Mouse Models of Cancer

Early History of mice in research

- Mice were originally studied by European zoologists as a means to test Galton's law of ancestral inheritance (latter half of the 19th century).
- “Fancy” mice were bred with unusual traits that could be quantified to test genetic laws.
- With the re-discovery of Mendel's laws of inheritance in the early 1900's, the mouse was repurposed to test whether Mendelian genetics also applied to mammals.
- In 1902, Lucien Cuenot confirmed that coat color inheritance followed Mendelian inheritance patterns.
- In 1904, Cuenot also proved the presence of two alleles at the same genetic locus.

Early History of mice in research

- In 1908, William Earnst Castle established the Bussey Institute at Harvard as a center for mammalian genetics. Castle and his undergraduate, Clarence Cook Little, expanded coat color studies to 9 genetic loci, and demonstrated the first lethal phenotype (A') in mammals. Inbred strains were established.
- As a grad student, C.C. Little joined the lab of Earnst Edward Tyzzer at Harvard, and studied the transplantation of spontaneous tumors between mice of different strains.
- From this work, Little and Tyzzer (1916) accurately predicted some basic concepts in immunology, specifically the multi-gene basis for immune recognition of “self”.
- C.C. Little went on to become the President of the University of Michigan, and later to found the Jackson Laboratories in Maine.
History of mice in cancer research

- Extensive efforts to identify mice with high or low cancer incidence culminated in 1933 with the discovery that the tendency to inherit mammary tumors in mice had both genetic components and non-genetic components.
- For more than 50 years, "forward genetics" was the mainstay. Mice prone to developing cancer were isolated or induced to understand the underlying mutations.
- The development of restriction enzymes and the isolation of embryonic stem cells in the 1970's led to the first engineering of mouse genes in the 1980, and the dawn of "reverse genetics" in mice. Mutations in specific genes could be induced and the resulting phenotype studied.

Forward genetics: The study of mutations that cause a known phenotype.
Reverse genetics: The study of phenotypes that are caused by a known mutation.

Uses of Mouse Models of Cancer

- Studies of cancer gene function (Mouse +/- Gene X)
- A source of genetically defined cells of many types (Isolate cell lines from different tissues)
- Understand the biology of specific diseases (Engineer mice with a type of cancer, and study how it works)
- Understand the role of environment in disease and its interaction with genetics
- Understand the interactions of drugs with cancer (Pharmacology and molecular biology of tumors treated with drugs)
- As final efficacy screens before clinical trials (pre-clinical trials)
- Better interpret the results of clinical trials (co-clinical and post-clinical trials)

Advantages/Disadvantages of Cancer Models

<table>
<thead>
<tr>
<th>Model</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Humans</td>
<td>Most accurate</td>
<td>Least manipulable</td>
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<tr>
<td></td>
<td></td>
<td>Extremely slow generation time (16 - 40 years)</td>
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<tr>
<td></td>
<td></td>
<td>Generally outbred</td>
</tr>
<tr>
<td>Bacteria</td>
<td>Extremely fast growth (minutes)</td>
<td>Most accurate</td>
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<tr>
<td></td>
<td></td>
<td>Powerfull genetic tools</td>
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<tr>
<td></td>
<td></td>
<td>Powerful biochemical techniques</td>
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<tr>
<td>Yeast</td>
<td>Very fast growth (100 minutes)</td>
<td>Haploid, unicellular</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Powerful biochemical techniques</td>
</tr>
<tr>
<td>Mammalian</td>
<td>Fast growth (6 - 8 hours)</td>
<td>II genets</td>
</tr>
<tr>
<td>Cell Culture</td>
<td></td>
<td>Only one or a few cell types (no stroma)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No immune system</td>
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<tr>
<td></td>
<td></td>
<td>Often inaccurate in predicting response to therapy</td>
</tr>
<tr>
<td>Drosophila</td>
<td>Multicellular</td>
<td>Significant evolutionary divergence</td>
</tr>
<tr>
<td></td>
<td>Powerfull genetic tools</td>
<td>Differences in organ composition/structure</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Not useful as efficiently screen</td>
</tr>
<tr>
<td>Zebrafish</td>
<td>Vertebrate</td>
<td>Significant evolutionary divergence</td>
</tr>
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<td>Differences in organ composition/structure</td>
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<td></td>
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<tr>
<td>Mice</td>
<td></td>
<td>Significant evolutionary divergence</td>
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<td>Differences in organ composition/structure</td>
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<tr>
<td></td>
<td></td>
<td>Not useful as efficiently screen</td>
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<tr>
<td></td>
<td></td>
<td>Few antibodies available</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Difficult to interpret histopathology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Very slow generation time (3-4 months)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Slow generation time (9 weeks)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice are not small, furry people</td>
</tr>
</tbody>
</table>
Classes of Mouse Models of Cancer

- **Viral Infection Models**
  - Infection with natural or engineered viruses
  - Treatment with cancer-causing viruses

- **Chemical Induced Carcinogen Models**
  - Treatment with cancer-causing chemicals

- **Transplantation Models**
  - Implantation/Injection with tumor tissues or cells

- **Engraftment Models**
  - Incorporation of tumorigenic cells into normal tissues

- **Transgenic Models**
  - Randomly inserting tumor-promoting DNA into the mouse genome

- **Genetically Engineered Models**
  - Targeted manipulation of specific sites in the mouse genome

- **Somatic Engineering Models**
  - Targeted manipulation of specific sites in the mouse

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### Chemical Carcinogen Models

- 1775 - Sir Percival Pott, a London surgeon, noticed that chimney sweepers frequently developed a peculiar form of scrotal cancer. He ascribed it to frequent, direct contact with coal tar. This launched 125 years of research into the chemical basis of cancer. 

- Treatment of mice with carcinogens is the basis of numerous mouse models of cancer.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Carcinogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>7,12-dimethylbenzanthracene (DMBA) + 2-O-tetradecanoyl-phorbol-13-acetate (TPA)</td>
</tr>
<tr>
<td>Lung</td>
<td>Nitrosamines</td>
</tr>
<tr>
<td>Liver</td>
<td>Vinyl chloride</td>
</tr>
<tr>
<td>Breast</td>
<td>4-nitroso-N-methylurea (NMU)</td>
</tr>
<tr>
<td>Colon</td>
<td>Dimethylhydrazine (DMH)</td>
</tr>
<tr>
<td>Bladder</td>
<td>Aromatic Amines</td>
</tr>
</tbody>
</table>

**Advantages**
- Very quick, cheap, and easy to use
- Some models use common environmental carcinogens

**Disadvantages**
- Challenging to identify the mutations induced by a chemical in a given cell or tumor

**Uses**
- Replicate exposure to complex environmental carcinogens such as cigarette smoke

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### Viral Infection Models

- **Cancer-causing viruses** such as the Rous Sarcoma Virus (RSV) were initially discovered in chickens in 1911 by Peyton Rous (Nobel Prize, 1966) at Rockefeller U.

- **Mouse Mammary Tumor Virus (MMTV)** was the first mouse virus, isolated at Jackson labs as the "non-chromosomal factor" that caused mammary tumors in the C3H strain of mice. Transmitted through milk - pups fostered to mothers of other strains did not develop breast tumors. (John Joseph Bittner, 1936)

- Some viruses cause cancer via random integration in certain cells

- Some viruses carry cellular oncogenes
  - Abelson murine leukemia virus - Abl
  - Moloney murine sarcoma virus - Raf
  - FBJ rat sarcoma virus - Fos
  - Friend murine leukemia virus - c-fms

- This was predicted by Ed Stadtman in 1973, and proven by Harold Varmus*, Mike Bishop*, Peter Vogt in 1976 (*1989 Nobel Prize in Physiology/Medicine)

- The finding that viruses simply regulate endogenous genes diminished interest. However, Gardasil (Merck) is a highly effective vaccine against HPV induced cervical cancer.

- Engineered viruses now used routinely in the laboratory to induce genetic changes that lead to cancer.
Transplantation Models
Tumor cells or tissues transplanted into a host mouse
- Pioneered in the 1960’s and 1970’s

Ectopic – Implanted into a different organ than the original (typically subcutaneous or kidney capsule)
Orthotopic – Implanted into the analogous organ of the original tumor

Advantages – Typically cheap and fast
Typically easy to use
Not covered by patents

Disadvantages – Inaccurate histopathology compared to human tumors
Lack of step-wise progression through pre-neoplastic stages
Evolution of tumor cells during passaging
Requirement for angiogenesis to support the newly transplanted tumor
Historically poor ability to predict response to therapy in humans

Transplantation Models
Cell line based xenografts

Advantages – Very easy to maintain cell lines indefinitely;
Very fast to generate large numbers of xenograft tumors
Relative homogeneity of tumors makes it easy to detect differences
Comparatively cheap

Disadvantages – Inaccurate histopathology compared to human tumors
Lack of an immune system
Selection of cells during adaptation to growth on plastic
Evolution of tumor cells during culture
Historically poor ability to predict response to therapy in humans

Transplantation Models
Cell line based syngeneic allografts

Advantages – Very easy to maintain cell lines indefinitely;
Very fast to generate large numbers of xenograft tumors
Relative homogeneity of tumors makes it easy to detect differences
Comparatively cheap
Intact immune system

Disadvantages – Mouse cells rather than human
Inaccurate histopathology compared to human tumors
Selection of cells during adaptation to growth on plastic
Evolution of tumor cells during culture
Historically poor ability to predict response to therapy in humans
Transplantation Models

Patient-derived xenografts (PDX models)

Advantages –
- Neoplastic cells are human and may better interact with drugs designed for human proteins
- Transplant both neoplastic cells and stroma
- Each PDX in a mouse represents the specific human patient
- No adaptation acquired during cell culture
- May be serially passaged in mice

Disadvantages –
- Lack of an immune system
- Stromal cells are rapidly depleted over 1-3 passages
- Requirement for angiogenesis to support the newly transplanted tumor
- Requires a major infrastructure to acquire and maintain human tumors
- Relatively new- unclear how accurate they are

Engraftment Models

Organ reconstitution models - cell engraft and create a normal organ, from which focal cancer then emerges

Advantages –
- Step-wise progression through pre-neoplastic stages
- Can engineer the donor cells to study gene function or add reporters
- Relatively rapid and cheap
- Amenable to powerful genetic screening approaches

Disadvantages –
- Lack of an immune system when using human donor cells
- Only available for a few organ systems: breast, liver, hematopoietic

- Culture 3D organoids in a specialized matrix
- Genetically modify organoid cells
- Inject into normal organ (pancreas, colon)
- Develop into normal cells, then premalignacies, then cancer.

Mammary reconstitution - (Karreman et al. 2008, PNAS 105(49):19635-71)
- Isolate mammary epithelial cells from donor mice
- Genetically manipulate donor cells (transfected)
- Inject into cleared fat pad
- Develop into normal cells, then premalignancies, then cancer.

Liver reconstitution - (Scott Lowe lab)
- Isolate fetal liver cells
- Genetically manipulate donor cells
- Inject fetal liver cells into newborn liver

Hematopoietic reconstitution - (many laboratories)
- Genetically manipulate donor cells
- Inject fetal liver cells into newborn liver
- Lymphoma, myeloid leukemias
Engineering Cancer in Mice

- Mice can be genetically engineered to develop cancer
- Use the human disease as a guide
- Mutations found in cancer can be programmed into mice
- Not all mice that develop cancer are “good” models of human disease
- Compare tumors in mice to human tumors- validation

Embryonic Stem Cell
- Mutant Embryonic Stem Cell
- Injection
- Chimera

This video has been kindly provided by Johannes Wilbertz, from the Karolinska Institute, in Stockholm (Sweden), and belongs to a large collection of educational videos available from the International Society for Transgenic Technologies (ISTT) web site. https://www.youtube.com/watch?v=1m9kQuKXxEA
Genetically Engineered mice

**Advantages** –
- Step-wise progression through pre-neoplastic stages
- Can engineer specific mutations to study gene function or add reporters
- Well-established and understood technology
- Amenable to powerful genetic screening approaches
- Powerful genetic tools for imaging and other applications
- Tumors develop in the presence of an intact immune system
- Can model both the neoplastic component and stroma cells
- Indications that the “best” engineered models are more accurate in predicting the response of human tumors to therapy

**Disadvantages** –
- Mice are not people; mouse cells are not human cells
- Laboratory strains fail to represent the genetic diversity of human population
- Mouse tumors typically grow very fast relative to human tumors
- Complicated engineering strategies typically have drawbacks
- Relatively slow and expensive
- By providing one or more “hits”, genetically engineered tumors are able to shortcut evolution, and therefore they develop less complex genomes

Transgenic Models

Randomly integrate DNA constructs into ES cells

- cDNA transgenic
- tissues-specific transgenic
- BAC transgenic
- BAC transgenic

**Uses** –
- Demonstrate what happens when you overexpress gene X
- Used to create tissue specific gene regulatory elements for more complex strategies

**Advantages** –
- Easy to engineer. Comparatively rapid and cheap

**Disadvantages** –
- Random site of integration can lead result in positional effects
- Overexpression of cDNA lacks the physiological gene regulation

**Advantages:** Rapid way to learn what reduced expression of a gene does. May mimic drug action.

**Disadvantages:** Never get zero expression.

**Advantages:** Extremely rapid generation of transgenic lines

**Disadvantages:** Random integration. Can be multiplicity. Silencing of viral LTR promoters is common
Genetically Engineered Mouse Models

Uses –
Precisely engineer gene loss, gene addition, subtle mutations, reporter constructs into specific places within the mouse genome.

Advantages –
Physiological gene regulation.

Disadvantages –
Slow and more difficult to carry out.
Targeted mutations can cause embryonic lethal phenotypes.

Targeted integration of DNA into specific sites within the genome through homologous recombination.

Mouse genomic locus

Area to be targeted

Targeting construct

Mouse genomic locus

Area to be targeted

Targeting construct

Types of GEM Models

Wild-type locus

Examples

Knockout mouse

Uses: Loss of function mutations in tumor suppressor genes.

Knockin mouse

Uses: Reporter alleles for developmental biology

Gene function analysis.

Targeted Mutant

Uses: Targeting subtle mutations into mice.

Latent mice rely on stochastic recombination in vivo

Uses –
Spontaneous tumor models.

Advantages –
Random cells are mutated, surrounded by wild-type cells.

Disadvantages –
Don’t know which cells underwent recombination.
Different cell types have different rates of recombination.
Alleles are null prior to recombination.

K-ras^{LA1} Lung Cancer Model

p53 \(+/-\): lymphomas, sarcomas
K-ras: pharyngeal tumors

K-ras^{H12}\textsuperscript{RAS}: ID differences between K-ras and H-ras

K-ras^{H12}\textsuperscript{RAS}: marking Shh cells

p53 \textsuperscript{R172H}(-): lymphomas, sarcomas, and carcinomas
Advanced genetic tools enable construction of complex gene regulation strategies

**Recombinases** – Enzymes that catalyze site specific recombination

**Inducible alleles** – Genes or proteins sensitive to the presence of a specific chemical

**Transposons** – Mobile genetic elements who excision and reinsertion into the genome is catalyzed by an enzyme (transposase)

**Reporters** – Genes that allow visualization through microscopy, histochemistry, or in vivo imaging

*Cre* is a site-specific bacterial recombinase

Cre catalyzes two types of reactions

- **Excision**
  - LoxP
  - LoxP
  - Cre
  - +

- **Inversion**
  - LoxP
  - LoxP
  - Cre
  - LoxP
  - LoxP
Variant Lox sites have been identified with useful properties

Unidirectional Inversion (10-fold weighting towards forward reaction)

Incompatible Sites

Conditional mouse models

Cre/lox technology is widely used to create conditional alleles

<table>
<thead>
<tr>
<th>Conditional knockout</th>
<th>Conditional activatable</th>
<th>Conditional mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-targeted Cells Before After</td>
<td>+/- +/-</td>
<td>+/- +/-</td>
</tr>
<tr>
<td>Targeted Cells Before After</td>
<td>+/- +/-</td>
<td>+/- +/-</td>
</tr>
</tbody>
</table>

Uses — Conditional loss of gene function in mice

Advantages — Spatial control can be achieved by expressing Cre from a tissue specific promoter
Temporal control can be achieved by expressing Cre from a stage specific promoter, or by using inducible alleles to control Cre expression
Gene is COMPLETELY lost from recombined cell

Disadvantages — Requires two alleles: conditional knockout, and Cre allele
Because deletion is a binary event, even very low-level expression of Cre yields a 100% deletion

Uses — Conditional expression of wild-type or mutant proteins under endogenous regulation

Advantages — Physiological control of gene expression
Spatial and temporal restriction of gene expression

Disadvantages — Requires two alleles: conditional knockout, and Cre allele
Allele is null prior to recombination
Sensitive to low-level Cre expression
Inducible alleles

Tetracycline inducible genes

\[ \text{rTA} \]

Tet inducible gene

Uses – Dose-dependent expression of gene products through administration of tetracycline

Advantages – Precise temporal and amplitude control of gene expression

Disadvantages – Requires two alleles: tet inducible allele and rTA

Little spatial control

Non-physiological gene expression

Tamoxifen inducible alleles

Uses – Regulation of protein activity through administration of Tamoxifen

Advantages – Can control time and place of expression

Tighter than tet-induced systems

Disadvantages – Tamoxifen is expensive

Targeted protein must tolerate an N- or C-terminal fusion

Reporter alleles

Activate expression of a marker gene under given conditions

Cre reporter: Activated when LoxP sites are removed

Pathway reporter: Activated proportional to the activity of a transcription factor

Fusion protein: Reporter protein is directly fused to another protein of interest

Reporter genes are typically optically based (visible, near-infrared)

May be imaged by luminescence, fluorescence

Instruments include 2-photon microscopy and whole animal imaging

May also include reporters for MRI, PET, and SPECT
Putting it together: the Brainbow Mouse

Creativity

Test in vivo

No Cre

x Cre

OPFP

M-OPFP

M-YPFP

M-YPFP

M-CFP
The coming revolution... has arrived, kinda

- CRISPR = Clustered Regularly Interspaced Short Palindromic Repeats
- Cas = CRISPR-associated

- Used by bacteria/archaea as an “adaptive immune defense”
- Involves targeted degradation of foreign nucleic acids using short RNA and Cas9. 
  [http://www.youtube.com/watch?v=M739wgbcKuA](http://www.youtube.com/watch?v=M739wgbcKuA)

Theme and variation
Somatically Engineered Mouse Models

Uses –
- Generating specific mutations on a wild-type background
- Engineering tumor mutations in mice with genetically altered stromal cells

Advantages –
- Specific mutations (mostly)
- Immune competent
- Stochastic alteration of cells - not every cell in a tissue is mutant!
- No background mutations
- Does not require years of breeding
- Cheap and fast relative to GEMMs

Disadvantages –
- Complex alleles are not yet possible
- Inefficient targeting in vivo - just 1-2% of cells are transduced.
- Not yet developed for all organ systems
- Lack of available reporter genes

Small animal imaging enables the study of mouse models

- Vevo 2100 Ultrasound
- IVIS Quantum FX Micro CT
- Bruker 9.4T MRI
- IVIS Spectrum
Ultrasound is a non-invasive imaging technique.

Small Animal Imaging SR

Tomography can be used to reconstruct 3D tumor volumes.

Pancreatic Tumor
Contrast ultrasound shows pancreatic tumors are poorly perfused.

Micro CT Imaging is ideal for bone and lung.

Co-registration of optical + CT data.