The Mouse Eye

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• 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
    • Sample collection & handling
    • Eye problems confounding your studies (e.g. behavioral tests)
    • Hopkins investigators in specific eye research areas

What to Take from the Lecture

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

Exophthalmos / Proptosis

Sample Preparation

• To perfuse or not to perfuse – that is the question
• Enucleation
  – Tissue of greatest interest? Globe vs optic nerve (ON)
  – Globe – proptose eye, forceps behind eye and gentle traction until ON tears
  – Optic Nerve – avoid traction, expose ON from lateral aspect, sever at optic foramen
    • In advanced ON disease models, brain dissection & fixation prior to collection of ONs
• In situ fixation with decalcification for young animals
Sample Preparation

- Fixation – varies depending on goal
  - Decent morphology with paraffin embedding following fixation in 10% neutral buffered formalin or an acidic fixative (Bouin’s, Davidson’s, etc.)
  - Plastic embedding following glutaraldehyde/paraformaldehyde mix for best morphology
  - 4% paraformaldehyde is a very good versatile fixative useful for morphology as well as immunohistochemistry & in situ hybridization

Whole Globe: Genetic Models

- Buphthalmos (whole eye)
- Axial myopia (single axis)
- Anophthalmos
- Microphthalmos


Anophthalmia & Microphthalmia

- C57BL mice known for small or absent eyes
- Frequency varies from 1 to 10% depending on background strain
  - Females and right eyes affected more often than males & left eyes
- Gene(s) have not been identified
- Also more susceptible to ocular infections because of abnormal flushing of ocular surface
- Microphthalmia and associated abnormalities in inbred black mice.
  

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

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)

Induced Corneal Injury

- **Fusarium keratitis model**
  - Contact lens with adherent *Fusarium* applied to abraded mouse eye

Genetic Corneal Diseases

- Fuchs endothelial corneal dystrophy (human)
  - corneal endothelial cell loss and abnormalities often leading to corneal transplantation
- Knock in mouse model
  - An 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 the alpha 2 collagen 8 gene
  - Progressive alterations in corneal endothelial morphology
  - Cell loss & basement membrane guttae


Lens diseases (cataracts)

- Induced
  - Unintentional - with corneal exposure and decreased body temperature during anesthetic procedures
  - Intentional
- Genetic
- Many different examples of both

Unintentionally induced cataracts

CAN be unilateral.
ARE reversible.


Intentionally induced cataracts

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

Genetic Cataract 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

Cataract Models

WT

C3H

Mut

ENU 988

What is glaucoma?

- More complicated than just elevated intraocular pressure
- Loss of retinal ganglion cells (RGCs) with optic nerve degeneration/atrophy

Induced Models

- Bead injection, dexamethasone injection

Genetic Models

- DBA/2J mouse, MYOC mutant mouse

Glaucoma

IOP Measurement in Mice

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

Calibration of the TonoLab tonometer in mice with spontaneous or experimental glaucoma. Pease ME, Cone FE, Gelman S, Son JL, Quigley HA. Invest Ophthalmol Vis Sci. 2011 Feb 22;52(2):858-64.

Induced glaucoma: bead injection

Calibration of the TonoLab tonometer in mice with spontaneous or experimental glaucoma. Pease ME, Cone FE, Gelman S, Son JL, Quigley HA. Invest Ophthalmol Vis Sci. 2011 Feb 22;52(2):858-64.

Induced glaucoma: dexamethasone

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Genetic glaucoma: DBA/2J mouse

- Model of iris atrophy leading to pigmentary dispersion and clogging of trabecular meshwork

Genetic glaucoma: DBA/2J mouse

- Pigmented cells occlude the filtration angle
- Iris atrophy

Genetic glaucoma: Myocilin mutant

- Transgenic strain carrying MYOC Tyr437His mutation
- Mutants have modest but significant IOP elevation and loss of axons in the optic nerve

(Neural) Retinal Degenerations

- Induced
  - Light induced retinal degeneration
  - N-methyl-N-nitrosurea (MNU)
- Genetic Models
  - Spontaneous
    • rd1 & rd8
  - Transgenic
  - Many examples of both
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

Retinal Structure: Optical Coherence Tomography (OCT)

Optical Coherence Tomography (OCT)

R-4300 for Greatest Depth, R-2200 for Greatest Resolution

Retinal Function: Electroretinogram (ERG)

ERG Intensity Series
Abnormal Electroretinogram


Visual function: Optokinetic Device


Induced Retinal Degenerations

- **Light induced retinal degeneration**
  - Bright light exposure with dilated pupils


- **N-methyl-N-nitrosurea (MNU)**
  - Single systemic dose causes photoreceptor apoptosis and retinal degeneration within days


Induced Retinal Degenerations

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Genetic retinal degeneration: Rd1 (**Pde6brd1**) mutation

- Develop normal photoreceptors that then rapidly degenerate in the 3rd post-natal week
  - Complete blindness by 4 weeks of age
- **C3H/HeJ**, **CBA/J**, **FVB/NJ**, **SJL/J**, **SWR/J**
  - Many others
  - FVB/N of particular concern because they are often used to make targeted mutants because of their large pronucleus
- http://eyemutant.jax.org/index.html
  - Complete list of strains carrying the rd1 mutation

Retinal Degeneration Models

C57BL/6J at 3 months of age  rd1/rd1 at 21 days of age

Rd8 Mutation Contamination

- *rd8* mutation is a single nucleotide deletion in the Crb1 gene, which results in a form of retinal degeneration
- Phenotype: multiple light colored spots in the fundus of the eye that correspond histologically to retinal folds, pseudorosettes, as well as focal retinal dysplasia and degeneration
- Autosomal recessive


Rd8 Mutants


Retinal Degeneration: Genetic Models

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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]

Vascular eye disease models

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

SOD1 deficient mice

A'M'D: Genetic Models

Diabetic Retinopathy

- C57BL/6Ins2Akita mice (mut 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
- Type 2 diabetes: http://jaxmice.jax.org/diabetes/comparison.html

Oxygen-induced Retinopathy (OIR)

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

Strain Differences

- Inbred mice can carry background disease that can influence ocular phenotype:
  - 1. Systemic disease can affect the eye
  - 2. Known genetic defects (such as Pde6b<sup>drd1</sup>)
  - 3. Congenital abnormalities that may be polygenic (microphthalmia in black mice)
  - 4. Susceptibility genes that alter the response to an external stimulus