Evaluation of Embryonic & Perinatal Lethality In Mice

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Outline of Presentation

• Normal development of the mouse embryo & placenta
• Abnormal development
• Neonatal and perinatal mortality
• Resources for evaluating mouse embryology

Normal Development of the Mouse Embryo

Mouse Development

Adapted from C. Stewart

TS 1-6
TS 8-14
TS 14-26
TS 20-26

Early Mouse Embryonic Development
E0.5 - E4.5
(Beddington & Robertson, Cell 96: 195, 1999)

Early Mouse Embryonic Development
E5.5 – E7.5
(Beddington & Robertson, Cell 96: 195, 1999)
Growth of the Mouse Embryo

Mouse Embryo Development

VE Papaioannou & RR Behringer
Veterinary Pathology, 49: 64, 2012

Use "embryo" for entire mouse gestation period
"fetus" after mineralization of bone (E15+)
(not usually used for mice)

Gross Analysis of The Pregnant Uterus, Placenta and Embryo

• Dissect out the uterus of the pregnant mouse
• Use a mouse uterus/placenta/embryo dissection form to record information
• Record numbers, location and size of each placental site
• Record appearance of placenta
• Record the appearance of the yolk sac & umbilical vessels
• Record size, shape and appearance of embryo – is the heart beating?, is the embryo viable?
• Record resorptions (any abnormal placental sites – abnormal in size and/or shape)

Dissection of the Mouse Uterus

Embryo DNA
Yolk sac
Tail
Limbs
Whole embryo

Extra-embryonic Embryonic Tissues
Fixation and Trimming

Embryos at most gestational stages are best fixed in Bouin’s fixative. Embryo fixation is dependent upon gestational age:
- E<12.5, fixed for 2 hrs,
- E12.5-E17, 4 hrs
- E17.5-18.5, 24-48 hrs
(Kaufman MH, 1992; LW Crawford et al, Tox Path 38: 872, 2010)

IHC, ISH paraformaldehyde or formalin
Imaging formalin or other fixatives

The intact placenta is fixed in formalin (48 hrs), not Bouin’s

A Suggested Optimal Embryo Trimming Protocol
for a screen for the histology of most tissues

E14-15

From Kaufman, 1992

Sectioning of Mouse Embryos
(M Kaufman, Bouins fixation, H&E stain)

Coronal Section E13.5
Longitudinal/Sagittal Section E12.5-13
Cross/Transverse Section E12.5-13

Pituitary Gland and Thalamus Development in Newborn T/ebp -/- Mice

Ward P 3
How To Identify Mouse Embryonic Tissues
Use The Kaufman Mouse Embryo Histology Atlas


Gene Expression in Mouse Embryos

ISH [S probe]
autoradiography (J. Chou)


Reporter Transgene

E14 BrdU

Mouse Embryo Gene Expression

Edinburgh Mouse Atlas Project
http://www.emouseatlas.org/emage/home.php

Online Mouse Brain Gene Expression Atlas
Allen Brain Atlas
http://www.brain-map.org

AA Sharov et al, Gene Expression Profiling of Mouse Embryos

Mouse Embryo Gene Expression

The placenta arises, in part, from the ectoplacental cone (EC), which expands and develops

Fri 2 Ward  Embryo

The Placenta

Pre-gastrulation

Gastrulation
(development of The 3 germ layers)

Fri 2 Ward  Embryo

Ward P 4

JH Ward JHU 2012
Placental Development  E7-12

From Ward and Devor-Henneman, 2000

The Pregnant Uterus

Courtesy of P. Treuting

The Mature Mouse Placenta

E12.5

Embryonic erythroid cells

Decidua

Yolk sac

Chorionic plate

Labyrinth

Uterus

The Mature Mouse Placenta

E12.5

Embryonic endothelium

Maternal rbc

Dam's rbc

Embryonic and the labyrinthine trophoblasts (STB)

Mature erythrocytes of embryo and dam

Fri 2 Ward  Embryo

Ward P 5
Genes Expressed in Placenta

- Endothelium - MECA-32, CD31
- TB giant cells - Prolactin
- Decidual cells – MAC2
- Spongiotrophoblast - SOS-2

How Many Embryonic Lethals?


472 knockout mouse lines created for secreted and transmembrane proteins by Genentech and Lexicon Pharmaceuticals

37/472 = 7.7% embryonic lethal

Abnormal Development can lead to Embryonic Mortality

- Abnormal development of the placenta, its membranes and embryo may occur at any stage of gestation
- The consequences of abnormal organ or tissue development may result in embryonic lethality at any stage of gestation, may occur after birth or may have no effects depending on the organ or tissue affected and functions of that organ
- Gene inactivation or modification may cause no or abnormal protein product synthesis in specific cells, tissues and organs, which can result in abnormal organ development and/or function
- Gene inactivation or modification may cause no development defect or even a functional defect, in part, due to gene redundancy
- A developmental defect in one organ or tissue may have consequences in other organs or tissues

The Jackson Laboratory Mammalian Phenotype Browser

http://www.informatics.jax.org/searches/Phat.cgi?id=MP:0001672
Identification of Developmental Defects in Mouse Embryos

- 0-15% of wild type (+/+) embryos developing in wild type or heterozygous dams can develop spontaneous embryonic defects which may result in *in utero* embryonic deaths (Peters, JM et al, 1996; Muralidhara, N et al, 1997)
Morphological Changes of The Mouse Embryo

- Decreased Growth (growth retardation, growth arrest, delay of development)
- Death
- Malformations
  - Head size and shape
  - Limb structure
  - Others
- Beating heart
- Pericardial sac distension
- Edema
- Hemorrhage
- Abdominal distension

Progressive Embryonic Death

3/12 Abnormal/hemorrhagic
Dead embryo
Early death
Resorption

Possible Causes of Embryonic Mortality E9-12

- Placental failure
- Membrane abnormalities
- Cardiovascular abnormalities
  - Abnormal embryonal blood vessel development
- Heart failure
- Abnormal erythropoiesis (in yolk sac/embryo)
- Loss of normal cell cycle regulation
- Cell adhesion/germ layer defects/patterning

The Placenta Is Important For Embryonic Growth From E9-12

If it develops abnormality or fails, the embryo will die due to lack of normal nutrition received from the dam through the placenta
How to Evaluate Placental Causes of Embryo Lethality

- Determine patterns of gene expression in placenta, membranes and embryo at various stages of gestation—ISH/IHC/Northern blotting
- Determine cells and anatomic placental/embryonal structures normally expressing the gene
- In null mice, these cells may not function normally for placental development
- Null mutation>abnormal placenta development->embryonic death

Developmental Abnormalities in The Mouse Placenta

- Labyrinth vasculogenesis (94 genotypes) - VHL, Tfeb, Dlx3, PPARγ, Mash-2, HGF/SF, EGFR, LIFR, IL11Ra, ERR-β, JunB, HSP90β, Cyr61(CCN1), RAP250
- Labyrinth trophoblasts - VHL, EGFr, Mash-2, RXRa, ARNT, LIFR, ERR-β, I-mfa, HSD17B2, Cx26
- Spongiotrophoblasts - ARNT
- Giant cell trophoblasts - I-mfa, Cdkn1c
- Giant placenta – cloned mice, Cdkn1c
- Decidua - IL11Ra

Abnormal Placenta Vascular Morphology

http://www.informatics.jax.org/searches/Phat.cgi?id=MP:0001711

Vasculogenesis of the Placental Labyrinth

- Angiogenesis – the formation of new blood vessels from pre-existing blood vessels (endothelium)
- Vasculogenesis - the formation of new blood vessels when there are no pre-existing ones; formation of new blood vessels from mesenchyme, in mouse embryo from the chorionic plate (CP)

Vascular Lesions in the Placenta

- Vascular lesions in various genotypes and stages of pregnancy
- Hemorrhagic placental sites 3/12 KO
- Lack of normal vasculogenesis >disruption of normal placental development

Abnormal Vasculogenesis of The Labyrinth

- CD31 (formalin, Santa Cruz M-20, goat anti-mouse, 1:500, antigen retrieval)
**Trophoblast Lesions**

- Degenerative Changes of Labyrinth Trophoblasts
- Eosinophilic Droplets in Trophoblastic Giant Cells
- Dysplasia Syncytial Labyrinth Trophoblasts

**Summary**

**How To Determine If Placental Failure Occurs**

- E9.5-10.5 determine if embryo is grossly normal
- No histological embryonic changes seen that could cause death
- Check especially heart, blood vessels, yolk sac, and any tissues where gene is expressed
- Beating heart
- Placenta – fusion of chorion and allantois, vasculogenesis into labyrinth, differentiation of trophoblasts
- Gene expression – placenta, membranes

**Knockout Mice: Lethality - E12-19**

- Rb - hematopoietic & neuronal abnormalities
- Tyrosine hydroxylase - abnormal cardiac myocytes
- Nf1 - Heart malformation
- PTH1R – dilation and hemorrhage in pericardial sac, myocardial necrosis
- Wt1 – edema, small heart, pericardial blood

**Embryonic Hemorrhage >E13**

- Yolk sac vessels empty
- No platelets
- Abnormal brain angiogenesis
- Cullen et al, PNAS 108: 5759, 2011
- Hemorrhage due to handling +/-

**Survival of Newborn Mice**

- Percent alive

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**Perinatal, Neonatal and Postnatal Deaths**
Determining Causes of Death of Neonatal and Older Mice

- Gene expression studies in wild type mice
- Age at death (embryonic->adult)
- Clinical signs
- Gross pathology
- Histopathology screen of many tissues (if cause not obvious or predictable)
- Lesion causing interference with vital cell, tissue or organ function
- Secondary effect of lesion that interferes with vital function

Major Causes of Neonatal Death in GEM

- Skeletal IKK1, p63, Egfr, Gdf11
- Skin integrin B4, integrin a6, LAMA3
- Cardiovascular abnormalities cn43, uMHc, NMHC-B, trkC, MLP, MnSOD, NMM II-b, TBX1
- CNS Pax2, Pax6, en-1, wnt-1, p53, Gadd45, NMHC-B, t/ebp
- Kidney BF2, Ieh, BHD
- Metabolic (includes breathing-respiratory distress) AhR, c/ebp, insr, CYP1A2, a2Vac, OAT, Hmg1, Ndh
- Others, Multiple Defects, Unknown COD
- Others in adults - Krauss R (Editor), Mouse Models of Developmental Genetic Disease, Volume 84 (Current Topics in Developmental Biology), Academic Press, 2008

Skin, Bones and Brain

Neonatal Lethality

Embryos can survive until birth with abnormal or no brain, lungs, kidneys, thyroid, pituitary, skin, gonads, and other tissues

Kidney

Exencephaly in 23% of p53 -/- Mice
Defect in Neural Tube Closure

Armstrong et al Current Biology 5: 931, 1995

C. Stewart 2000

Fri 2 Ward  Embryo

Fri 2 Ward  Embryo

Ward P 11

JM Ward JHU 2012
Birt-Hogg-Dubé Syndrome (BHD)

- Humans - Germline mutations in the BHD/FLCN tumor suppressor gene predisposes patients to develop renal tumors in the hamartoma syndrome, Birt-Hogg-Dubé (BHD).
- BHD encodes folliculin, a protein with unknown function that may interact with the energy- and nutrient-sensing AMPK-mTOR signaling pathways.

BHD (Birt-Hogg-Dubé)


Heart Development

- E9-11 looping and chamber formation of primitive heart tube
- E11-13 septation
- E12-birth cardiac conduction system
- Birth adaptation to adult life/birth, close interarterial and aorta-pulmonary trunk shunts
- Edema – classic gross finding
- Studied best by MRI or other imaging

Neonatal Lethality: Heart Failure

(Tullo et al, PNAS 94:12407, 1997)

nonmuscle myosin heavy chain B (NMHC-B)

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Liver
Congestive heart failure
Null Neonatal Deaths in New GEM

Right-sided failure

Backward failure of the right ventricle leads to congestion of systemic capillaries. This generates excess fluid accumulation in the body. This causes swelling under the skin (peripheral edema or anasarca).

Histological findings

- Edema in various tissues
- Lung – macrophages and pigment in alveoli
- Liver – congestion and hemorrhages

Known cardiac expression of the null gene

Shown by MRI to have multiple developmental heart defects

Bhattacharya S
Genetic mechanisms controlling cardiovascular development.


Lung

Respiratory Distress

Neonatal lethality – defect in lung development?

A hidden subtle cause of death?

CYP1A2


Pathology of Mouse Development

CYP1A2

Ndn<sup>tm25tw</sup> mutant mice

Nedrin (neurally differentiated embryonal -cell derived factor)

Deficient in human Prader-Willi syndrome

A developmental neurobehavioral disorder with respiratory abnormalities

Mice – neonatal death, no histopath lesions

"pups gasped for air, turned cyanotic, & died over a postnatal time course of a few hours" (J Ren et al, J Neuroscience 23: 1569, 2003)
**Summary**

- Dissect carefully placenta, membranes and embryo
- Evaluate each embryo grossly, record findings
- Evaluate histologically
- Perform ISH or other methods for gene expression
- Correlate all gross and histological abnormalities with gene expression patterns and possible gene functions
- Get help from a mouse developmental biologist
- Conclude the mechanism(s) of the developmental defect(s) and its consequences

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**Mouse Placenta References**

- Comparative Placentation (K. Benirschke)  
  [http://medicine.ucsd.edu/cpa](http://medicine.ucsd.edu/cpa)

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**Mouse Embryo References**


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**Thanks for Listening**