and luciferase under control of a bidirectional transactivator (tTA)-responsive promoter.
  – Bidirectional promoter ensures co-regulation of Tau and luciferase; luciferase expression serves as a direct measure of human Tau expression.
  – Expression in forebrain neurons (CamkIIa promoter).
  – Inducible expression controlled by application of doxycycline.

Tau-induced phenotypes are reversed when mutant Tau is turned off

Summary

• Mouse models are a powerful tool for dissecting mechanisms that contribute to neurodegeneration, and provide a system for early testing of therapies.

• Mouse models of neurodegeneration are not perfect, but some of the phenotypic discrepancies between mice and people expressing the same mutant genes could provide important insights – understanding why are mice sometimes unaffected could reveal therapeutic targets?