Mouse Eye Disease Models, Imaging, Clinical and Histological Evaluation

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What to Take From the Lecture

- 2 types of people:
  - Those focusing on ocular disease
  - Those who happen to notice something funky with their mouse’s eye(s) (e.g. cloudy cornea; cataract; not there!)
- Aims:
  - Notice something wrong with your mice:
    - How to investigate without killing the mouse (in-vivo)
    - How to further investigate in detail post-sacrifice (ex-vivo)
  - Genetic & Environmental/inducible eye disease models:
    - Whole Globe, Cornea, Glaucoma, Lens, Retina, Neoplasia
  - Specific considerations in mouse ocular phenotyping
  - Eye problems confounding your studies (e.g. behavioral tests)
  - Hopkins investigators in specific eye research areas

Animal Eye Anatomy (Human & Mouse)

- Newborn's eye: about 18mm axial length
- Infant’s eye: 19.5mm axial length
- Adult human’s eye: 24-25mm (approx. 1 inch)
- Mouse eye: 3-3.5mm

Telorism: Cranio-Facial Structure

Exophthalmos / Proptosis

Whole Globe: Genetic Models

- Anophthalmos
- Microphthalmos
- Buphthalmos (whole eye)
- Axial myopia (single axis)
**Whole Globe: Genetic Models**

- **Anophthalmos and microphthalmos**
- C57BL mice known for small or absent eyes
- Frequency from 1 to 10% depending on background strain
  - Females and right eyes affected more
- Gene(s) have not been identified
- Also more susceptible to ocular infections because of abnormal flushing of ocular surface

**Whole Globe: Genetic Models**

- **Axial myopia (single axis)**
  - Axial elongation vs short sightedness – physical vs optical measures
  - ZENK/Egr-1 model

**Axial Myopia: Environmental Models**

- Occluders / Lenses, suturing lid closed
- Issues over effect of defocus in mouse (as no macula/fovea)

**In-vivo: US (A,B,M)**

**Magnetic Resonance Imaging (MRI)**
Corneal Diseases

- Induced
  - Corneal injury models
    - Used to study corneal inflammation and healing
    - Examples: alkali burn, fungal keratitis, decreased lacrimation (dry eye)
  - Genetic
    - Fuchs endothelial corneal dystrophy

Ex-vivo Sample Preparation

- Enucleation: Tissue of greatest interest? Globe vs optic nerve
  - Globe: Proptose eye, forces behind, gentle ON traction
  - Optic Nerve: Avoid traction, expose ON from lateral aspect, sever at optic foramen
    - In advanced ON disease, brain dissection & fixation 1st
    - In young, in situ fixation with decalcification
- Fixation: Varies depending on goal
  - Plastic embedding following glutaraldehyde / PFA mix for best morphology
  - Decent morphology with paraffin embedding after fixation in 10% neutral buffered formalin or an acidic fixative (Bouin’s, Davidson’s, etc.)
  - 4% PFA versatile fixative for morphology plus IHC & ISH
- Perfusion: To do, or not to do

Induced Corneal Injury

- Alkali burn
  - Button of filter paper soaked with 1N NaOH applied to cornea of anesthetized mice

Modeling Dry Eye (keratoconjunctivitis sicca)

- Experimental reduced lacrimation
  - Scopolamine patch applied to tail
  - Botulinum toxin injection into lacrimal gland
  - With or without environmental change (low humidity, increased air movement)

Fluorescein staining (punctate green) on damaged corneal surface (A, B, C) compared to normal (D, E, F) after botulinum toxin induced dry eye
**Genetic Corneal Diseases**

- Fuchs endothelial corneal dystrophy (human)
  - Corneal endothelial cell loss and abnormalities often leading to corneal transplantation
- Knock in mouse model
  - Alpha 2 collagen VIII transgenic knock-in mouse model of Fuchs endothelial corneal dystrophy

**Fuchs Endothelial Corneal Dystrophy Model**

- Mice with knock-in mutation of alpha 2 collagen 8 gene
  - Progressive alterations in corneal endothelial morphology
  - Cell loss & basement membrane guttae


**Lens: Genetic Models**

- Most genetic mouse cataracts congenital (humans: age-related)
- Understanding of lens development rather than ageing
- Many mouse models:
  - Most commonly
    - Gamma-crystallins (Cryg)
  - Some postnatal, progressive
    - Beta-crystallins (Cryb)
  - Membrane proteins
    - MIP or connexins
  - Transcription factors
    - FoxE3, Maf, Sox1, Six5
  - Systemic disease models:
    - Galactosemia, SDH, perlecan

**Lens: Environmental Models**

**Unintentionally induced cataracts**

CAN be unilateral.

ARE reversible.


**Intentionally induced cataracts**

- N-acetyl-p-benzoquinone imine (NAPQI) = acetaminophen metabolite
  - Intracameral (AC) injection of elicits increase in free Ca2+ in lens epithelium, calpain activation & lens opacification
- UVR-B induced cataract


**Glaucoma: Genetic Models**

- What is glaucoma? How to investigate?
- Half of patients have increased IOP
- Half ONH cupping without raised IOP
- In mice: Increased IOP +/- ONH fiber loss
- DBA2/J is genetic model. Available at Jax
- Compound heterozygote in 2 genes

The lens is prone to artifact in processing for histologic imaging, so imaging the lens antemortem with a slit lamp and/or imaging dissected lenses immediately after euthanasia is most reliable.
Glaucoma: Genetic: DBA/2J Mouse

- Iris atrophy, pigmentary dispersion and clogging of TM

Transgenic strain carrying MYOC Tyr437His mutation

MYOC = heavily expressed in and secreted by the trabecular meshwork

Mutants have modest but significant IOP elevation and loss of axons in the optic nerve

Glaucoma: Genetic: Myocilin Mutant


Glaucoma: Environmental: Dexamethasone


IOP Measurement in Mice

- AC needle vs tonolab
- Anesthetics affect IOP (inhalational)
- Hold mouse too tight affects IOP
- Corneal issues affect Tonolab

Retinal Disease

- Neural retina (retinal degeneration, RD)
  - Direct effect on retinal neurons
  - RPE changes with secondary effects on neural retina
- Vascular diseases
  - Diabetic retinopathy (DR)
  - Age-related macular degeneration (AMD)

http://www.bio.miami.edu/tom/courses/protected/bi265/retina.jpg
Neural Retina: Degeneration: Genetic Models

Genetic RD: Rd1 (Pde6b<sup>rd1</sup>) Mutation
- Develop normal photoreceptors that then rapidly degenerate in the 3rd post-natal week – complete blindness by 4 weeks

Genetic RD: Rd8 Mutant Contamination
- AR; single nucleotide deletion in Crb1 gene, causes form of RD
- Multiple light colored spots in fundus; histologically get retinal folds, pseudorosettes, focal retinal dysplasia and degeneration

Retinal Degeneration: Environmental
- Many models induced by physical insults:
  - Exposure to strong light
  - Intravitreal insertion of iron particles
  - Intravitreal chemical agents
    - Iodoacetic acid
    - Sodium iodate
    - Cobalt chloride
    - L-ornithine chloride
    - N-methyl-N-nitrosourea (MNU)
  - MNU model: single systemic injection
    - Selective & progressive degeneration of photoreceptors in a variety of animals, including non-human primates
- Remember: Retina not fully developed until 2 weeks of age!

Induced Retinal Degenerations
- Light induced retinal degeneration
  - Bright light exposure with dilated pupils

Induced Retinal Degenerations
- N-methyl-N-nitrosourea (MNU)
  - Single systemic dose causes photoreceptor apoptosis and retinal degeneration within days

Vascular Diseases of the Retina

- Age-related macular degeneration
  - Dry = drusen deposition between choroid & RPE
  - Wet = vascular problem caused by unhappy (hypoxic) cell signaling
- Diabetic retinopathy
- Retinopathy of prematurity
  - Pathogenesis similar to wet AMD

Age-Related “Macular” Degeneration (AMD) models

- Mice make imperfect animal models of AMD because they lack a macula
  - i.e. no perfect single model available
- But many hallmarks of AMD in humans can be modeled in various mouse models
- There are many examples of genetic and induced (and combinations of the two) models
  - Example: SOD1-/ mouse [superoxide dismutase] [wet AMD]

SOD1 Deficient Mice

Choroidal neovascularization (CNV) = vessels invade through Bruch’s membrane and into the RPE & retina. CNV vessels are abnormal and leaky.

Diabetic Retinopathy: Genetic Model

- CS7BL/6JIns2Akita mice (mutation in insulin 2 gene)
- Diffusion of fluorescently labeled BSA into the adjacent parenchyma of a blood vessel
- (left image = cross section, 2 right images = retinal flat mounts)

Diabetic Retinopathy: Genetic Models

- Type 1 diabetes: e.g. http://jaxmice.jax.org/list/ra64.html ; e.g.
- Vary: age of onset, mechanism, resistance, gender/strain variation

A’M’D: Genetic Models

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<tr>
<th>Mouse Models</th>
<th>Strain Name</th>
<th>Standard Supply</th>
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Untreated Akita mouse
Treated Akita mouse

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Diabetic Retinopathy: Genetic Models

- **Type 2 diabetes**: [http://jaxmice.jax.org/diabetes/comparison.html](http://jaxmice.jax.org/diabetes/comparison.html)

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Diabetic Retinopathy: Environmental Models

- Streptozotocin (STZ) – induced diabetes (Type 1)
- Alkylating agent; originally developed as antibiotic
- Similar enough to glucose to be transported into cells by GLUT2
- Not recognized by any other GLUT
- Hence toxic just to islet beta cells
- I.v. injection: 100 mg/kg body wgt
- Glucose levels, HbA1c, body weight
- NPH insulin given to prevent critical weight loss

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Oxygen-Induced Retinopathy (OIR)

- P7 mice exposed to 75% oxygen
- Induces loss of immature retinal vessels and slows development of the normal retinal vasculature
- Results in central zone of vaso-obliterating (VO)
- After returning mice to room air at P12, central avascular retina becomes hypoxic, triggering both normal vessel regrowth and a pathologic formation of extraretinal neovascularization (NV)

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Retinal Analysis

- Retinal Imaging Microscopy System allows ‘in-vivo microscopy’
- White light imaging mice and rats, fluorescein angiography, diabetic retinopathy, retinoblastoma, retinitis pigmentosa, choroidal neovascularization & anterior segment slit-lamp
- Live animal GFP & YFP fluorescent studies also possible

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Retinal Structure: Optical Coherence Tomography (OCT)

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Retinal Function: Electroretinogram
Visual Function: Optokinetic Device

Neoplasia: Uveal Melanoma etc.

- Too broad to cover in 2 minutes
- Primary vs metastases
- Genetic vs inducible
- Location: anterior (more obvious)
- USS, MRI, microCT
- Enucleation, transillumination
  - Depends on mouse strain
- Histological analysis

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